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Comparative Study on Free Amino Acid Composition of Wild Edible Mushroom Species

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A comparative study on the amino acid composition of 11 wild edible mushroom species (*Suillus bellini*, *Suillus luteus*, *Suillus granulatus*, *Tricholomopsis rutilans*, *Hygrophorus agathosmus*, *Amanita rubescens*, *Russula cyanoxantha*, *Boletus edulis*, *Tricholoma equestre*, *Fistulina hepatica*, and *Cantharellus cibarius*) was developed. To define the qualitative and quantitative profiles, a derivatization procedure with dabsyl chloride was performed, followed by HPLC-UV−vis analysis. Twenty free amino acids (aspartic acid, glutamic acid, asparagine, glutamine, serine, threonine, glycine, alanine, valine, proline, arginine, isoleucine, leucine, tryptophan, phenylalanine, cysteine, ornithine, lysine, histidine, and tyrosine) were determined. *B. edulis* and *T. equestre* were revealed to be the most nutritional species, whereas *F. hepatica* was the poorest. The different species exhibited distinct free amino acid profiles. The quantification of the identified compounds indicated that, in a general way, alanine was the major amino acid. The results show that the analyzed mushroom species possess moderate amino acid contents, which may be relevant from a nutritional point of view because these compounds are indispensable for human health. A combination of different mushroom species in the diet would offer good amounts of amino acids and a great diversity of palatable sensations.

KEYWORDS: Wild edible mushrooms; free amino acids; nutritional value

INTRODUCTION

Mushrooms are of increasing importance in modern nutrition and medicine (*1*). They are appreciated not only for their texture and high content of flavor components but also because they are rich in proteins and amino acids and poor in calories (*2, 3*).

Amino acid composition is a reliable indicator of the nutritional value of food, including mushrooms (*1, 2*). It is also known that some amino acids contribute to the delicious taste of mushrooms, making them attractive for consumption (*4*).

In addition to their role as protein monomeric units, α-amino acids are energy metabolites and precursors of many biologically important nitrogen-containing compounds, such as heme, glutathione, various hormones, nucleotides, nucleotide coenzymes, physiologically active amines (neurotransmitters) (*5*), and alkaloids (*6, 7*). The excess dietary amino acids in mammals is neither stored for future use nor excreted. Rather, they are converted to common metabolic intermediates, such as pyruvate, oxaloacetate, acetyl-CoA, and α-ketoglutarate. Consequently, amino acids are also precursors of glucose, fatty acids, and ketone bodies and are, therefore, metabolic fuels (*5*).

A failure in nonessential amino acid production or in dietary amino acid intake can lead, directly or indirectly, to damages to health, namely, compromising neurotransmitter biosynthesis, allergic responses, and some blood functions (*5*).

The Trás-os-Montes region (northeastern Portugal) is known for the variety of its soils and diversity of climate conditions. This variability assumes an important role in mushroom production, explaining why this region is recognized as one of the richest ones in wild edible species. The mushroom species growing there are usually consumed fresh, dried, during the off-season, or canned, offering a diversity of organoleptic characteristics and palatable sensations to the consumer.

In the present work, 11 wild edible mushroom species spontaneously occurring in this region were studied: *Tricholomopsis rutilans*, *Hygrophorus agathosmus*, *Amanita rubescens*, *Russula cyanoxantha*, *Boletus edulis*, *Tricholoma equestre*,*Suillus bellini*, *Suillus luteus*, *Suillus granulatus*, *Fistulina hepatica*, and *Cantharellus cibarius*. Despite the high consumption of these species, as far as we know, nothing has been reported about the free amino acid profiles of *S. bellini*, *H. agathosmus*, *R. cyanoxantha*, *T. equestre*, and *F. hepatica*. For the remaining

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Table 1. Characterization of Mushroom Samples

sample	species	origin	orchard	date of collection
1	*Tricholomopsis rutilans*	Bragança	*Pinus pinaster*	Dec 2004
2	*Hygrophorus agathosmus*	Vinhais	*Pinus pinaster* + *Castanea sativa*	Dec 2005
3	*Amanita rubescens*	Bragança	*Castanea sativa*	June 2005
4	*Russula cyanoxantha*	Bragança	*Quercus pyrenaica*	May 2006
5	*Boletus edulis*	Bragança	*Castanea sativa*	June 2005
6	*Tricholoma equestre*	Carrazeda de Ansiães	*Pinus pinaster*	Nov 2005
7	*Suillus bellini*	Bragança	*Pinus pinaster*	Nov 2004
8	*Suillus luteus*	Vinhais	*Pinus pinaster* + *Castanea sativa*	Nov 2005
9	*Suillus granulatus*	Bragança	*Pinus pinaster*	Nov 2005
10	*Fistulina hepatica*	Bragança	*Castanea sativa*	Sept 2004
11	*Cantharellus cibarius*	Bragança	*Castanea sativa*	May 2005

analyzed species, data found in the literature are scarce, and only a few amino acids were described.

For *T. rutilans* previous works concerned the isolation of L-3-(3-carboxy-4-furyl)alanine (*8*) and L-2-aminohex-4-ynoic acid (*9*) and the identification of 2-amino-3-hydroxyhex-4-ynoic acid as a mixture of *threo*- and *erythro*-2-amino-3-hydroxyhex-4-ynoic acid forms (*10*). For *R. cyanoxantha* species, L-2-amino-7-hydroxyoctanoic acid was isolated from its fruiting bodies (*11*). Early studies on *A. rubescens* (*2*), *S. luteus* (*12*), and *S. granulatus* (*1, 12*) reported the occurrence of some free amino acids. Regarding *B. edulis*, its free amino acid profiles were determined (*13*−*15*), as well as the contents of proline in the presence of exceeding metals (*16*). Studies were also conducted on some new combined forms of glutamic acid (*17*) and on the identification of selenocystine and selenomethionine in this species (*18*). Some studies about the free and bound amino acid composition of *C. cibarius* (*1, 19*) and concerning the exudation and storage of those compounds (*20*) have already been performed.

In the sequence of the chemical characterization that we have been developing on these mushroom species, namely, involving phenolics (*21*−*24*), organic acids (*21*−*24*), alkaloids (*24*), and aroma compounds (*3*), the study of their free amino acid composition was conducted with the purposes of finding the qualitative and quantitative profiles of *T. rutilans*, *S. bellini*, *H. agathosmus*, *R. cyanoxantha*, *T. equestre*, and *F. hepatica* for the first time, of improving the knowledge about *A. rubescens*, *S. luteus*, *S. granulatus*, *B. edulis*, and *C. cibarius* species, and, ultimately, to compare the different species.

Thus, we proceeded to a derivatization method with dabsyl chloride, prior to the HPLC-UV−vis determination of the resulting dabsyl amino acids. Dabsyl derivatives have a number of advantages over other derivatives, including simple derivatization procedure, good reproducibility, low detection limit, and good HPLC separation of all the amino acids (*25*).

MATERIALS AND METHODS

Standards and Reagents. All L-amino acid standards, sodium phosphate, triethylamine, *N*,*N*-dimethylformamide, and sodium bicarbonate were purchased from Sigma (St. Louis, MO). Acetonitrile, orthophosphoric acid, ethanol, and acetone were obtained from Merck (Darmstadt, Germany). Hydrochloric acid was purchased from Panreac Quimica SA (Barcelona, Spain). Dabsyl chloride (4-dimethylaminoazobenzene-4′-sulfonyl chloride) was from Fluka (Buchs, Switzerland). Sodium hydroxide was obtained from Pronalab (Lisboa, Portugal). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA).

Sampling. Samples of wild edible mushroom species were collected in the Trás-os-Montes region (northeastern Portugal), and their characterization is described in **Table 1**. Each sample corresponds to the mixture of three to five individuals. After harvesting, the mushrooms were immediately transferred to the laboratory. Taxonomic identification followed that of several authors (*26*−*31*), and representative voucher specimens were deposited at the herbarium of Escola Superior Agrária of Instituto Politécnico de Bragança. Samples were dehydrated in a ventilated oven at 30 °C for 7 days. All materials were kept in the dark, in hermetically sealed bags, and powdered (910 μm) before analysis.

Extracts Preparation. For the amino acid profile characterization, an aqueous extract was prepared: ca. 10 g of powdered mushroom was boiled in 500 mL of water during 30 min, followed by filtration over a Büchner funnel. The resulting extract was then lyophilized in a Labconco 4.5 Freezone apparatus (Kansas City, MO) and a yield of ca. 0.713 g, on average, was obtained. The lyophilized extracts were kept in a desiccator, in the dark. For amino acid derivatization, the lyophilized extracts were redissolved in 0.1 M hydrochloric acid.

Derivatization Procedure. *Dabsyl Chloride Solution (12.4 mM).* Dabsyl chloride was dissolved in acetone and then filtered and stored at −20 °C.

Elution Buffer. It consisted of a mixture of 9 mM sodium phosphate, 4% dimethylformamide, and 0.15% triethylamine (pH 6.55 with phosphoric acid).

Reaction Buffer (0.15 mol/L $NaHCO_3$, pH 8.6). Sodium bicarbonate was dissolved in Milli-Q-Water, adjusted to pH 8.6 with diluted NaOH.

Dilution Buffer. A mixture of 50 mL of acetonitrile, 25 mL of ethanol, and 25 mL of elution buffer was prepared.

Assay. Aliquots of 20 μL of standard solution or mushroom lyophilized extract in 0.1 M HCl were diluted with 180 μL of reaction buffer. After mixing, 200 μL of dabsyl chloride solution was added. The vials were mixed again and incubated at 70 °C for 15 min. The reaction was stopped by placing the vials in an ice bath for 5 min. Four hundred microliters of dilution buffer was added, followed by mixing and centrifugation (5 min at 5000 rpm). The clear supernatants were stored at −20 °C until analysis (*32*).

HPLC Analysis of Amino Acids. After a precolumn derivatization of the free amino acids, the dabsyl derivatives were separated on a HPLC unit (Gilson), using a reversed-phase Spherisorb ODS2 (25.0 × 0.46 cm; 5 μm particle size) column. Twenty microliters of the derivatized standard or samples was injected. The solvent system was composed of two eluents: elution buffer (A) and acetonitrile 80% (B). Elution was performed at a flow rate of 1 mL/min, starting with 20% B until 7 min and installing a gradient to obtain 35% B at 35 min, 50% B at 45 min, and 100% B at 66 min, maintaining 100% B until 76 min. Detection was achieved with a UV−vis detector at 436 nm. Free amino acid quantification was accomplished by the absorbance recorded in the chromatograms relative to external standards. The peaks in the chromatograms were integrated using a default baseline construction technique.

RESULTS AND DISCUSSION

Dabsyl chloride is a useful chromophoric labeling reagent that allows the detection of amino acids in visible light by reversed-phase HPLC. It reacts readily with all amino acids to form dabsyl derivatives, most of which exhibit absorption maxima at approximately 436 nm (*25*). This method was used for the determination of the amino acid composition of *T.*

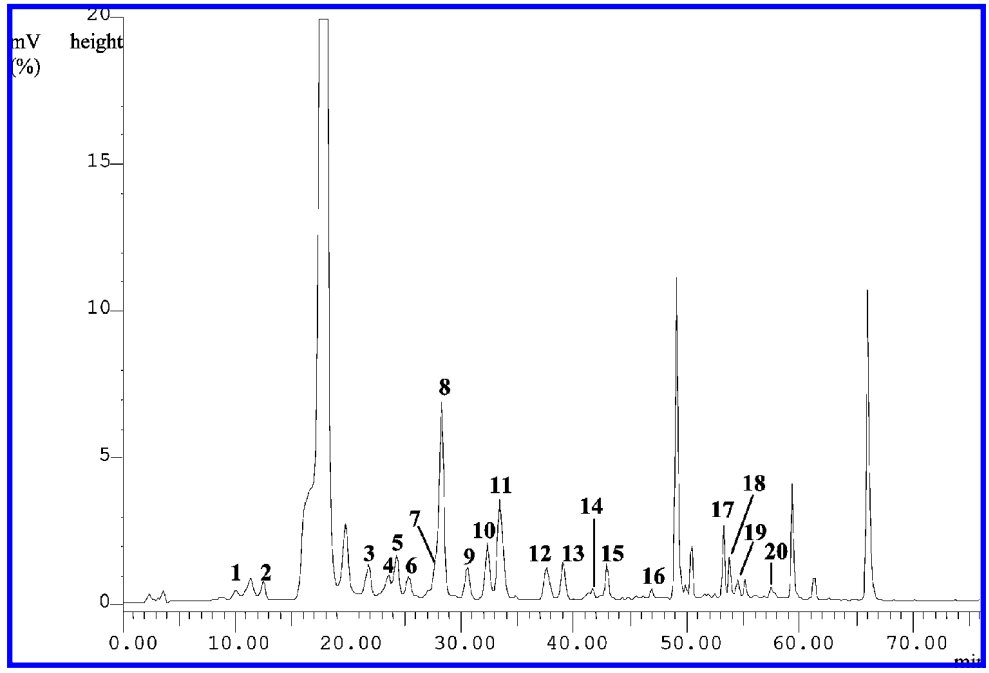

Figure 1. HPLC-UV amino acids profile of *R. cyanoxantha* mushroom. Detection at 436 nm: (MP) mobile phase; (1) aspartic acid; (2) glutamic acid; (3) asparagine; (4) glutamine; (5) serine; (6) threonine; (7) glycine; (8) alanine; (9) valine; (10) proline; (11) arginine; (12) isoleucine; (13) leucine; (14) tryptophan; (15) phenylalanine; (16) cysteine; (17) ornithine; (18) lysine; (19) histidine; (20) tyrosine.

rutilans, *H. agathosmus*, *A. rubescens*, *R. cyanoxantha*, *B. edulis*, *T. equestre*, *S. bellini*, *S. luteus*, *S. granulatus*, *F. hepatica*, and *C. cibarius*. In almost all of the species it was possible to determine 20 free amino acids: aspartic acid, glutamic acid, asparagine, glutamine, serine, threonine, glycine, alanine, valine, proline, arginine, isoleucine, leucine, tryptophan, phenylalanine, cysteine, ornithine, lysine, histidine, and tyrosine (**Figure 1**; **Table 2**). As far as we know, this is the first work revealing the presence of 20 essential and nonessential free amino acids in the referred wild edible mushroom species, which is very important considering their nutritional value. However, few amino acids were not detected in some species, as in *T. rutilans*, which presented the lowest number of these compounds. The total amino acid contents in the analyzed samples ranged from ca. 1531 to 22673 mg/kg (**Table 2**). *B. edulis*, followed by *T. equestre*, proved to be the mushroom species with the highest amino acid amounts. On the contrary, *F. hepatica* showed the minor ones (**Table 2**). In previous works, considering other edible mushroom species, the total amino acid amounts found ranged from ca. 0.1 to 50.1 mg/kg (*19*) and from ca. 9540 to 14910 mg/kg (*15*). This suggests that, as in our work, the amino acid contents in mushrooms are considerably divergent. With regard to essential amino acids, their contents in the analyzed species varied between ca. 551 and 6309 mg/kg. For an intake of about 100 g of mushroom, this would provide from 1 to 15% of the FAO daily recommended doses (*33*), *T. equestre* being the most interesting species. The quantification of the identified compounds indicated that alanine was the main compound in almost all of the analyzed species (*H. agathosmus*, *A. rubescens*, *R. cyanoxantha*, *T. equestre*, *S. bellini*, *S. luteus*, and *S. granulatus*), representing from ca. 18 to 45% of total compounds (**Figure 2**). Alanine is a nonessential amino acid required for the metabolism of glucose and tryptophan (*5*). It was demonstrated to have a cholesterol-reducing effect in rats (*34*). Glutamine also seemed to have a great importance, and it was the most abundant compound in *C. cibarius*, *B. edulis*, and *T. rutilans* species (ca. 25−31% of total amino acids) (**Figure 2**). Glutamine is also a nonessential amino acid, which is biosynthesized from glutamate (*5*), being converted to glutamic acid in the brain; it is essential for cerebral functions and increases the amount of GABA required for brain functioning and mental activity. Additionally, it is used in the muscles for the synthesis of muscle proteins and is applied in the treatment of wasting muscles after illness or postoperative care. It is further used in the body to balance the acid/alkaline level and in building blocks of RNA and DNA (*34*).

Valine was the principal component in *F. hepatica*, representing ca. 18% of total compounds. Valine is an essential amino acid deriving from pyruvate and is degraded to succinyl-coA (*5*). It is needed for muscle metabolism and repair and tissue growth and for the maintenance of the nitrogen balance in the body. It can be utilized as an energy source in the muscles, preserving the use of glucose (*34*). Deficiency in valine caused by maple syrup urine disease may affect the myelin covering of the nerves (*5*). Furthermore, it could be noted that all of the analyzed species revealed distinct quantitative profiles (**Figure 2**; **Table 2**). The minor amino acids differed among the 11 mushroom species, with cysteine, tyrosine, arginine, isoleucine, histidine, ornithine, tryptophan, and phenylalanine found in the lowest amounts.

The amino acid profiles found in the literature for edible mushrooms are very distinct (*1, 15*). However, it seems that glutamic acid, glutamine, and aspartic acid are, more often, the major compounds, whereas phenylalanine and tryptophan are usually present in reduced amounts. In the present work glutamine was also one of the major amino acids in the mushroom species, except for *S. luteus*, *S. bellini*, and *T. equestre*. Glutamic acid and aspartic acid were revealed to be the preponderant amino acids in *C. cibarius* and *S. luteus*, respectively.

As far as we know, this is the first work describing the presence of 18 amino acids in *C. cibarius* (**Table 2**): in addition to those already mentioned in the literature (*1, 19*), we are now reporting the occurrence of asparagine, glutamine, serine, proline, cysteine, ornithine, and tyrosine. Glutamic acid and alanine were among the main compounds, which is in conso-

Table 2. Free Amino Acid Composition of Mushroom Species (Milligrams per Kilograms of Dry Matter)[a]

	samples									
amino acid	*T. rutilans*	*H. agathosmus*	*A. rubescens*	*R. cyanoxantha*	*B. edulis*	*T. equestre*	*S. bellini*	*S. luteus*	*S. granulatus*	*F. hepatica*	*C. cibarius*
aspartic acid	nd	371.3 (0.1)	285.6 (0.2)	306.7 (3.2)	535.8 (13.0)	550.6 (1.0)	280.8 (0.3)	1584.0 (0.2)	nd	nd	nd
glutamic acid	274.4 (2.1)	606.9 (0.5)	292.7 (0.2)	274.8 (2.4)	212.9 (0.2)	505.9 (1.1)	322.9 (0.2)	nd	168.0 (1.0)	nd	888.2 (4.5)
asparagine	212.7 (2.3)	385.6 (0.1)	635.5 (4.0)	837.1 (0.1)	939.5 (7.0)	595.2 (1.2)	329.9 (0.4)	237.6 (0.1)	102.0 (0.5)	16.2 (0.0)	222.0 (0.2)
glutamine	1461.2 (3.4)	1956.4 (13.4)	1378.0 (20.3)	1386.6 (15.0)	5894.0 (0.1)	647.3 (1.2)	351.0 (0.2)	369.6 (1.0)	546.0 (1.3)	210.6 (1.2)	1863.6 (21.3)
serine	116.6 (0.0)	421.3 (4.1)	321.3 (0.0)	511.2 (3.1)	1123.0 (0.1)	684.5 (2.0)	329.9 (0.0)	184.8 (0.2)	288.0 (0.1)	40.5 (0.0)	253.8 (1.0)
threonine	178.4 (0.0)	814.0 (4.3)	478.4 (0.0)	485.6 (0.3)	954.2 (0.1)	677.0 (2.1)	337.0 (0.1)	138.6 (0.0)	300.0 (0.2)	32.4 (0.1)	182.4 (0.5)
glycine	102.9 (0.1)	378.4 (0.1)	299.9 (2.3)	485.6 (0.5)	1504.7 (0.2)	721.7 (1.3)	547.6 (1.2)	39.6 (0.1)	102.0 (0.0)	64.8 (0.1)	404.3 (1.5)
alanine	939.8 (0.6)	2392.0 (20.1)	3098.8 (0.9)	2607.1 (19.9)	5688.5 (0.1)	6874.6 (19.9)	1516.3 (13.1)	3986.4 (21.0)	1092.0 (10.1)	210.6 (1.2)	1356.0 (8.7)
valine	nd	642.6 (0.4)	164.2 (4.0)	1194.9 (12.7)	447.7 (0.0)	416.6 (0.0)	1039.0 (4.3)	541.2 (2.2)	648.0 (2.0)	275.4 (0.0)	nd
proline	322.4 (0.3)	806.8 (0.3)	685.4 (3.0)	875.4 (1.3)	1005.6 (0.1)	1294.6 (12.3)	456.3 (0.0)	310.2 (1.1)	216.0 (0.1)	160.7 (0.1)	253.8 (0.1)
arginine	89.2 (0.0)	207.1 (0.0)	449.8 (2.1)	1060.7 (30.3)	396.4 (0.0)	699.4 (7.3)	217.6 (0.1)	396.0 (1.1)	210.0 (1.5)	97.2 (0.5)	95.2 (0.0)
isoleucine	nd	214.2 (0.1)	249.9 (2.1)	447.3 (1.7)	293.6 (0.0)	714.2 (6.2)	301.9 (0.2)	112.2 (1.0)	498.0 (2.2)	56.7 (0.6)	95.2 (0.0)
leucine	102.9 (0.0)	264.2 (0.0)	499.8 (0.0)	364.2 (1.4)	425.7 (0.0)	1019.3 (101.0)	252.7 (1.0)	66.0 (0.0)	132.0 (0.1)	64.8 (0.5)	142.7 (0.1)
tryptophan	164.6 (0.1)	371.3 (0.0)	178.5 (0.0)	166.1 (0.0)	719.3 (0.2)	200.9 (2.3)	224.6 (0.3)	132.0 (1.1)	216.0 (0.1)	40.5 (0.3)	150.7 (0.1)
phenylalanine	89.2 (0.1)	485.5 (0.2)	285.6 (1.9)	396.2 (0.0)	565.2 (2.1)	758.9 (3.4)	308.9 (0.1)	184.8 (1.1)	192.0 (0.0)	56.7 (0.1)	95.2 (0.4)
cysteine	nd	157.1 (0.1)	264.2 (0.2)	95.9 (0.0)	491.8 (3.1)	133.9 (0.1)	nd	52.8 (0.0)	nd	17.5 (0.0)	190.3 (1.2)
ornithine	260.7 (0.0)	871.1 (10.2)	85.7 (0.0)	242.8 (0.1)	462.4 (4.1)	558.0 (1.2)	294.8 (0.9)	138.6 (2.0)	54.0 (0.1)	16.2 (0.1)	166.5 (1.3)
lysine	96.0 (0.0)	699.7 (0.9)	164.2 (0.1)	198.1 (0.1)	521.1 (0.1)	2522.2 (17.4)	266.8 (1.7)	145.2 (0.1)	120.0 (0.2)	24.3 (0.0)	364.8 (0.3)
histidine	185.2 (0.9)	556.9 (1.2)	314.2 (1.8)	153.4 (0.0)	345.0 (0.1)	223.2 (0.1)	182.5 (0.1)	132.0 (0.0)	156.0 (0.1)	97.2 (0.1)	341.0 (0.2)
tyrosine	89.2 (0.0)	806.8 (9.2)	178.5 (0.1)	147.0 (0.1)	146.8 (0.0)	505.9 (0.1)	245.7 (0.1)	191.4 (0.1)	168.0 (0.5)	48.6 (0.0)	301.3 (0.0)
total	4685.4	13409.2	10310.2	12236.7	22673.2	20303.9	7806.2	8943.0	5208.0	1530.9	7367.0

[a] Results are expressed as mean (standard deviation) of three determinations. nd, not detected.

nance with a previous work (*1*). However, in the present study glutamine was the major amino acid (**Figure 2**), whereas data from the literature (*19*) gave valine and histidine as the principal components. Furthermore, as far as we know, the total amino acid amounts obtained in our study were approximately 4000 times higher than that reported before (ca. 7367 > 1.8 mg/kg) (*19*).

The amino acid analysis of *B. edulis* revealed four compounds not previously found in the literature (asparagine, glutamine, cysteine, and ornithine) (*15*) (**Table 2**). The present study showed glutamine and alanine as the principal amino acids (ca. 26 and 25% of total compounds, respectively) (**Figure 2**), although lysine has also been classified as a preponderant compound in this species (*15*). Furthermore, the total amino acid content (**Table 2**) is much higher than that obtained from the literature (ca. 22673 > 8970 mg/kg).

With regard to *A. rubescens*, our work revealed asparagine, glutamine, arginine, tryptophan, cysteine, and histidine for the first time, besides another 14 amino acids (**Table 2**). On the basis of the results obtained, it could be noted that alanine and glutamine were the major compounds in this species, corresponding to ca. 30 and 13% of the total amino acid contents, respectively (**Figure 2**). However, glutamic acid and lysine were the major compounds in a previous study (*2*).

The analysis of the amino acid composition of *S. luteus* showed a total of 19 compounds (**Table 2**). Alanine and then aspartic acid were the principal components, corresponding to ca. 45 and 18% of total compounds, respectively (**Figure 2**). Nevertheless, glutamic acid, leucine, lysine, ornithine, and arginine were considered to be the major amino acids in *S. luteus* species, so far (*12*).

The determination of the amino acid profile of *S. granulatus* showed 18 free compounds (**Table 2**). The principal ones were alanine, valine, glutamine, and isoleucine, corresponding to ca. 21, 12, 10, and 10% of total contents, respectively (**Figure 2**). The main amino acids found in the literature for this species were glutamic acid, leucine, lysine, ornithine, arginine (*12*), and serine (*1*); only glutamine was present as a major compound in common with our work (*1*). As we can see, the quantitative profiles found for *S. granulatus* are very different, so we suggest that the amino acid profile could not be adequate for use as a biomarker of this species.

Concerning the species described above, their qualitative and quantitative amino acid profiles were revealed to be different from those described before. This could be explained by the diversity of extraction, derivatization, or quantification methods used in the different studies. In addition, the different geographical origin and/or stage of development of the analyzed species cannot be excluded.

Comparison of the results obtained with the three analyzed *Suillus* species (*S. luteus*, *S. granulatus*, and *S. bellini*) showed some differences (**Table 2**). Glutamic acid was not detected in *S. luteus* nor was aspartic acid in *S. granulatus*. Cysteine was not detected in either *S. bellini* or *S. granulatus*. In addition, and from a quantitative point of view, although alanine was the main compound in the three species, it was more important in *S. luteus* (**Figure 2**). *S. luteus* also presented a higher aspartic acid relative amount, whereas *S. granulatus* showed valine, glutamine, and isoleucine as other principal compounds. Furthermore, concerning the remaining compounds, their relative abundance is different among the three species (**Figure 2**). According to these observations, the amino acid distribution pattern found does not seem to reflect any phylogenetic relationship among the three *Suillus* species.

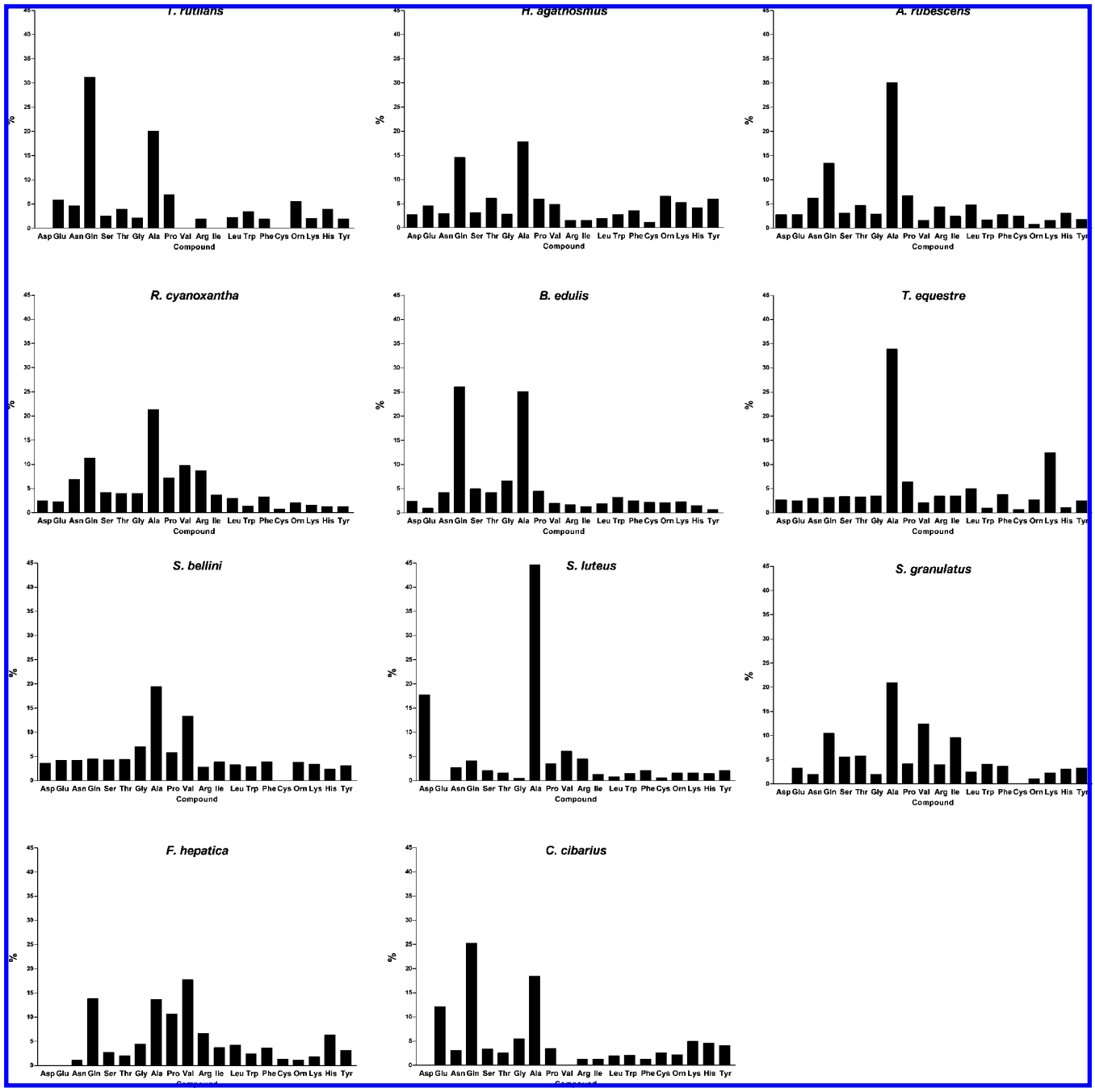

Figure 2. Amino acids profiles of *T. rutilans*, *H. agathosmus*, *A. rubescens*, *R. cyanoxantha*, *B. edulis*, *T. equestre*, *S. bellini*, *S. luteus*, *S. granulatus*, *F. hepatica*, and *C. cibarius* edible mushroom species. Abbreviations: (Asp) aspartic acid; (Glu) glutamic acid; (Asn) asparagine; (Gln) glutamine; (Ser) serine; (Thr) threonine; (Gly) glycine; (Ala) alanine; (Val) valine; (Pro) proline; (Arg) arginine; (Ile) isoleucine; (Leu) leucine; (Trp) tryptophan; (Phe) phenylalanine; (Cys) cysteine; (Orn) ornithine; (Lys) lysine; (His) histidine; (Tyr) tyrosine.

Free amino acids are known to be precursors of secondary metabolites such as alkaloids (*6, 7*) and phenolic compounds (*35*). It is known that flavonoids are synthesized by the phenylpropanoid metabolic pathway in which phenylalanine is used to produce 4-coumaroyl-CoA, then following many pathways that lead to the different flavonoids (*35*). However, the literature concerning the relationship between phenolics and amino acids is almost nonexistent. With regard to alkaloids, it is interesting to note that *B. edulis*, which was the species with the highest amino acid contents in the present work, was also the one that presented the highest total alkaloid amounts in a previous work involving some of the analyzed species (*24*). A deeper discussion is not possible once no alkaloid is identified, and alkaloid families arise from different metabolic pathways, in which individual amino acids are intervenient (*6, 7*).

The reason mushrooms are so appreciated and have been used as food and food-flavoring materials resides, in part, in their highly intense and good taste (*4*). The taste of edible mushrooms is primarily due to the presence of amino acids and other small molecules. Among amino acids, aspartic and glutamic acids are those contributing the most to the typical mushroom taste, the "umami" taste or "perception of satisfaction", which is an overall food flavor sensation induced or enhanced by monosodium glutamate (*4*). In humans, the taste receptor is far more sensitive to glutamate than to other amino acids (*36*). Among the studied species, *S. luteus* and *C. cibarius* were those containing the

highest contents of aspartic and glutamic acids, respectively. Other tasty compounds appear to give sweet, bitter, or less intense sensations. For example, alanine, serine, glycine, and threonine are known to have sweet tastes, whereas leucine, phenylalanine, isoleucine, valine, histidine, arginine, and tryptophan present bitter flavors. Lysine, cysteine, and tyrosine are considered to be tasteless amino acids (*4*). The taste of mushrooms depends, in part, on the combination of the amino acids described in each species. However, because alanine was the principal component in almost all of the species (*H. agathosmus*, *A. rubescens*, *R. cyanoxantha*, *T. equestre*, *S. bellini*, *S. luteus*, and *S. granulatus*), it seems that sweet taste contributes to their flavor, on a large scale. On the contrary, *S. bellini*, *S. granulatus*, and *F. hepatica* were richer in valine, which can turn these species more bitter and less accepted by the consumers. The umami and sweet tastes will be important to stimulate the consumption of these nutritious mushroom species by individuals with low appetite, such as the elderly and those with diabetes.

In addition, it is known that amino acids display antioxidant activity, namely, in mushrooms (*37, 38*). The antioxidant capacities of the different amino acids may be rather different. Under suitable conditions, extremely low amino acid concentrations may have a rather strong effect (*37*). Thus, it may be suggested that the amino acids could contribute to the antioxidant capacity of the analyzed species, especially to *B. edulis* and *T. equestre*, which contained the highest contents of these compounds. In fact, in previous works the aqueous lyophilized extracts of *B. edulis* (*23, 24*) and *T. equestre* (*23*) were revealed to have antioxidative properties.

It is also known that some amino acids (tryptophan, cysteine, alanine, and glycine) exert a synergistic effect with vitamin C on the antioxidant activity of vitamin E and that their effectiveness is partially related to their lipophilicity (*39*). All of the discussed amino acids were found in the analyzed species, alanine being one of the major compounds. This could mean that those mushroom species might be important sources of coadjuvants on the antioxidant activity of vitamin E.

Proline has been shown to accumulate in plants under heavy metal exposure: it is suggested that proline could effectively protect plants from heavy metal attack (*40*). This protective effect seems to be explained by heavy metal detoxification through the formation of a nontoxic heavy metal−proline complex. The *F. hepatica* species seems to be the best protected against heavy metal attack, because it showed the highest relative amount of proline (11% of the total compounds) (**Table 2**).

The aim of this work was to provide the amino acid compositions of *T. rutilans*, *H. agathosmus*, *R. cyanoxantha*, *T. equestre*, *S. bellini*, and *F. hepatica* for the first time and to achieve a deeper knowledge on the amino acid profiles of *A. rubescens*, *B. edulis*, *S. luteus*, *S. granulatus*, and *C. cibarius*. All 11 analyzed species presented essential and nonessential free amino acids, which is really important for their nutritional value. With regard to the quantitative profile, the different mushroom species were very distinct. In this way, it would be important to combine different species in the diet to obtain good amounts of amino acids and a great diversity of palatable sensations. Further studies regarding the influence of different stages of ripening and/or conservation procedures on the amino acid composition of the several species would allow an evaluation of whether these factors might have an impact on their nutritional value. In addition, they may have a great importance as a source of biologically active compounds.

LITERATURE CITED

(1) Mdachi, S. J. M.; Nkunya, M. H. H.; Nyigo, V. A.; Urasa, I. T. Amino acid composition of some Tanzanian wild mushrooms. *Food Chem.* **2004**, *86*, 179–182.

(2) Leon-Guzmán, M. F.; Silva, I.; López, M. G. Proximate chemical composition, free amino acid contents, and free fatty acid contents of some wild edible mushrooms from Querétaro, México. *J. Agric. Food Chem.* **1997**, *45*, 4329–4332.

(3) Guedes de Pinho, P.; Ribeiro, B.; Gonçalves, R. F.; Baptista, P.; Valentão, P.; Seabra, R. M.; Andrade, P. B. Correlation between the pattern volatiles and the overall aroma of wild edible mushrooms. *J. Agric. Food Chem.* **2008**, *56*, 1704–1712.

(4) Tsai, S.-Y.; Wu, T.-P.; Huang, S.-J.; Mau, J.-L. Nonvolatile taste components of *Agaricus bisporus* harvested at different stages of maturity. *Food Chem.* **2007**, *103*, 1457–1464.

(5) Voet, D.; Voet, J. G. *Biochemistry*, 3rd ed.; Wiley: Hoboken, NJ 2004.

(6) Robertson, A. V.; Mario, L. The biogenesis of alkaloids. XXIII. The role of proline and ornithine in the biogenesis of stachydrine. *Can. J. Chem.* **1959**, *37*, 1197–1200.

(7) Nyman, J. Incorporation of arginine, ornithine and phenylalanine into tropane alkaloids in suspension-cultured cells and aseptic roots of intact plants on *Atropa belladonna*. *J. Exp. Bot.* **1994**, *45*, 979–986.

(8) Hatanaka, S.-I.; Niimura, Y. L-3-(3-carboxy-4-furyl)alanine from *Tricholomopsis rutilans*. *Phytochemistry* **1975**, *14*, 1436.

(9) Hatanaka, S.-I.; Niimura, Y.; Taniguchi, K. L-2-Aminohex-4-ynoic acid: a new amino acid from *Tricholomopsis rutilans*. *Phytochemistry* **1972**, *11*, 3327–3329.

(10) Niimura, Y.; Hatanaka, S.-I. L-*threo*- and L-*erythro*-2-amino-3-hydroxyhex-4-ynoic acids: new amino acids from *Tricholomopsis rutilans*. *Phytochemistry* **1974**, *13*, 175–178.

(11) Aoyagi, Y.; Nakamura, N.; Sugahara, T. L-2-amino-7-hydroxy-octanoic acid: an amino acid from *Russula cyanoxantha*. *Phytochemistry* **1988**, *27*, 3305–3306.

(12) Rózycki, H.; Strzelczyk, E. Free amino acids production by ectomycorrhizal fungi of pine (*Pinus sylvestris* L.). *Acta Microbiol. Pol.* **1985**, *34*, 59–66.

(13) Mlodecki, H.; Lasota, W.; Trela, A. Chemical evaluation of the nutritional value of *Boletus edulis*. II. Levels of exogenous amino acids, calcium, phosphorus, iron and fats in *Boletus edulis*. *Rocz. Panstw. Zakl. Hig.* **1968**, *19*, 85–88.

(14) Touze-Soulet, J. M. The free amino acids of *Boletus edulis* Fr. Ex bull. and more related species. *C. R. Hebd. Seances Acad. Sci.* **1961**, *252*, 208–210.

(15) Tsai, S.-Y.; Tsai, H.-L.; Mau, J.-L. Non-volatile taste components of *Agaricus blazei, Agrocybe cylindracea* and *Boletus edulis*. *Food Chem.* **2008**, *107*, 977–983.

(16) Collin-Hansen, C.; Pedersen, S. A.; Andersen, R. A.; Steinnes, E. First report of phytochelatins in a mushroom: induction of phytochelatins by metal exposure in *Boletus edulis*. *Mycologia* **2007**, *99*, 161–174.

(17) Touze-Soulet, J. M.; Montant, C. Study of new combined forms of glutamic acid in *Boletus edulis* Fr. ex Bull. *Bull. Soc. Chim. Biol.* **1962**, *44*, 451–459.

(18) Slejkovec, Z.; van Elteren, J. T.; Woroniecka, U. D.; Kroon, K. J.; Falnoga, I.; Byrne, A. R. Preliminary study on the determination of selenium compounds in some selenium-accumulating mushrooms. *Biol. Trace Elem. Res.* **2000**, *75*, 139–155.

(19) Agrahar-Murugkar, D.; Subbulakshmi, G. Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya. *Food Chem.* **2005**, *89*, 599–603.

(20) Rangel-Castro, J. I.; Danell, E.; Pfeffer, P. E. A C-13-NMR study of exudation and storage of carbohydrates and amino acids in the ectomycorrhizal edible mushroom *Cantharellus cibarius*. *Mycologia* **2002**, *94*, 190–199.

(21) Valentão, P.; Andrade, P. B.; Rangel, J.; Ribeiro, B.; Silva, B. M.; Baptista, P.; Seabra, R. M. Effect of the conservation procedure on the contents of phenolic compounds and organic acids in

chanterelle (*Cantharellus cibarius*) mushroom. *J. Agric. Food Chem.* **2005**, *53*, 4925–4931.
(22) Ribeiro, B.; Valentão, P.; Baptista, P.; Seabra, R. M.; Andrade, P. B. Phenolic compounds, organic acids profiles and antioxidative properties of beefsteak fungus (*Fistulina hepatica*). *Food Chem. Toxicol.* **2007**, *45*, 1805–1813.
(23) Ribeiro, B.; Rangel, J.; Valentão, P.; Baptista, P.; Seabra, R. M.; Andrade, P. B. Contents of carboxylic acids and two phenolics and antioxidant activity of dried portuguese wild edible mushrooms. *J. Agric. Food Chem.* **2006**, *54*, 8530–8537.
(24) Ribeiro, B.; Lopes, R.; Andrade, P. B.; Seabra, R. M.; Gonçalves, R. F.; Baptista, P.; Quelhas, I.; Valentão, P. Comparative study of phytochemicals and antioxidant potential of wild edible mushroom caps and stipes. *Food Chem.* **2008**, *110*, 47–56.
(25) Krause, I.; Bockhardt, A.; Neckermann, H.; Henle, T.; Klostermeyer, H. Simultaneous determination of amino acids and biogenic amines by reversed-phase high-chromatography of the dabsyl derivatives. *J. Chromatogr., A* **1995**, *715*, 67–79.
(26) Bas, C.; Kuyper, T. H. W.; Noordeloos, M. E.; Vellinga, E. C. In *Flora Agaricina Neerlandica:* Critical Monographs on Families of Agarics and Boleti Occuring in the Netherlands; A. A. Balkema: Rotterdam, The Netherlands, 1990−2001; Vol. 1−5.
(27) Bon, M. In *Guia de Campo de los Hongos de Europa*; Ediciones Omega: Barcelona, Spain, 1988.
(28) Courtecuisse, R.; Duhem, B. In *Mushrooms and Toadstools of Britain and Europe*; HarperCollins Publishers: London, U.K., 1995.
(29) Courtecuisse, R. In *Mushrooms of Britain and Europe*; HarperCollins Publishers: London, U.K., 1999.
(30) Marchand, A. In *Champignons du Nord et du Midi*; Soc. Mycol. Pyrénées Mediterranéenes: Perpignan, 1971−1986; Vol. 1−9.
(31) Moser, M. In *Keys to Agarics and Boleti (Poliporales, Boletales, Agaricales, Russulales)*; Roger Phillips: London, U.K., 1983.
(32) Silva, B. M.; Silva, L. R.; Valentão, P.; Seabra, R. M.; Andrade, P. B.; Trujillo, M. E.; Velásquez, E. HPLC determination of free amino acids profile of Dão red wine: effect of *Dekkera bruxellensis* contamination. *J. Liq. Chromatogr. Relat. Technol.* **2007**, *30*, 1371–1383.
(33) FAO/WHO/UNU. Energy and protein requirements. Report of a Joint FAO/WHO/UNU expert consultation, October 1981; WHO Technical Report Series 724; WHO: Geneva, Switzerland, 1985.
(34) Belitz, H.-D.; Grosch, W. Amino acids, peptides and proteins In *Food Chemistry*; Springer-Verlag,: Berlin, Germany, 1999; pp 8−34.
(35) Filippos, V.; Emmanouil, D. C.; Guenter, V.; Georg, K.; Nickolas, P. Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: Chemical diversity, impacts on plant biology and human health. *Biotechnol. J. 2.* **2007**.
(36) Zhang, Y.; Hoon, M. A.; Chandrashekar, J.; Mueller, K. L.; Cook, B.; Wu, D.; Zuker, C. S.; Ryba, N. J. P. Coding of sweet, bitter, and umami tastes different receptor cells sharing similar signaling pathways. *Cell* **2003**, *112*, 293–301.
(37) Marcuse, R. The effect of some amino acids on the oxidation of linoleic acid and its methyl ester. *J. Am. Oil Chem. Soc.* **1962**, *39*, 97–103.
(38) Fu, H.-Y.; Shieh, D.-E.; Ho, C.-T. Antioxidant and free radical scavenging activities of edible mushrooms. *J. Food Lipids* **2002**, *9*, 35–46.
(39) Carlotti, M. E.; Gallarate, M.; Gasco, M. R.; Morel, S.; Serafino, A.; Ugazio, E. Synergistic action of vitamin C and amino acids on vitamin E in inhibition of the lipoperoxidation of linoleic acid in disperse systems. *Int. J. Pharm.* **1997**, *155*, 251–261.
(40) Sharma, S. S.; Schat, H.; Vooijs, R. In vitro alleviation of heavy metal-induced enzyme inhibition by proline. *Phytochemistry* **1998**, *49*, 1531–153.

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