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Phytochemical screening and amino acids analysis of mushrooms from Burkina Faso

Elise Sanon[1]*, Jean Claude W. Ouédraogo[2,3], Sylvain Ilboudo[3], Marie K. Laure Guissou[4], Pierre I. Guissou[3] and Philippe Sankara[1]

[1]Laboratoire Biosciences, équipe de Phytopathologie, Département de Biologie et Physiologie Végétales, UFR/SVT, Université Ouaga 1 Pr Joseph KI-ZERBO, 03 BP: 7021 Ouagadougou 03, Burkina Faso.
[2]Département de Chimie, UFR/SEA, Université Ouaga 1 Pr Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso.
[3]Institut de Recherche en Sciences de la Santé (IRSS/CNRST), 03, BP 7192, Ouagadougou, Burkina Faso.
[4]École Normale Supérieure, Université de Koudougou, BP 376, Koudougou, Burkina Faso.

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Mushrooms play an important social, economic and ecological role in the world. In this study, the proximate compositions, minerals and amino acids contents of *Russula alveolata*, *Russula* cf. *compressa*, *Russula flavobrunnea* var. *aurantioflava* and *Russula ochrocephala* from Burkina Faso were investigated. The chemical analysis showed the presence of volatile oil, sterols and triterpenes, carotenoids, saponosides, reducing compounds and cardenolides. Several amino acids including aspartate, glutamic acid, serine, glycine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine and lysine were identified in mushroom species. Among the identified amino acids, seven are essential for human body: Phenylalanine, valine, threonine, isoleucine, methionine, leucine and lysine.

Key words: Chemical composition, amino acids, *Russula*, mushrooms, Burkina Faso.

INTRODUCTION

Mushrooms are of fungus type with a large variety of them. Each component is closely linked to its physiological nature. Their lifestyle shows their identity: no pigment (chlorophyll) assimilation comparable to higher plants, no opportunity to draw their energy from the carbonic acid of the air. Mushroom has necessarily one of these lifestyles: saprotrophism, parasitism and symbiotic parasitism (Heim, 1984). The russules are macromycetes of the genus *Russula* Pers. Fr., in the family Russulaceae Lotsy and order Russulales Kreisel. This family consists primarily of symbiotic species of ectomycorrhizal types (ECM), involving mainly the roots of woody plants in wooded regions (Bâ et al., 2012). This group of macroscopic fungi differs from the others of Russulaceae family by the absence of latex when broken.

The literature shows that several taxonomic studies

*Corresponding author. E-mail: wonnadia@yahoo.fr. Tel: (00226) 74457272 or 72822035.

were carried out on the *Russula* genus in Africa (Buyck, 1993, 1994, 1997). More recently, new taxa were described in Zimbabwe (Buyck and Sharp, 2007), Tanzania (Buyck, 1995, 2005; Härkönen et al., 2003), Zambia (Buyck, 1999, 2004), Burundi (Buyck, 1995, 1999), Cameroon (Douanla-Meli and Langer, 2009), Madagascar (Heim, 1936; Buyck, 2008) and Burkina Faso (Sanon et al., 2014; Sanon, 2015).

Nowadays, up to 197 taxa of *Russula* are described in Africa (Sanon et al., 2014). Despite the importance of taxonomic studies on russules, it should be noted that very few chemical and biochemical studies have been conducted on them and their food value is not evaluated. Indeed, determining the nutritional value of russules requires measuring the rate and analyzing the composition and spectrum of amino acids, fatty acids, vitamins and minerals present.

The present study was aimed to investigate the nutritional value of four species of russules namely *Russula alveolata*, *Russula compressa*, *Russula flavobrunnea var. aurantioflava* and *Russula ochrocephala* by analyzing the composition of the different spectra of the amino acids contained in these fungal samples using high performance liquid chromatography (HPLC) method.

MATERIALS AND METHODS

Fungal material/biological material

The fungal material was composed of the carpophores of four mushroom species: *R. flavobrunnea var. aurantioflava, R. ochrocephala, R.* cf. *compressa* and *R. alveolata*. The mushroom samples were collected during each rainy season (2012-2014) following a standard protocol (Sanon, 2015). The first three mushrooms were collected in the forest of Dan which is a forest gallery at about fifteen kilometer from the city of Orodara at 10°52'28''N and 04°50'10''W, while the last one was collected in the forest gallery of Kou (western part of Burkina Faso). Morphological features were recorded using fresh materials that were dried afterward using a propane gas field dryer (De Kesel, 2001).

The species were then identified and voucher materials were stored at the herbarium of the University of Ouagadougou (Thiers, 2013) under the following reference numbers: SE009 (holotype OUA, MNHN Paris); 014b; 015; 145; 166; 167; 174; 178 (paratype OUA, MNHN Paris) for *R. flavobrunnea var. aurantioflava*, SE002 (holotype OUA, MNHN Paris); SE003a, SE005, SE146 (paratype OUA, MNHN Paris) *for Russula alveolata ;* SE144 (holotype OUA, MNHN Paris); SE147, 148, 151, 165, 173 (paratype OUA, MNHN Paris) for *R.* cf. *compressa* and SE 155 (holotype OUA, MNHN Paris) ; SE 157 ; SE 158 ; SE 162 ; SE 163 ; and SE 175 (paratype OUA, MNHN Paris) for *R. ochrocephala*.

Technical material

Phytochemical screening

The extracts from individual mushroom powders were prepared by maceration of 10 g of fungal powder in 50 mL of dichloromethane (DCM) for 24 h in a sealed pyrex bottle at room temperature. After filtration, each mark was extracted with methanol 80% (v/v) for 24 h and filtered. The mark was then extracted by infusion with 50 mL of distilled water. The organic extracts and the aqueous one were dried by evaporation under reduced pressure. Aliquots (1 g) of the aqueous extracts were redissolved in 30 mL of distilled water, and hydrolyzed with chlorhydric acid (10%) under reflux for 30 min and then extracted (liquid/liquid extraction) with dichloromethane. All these extracts were used for the phytochemical screening. The phytochemical screening was performed according to Cieuli (1982). Water, ethanol and dichloromethane extracts were used to assess the presence of: volatile oils, emodols, alkaloid bases, salt alkaloids, carotenoids, coumarins, flavonoid aglycons, oses and polysaccharides, reducing compounds, polyphenolic compounds, saponosids and sterols and triterpenes.

HPLC conditions for amino acids analyses

The profile and the amount of total amino acids were determined by reverse phase HPLC, using the Pico-Tag system described by Bidingmeyer et al. (1984). Samples were first defatted and hydrolyzed. For hydrolysis, 0.4 g of defatted powder of mushrooms was introduced into a hydrolysis pyrex bottle and 15 mL of 6 M chlorhydric acid were added. The bottle was flushed for 30 s with nitrogen and sealed immediately with cap. The bottle was then placed in an electric oven for 24 h at 110°C for sample hydrolysis. The sample solution was cooled down until room temperature and transferred into a 50 mL volumetric flask. Some mili-Q water was added until the 50 mL level was reached, and mixed (Hagen, 1989). Approximately, 1 mL of the diluted solution is homogenized and filtered through a filter of 0.45 µm. An aliquot of 10 µL of the solution is placed in a reaction tube and dried for 15 min with the Picotag Workstation.

The sample was then re-dissolved in 10 µL of re-drying solution ethanol-water triethylamine (2:2:1). They were dried again for 15 min and finally derivatized with 20 µL phenylisothiocyanate reagent (ethanol, water : triethylamine : phenylisothiocyanate 7:1:1:1) for 20 min at room temperature. Excess reagent was removed using a vacuum for 45 min in Pico-Tag Work station. Derivatized samples were dissolved in 100 µL Pico-Tag Sample diluent solution (WAT088119).

The HPLC analyses (identification and quantification) of the amino acids were performed under the conditions described by Bidlingmeyer et al. (1984). The system consists of Waters HPLC including degasser, controller - model 600, pump and detector - model 2487. Sample volume of 4 µL was injected. Chromatographic separation was performed on a PICO•TAG Column (3.9 × 150 mm), previously equilibrated for at least 8 min with Eluent A which consists of sodium acetate and triethylamine in water at pH 6.4-Acetonitrile (94:6 v/v). Solvent gradients were formed by varying the proportion of eluent A to eluent B (acetonitrile-water 60:40 v/v). Eluent B was increased to 46% in 10 min and subsequently increased to 100% in 10.5 min at a flow rate of 1 mL/min. The elution was performed at 254 nm with the column conditions set at 38°C. The areas under the peaks were used to calculate the concentrations of the amino acids using a Pierce Standard H amino acid calibration mixture (Rockford, IL).

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening was performed for the water, methanol and dichloromethane extracts. The results are summarized in Table 1. The phytochemical analysis revealed (Table 1) the presence of volatile oils,

Table 1. Phytochemical analyses extracts from mushroom species.

Extracts	Chemical groups	*R. alveolata*	*R. compressa*	*R. flavobrunnea var. aurantioflava*	*R. ochrocephala*
DCM	Volatile oil	++	++	+++	+++
	Sterols and triterpenes	+	+	++	++
	Emodols	-	-	-	-
	Carotenoids	+	+	++	++
Methanol	Polyphenols	-	-	-	-
	Saponosides	+++	+++	++	+++
	Reducing sugars	+	+	+	+
	Gallic tannins	-	-	-	-
Water	Anthracenosides	-	-	-	-
	Cardenolides	+	+	+	+

- = Absence; + = present in small amount (concentration); ++ = moderately present; +++ = present in large amount.

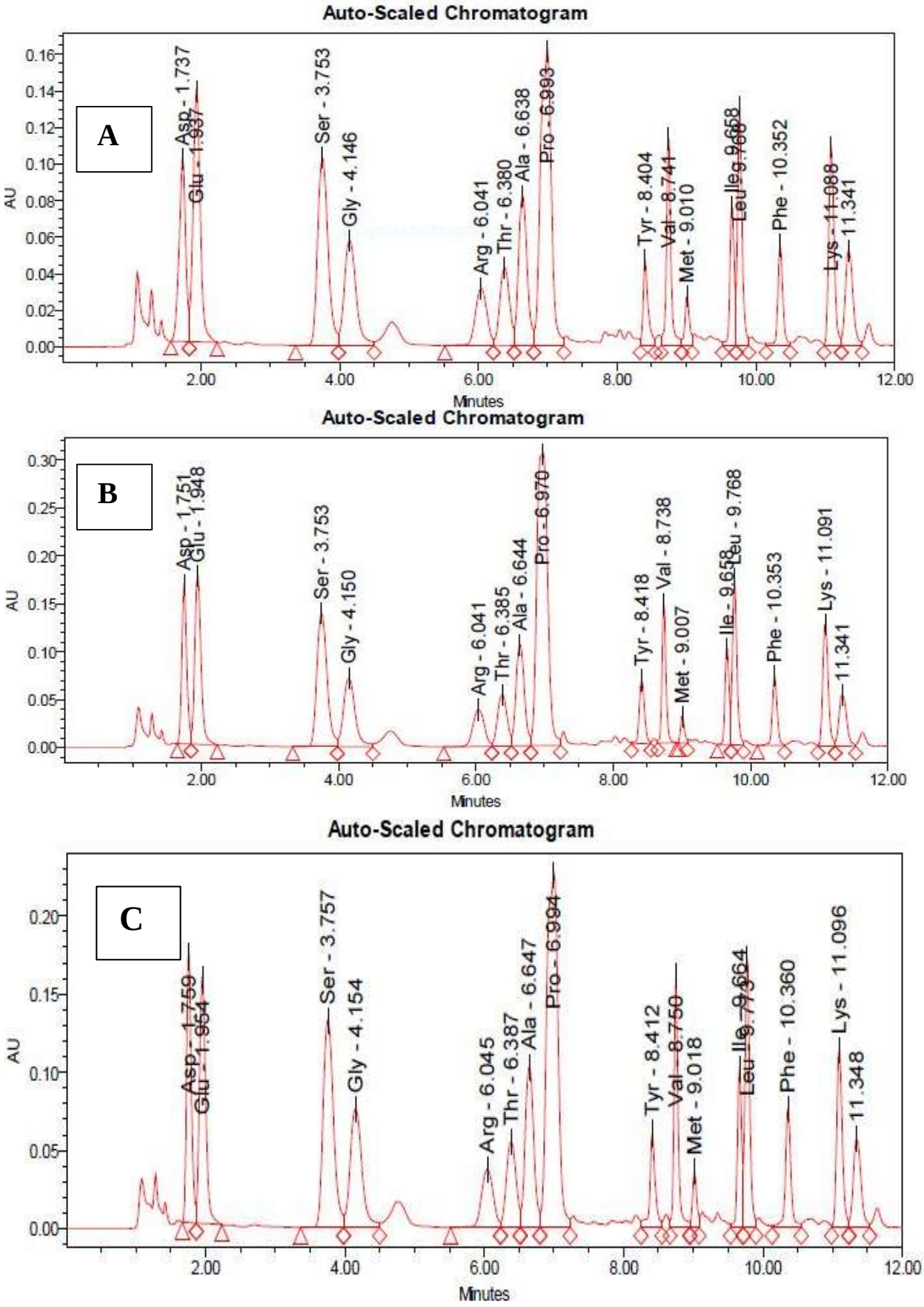
sterols and triterpenes, carotenoids, saponosides, reducing sugars and cardenolids. In the dichloromethane extract, volatile oils were in large amount in *R. flavobrunnea var. aurantioflava* and *R. ochrocephala* whilst. But in *R. alveolata* and *R. compressa*, they were moderately present. Sterols and triterpenes, and carotenoids are moderately found in *R*. *flavobrunnea var. aurantioflava* and *R. ochrocephala*, while they were in small amount in *R. alveolata* and *R. compressa.* In the ethanol extracts, reducing sugars were detected in the four species in small amount. It can be noticed that saponosides were in large amount in *R. alveolata*, *R. compressa* and *R. ochrocephala* but moderately present in *R. flavobrunnea var. aurantioflava*. In the aqueous extract, cardenolides were present in small amount in all the samples. The phytoconstituents such as emodols, polyphenols, gallic tannins and anthracenosides were absents in all the samples. The literature revealed that mushrooms contain saponins, polyphenols and terpenoids (Wandati et al., 2013) as found in the present study. In addition to the cited phytoconstituants, the mushrooms in the present study contain carotenoids, reducing sugars and trace of cardenolids. In a phytochemical study, Sanjay et al. (2015) found that edible mushrooms can also contain alkaloids and flavonoids.

Amino acids analysis by HPLC

The HPLC chromatograms of amino acids are presented in Figure 1A to D and the summary in Table 2. Figure 1 and Table 2 indicate that the mushrooms contain various amino acids. A total of fifteen amino acids have been detected in all the mushrooms species: aspartate, glutamic acid, serine, glycine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine and lysine. Among these amino acids, seven are regarded as essential to human body: phenylalanine, valine, threonine, isoleucine, methionine, leucine and lysine. Histidine and cysteine were absent in all the species.

Chemical analysis showed that the four species of russules namely *R. alveolata*, *R. compressa* aff., *R. flavobrunnea var. aurantioflava* and *R. ochrocephala* contain steroid and/or triterpene compounds (sterols and triterpenes, saponins), carotenoids, reducing compounds, cardenolides in trace and fifteen amino acids. Among the fifteen amino acids, it was observed that the proline had the highest concentration with a value of 4.082 g/100 g in *R. compressa*, followed by glutamic acid with the highest concentration in general and particularly in R. *ochrocephala* (2.202 g/100 g). The less concentrated amino acid is methionine in all the samples. The literature showed that mushrooms are deficient in cysteine and methionine (Agrahar-Murugkar and Subbulakshmi, 2005; Mdachi et al., 2004), isoleucine, leucine and lysine (Nakalembe and Kabasa, 2013), tryptophan (Agrahar-Murugkar and Subbulakshmi, 2005) and phenylalanine (Nakalembe and Kabasa, 2013). On the contrary, leucine was reported as the most abundant amino acid in wild mushrooms (Agrahar-Murugkar and Subbulakshmi, 2005). In the present study, cysteine and histidine were not detected and methionine was the less concentrated amino acid. Within the 7 essential amino acids, leucine had the highest concentration (1.062 g/100 g) in R. *flavobrunnea* var. *Aurantioflava*, while methionine remains the less concentrated one. The retention time of the amino acids ranged from about 1.7 to 11 min. Lysine has the maximal retention time and the smaller one is obtain for Asp.

Amino acids are very important for the regular


Auto-Scaled Chromatogram
A
AU
Minutes
Asp - 1.737
Glu - 1.937
Ser - 3.753
Gly - 4.146
Arg - 6.041
Thr - 6.380
Ala - 6.638
Pro - 6.993
Tyr - 8.404
Val - 8.741
Met - 9.010
Ile - 9.658
Leu - 9.768
Phe - 10.352
Lys - 11.088
11.341
Auto-Scaled Chromatogram
B
AU
Minutes
Asp - 1.751
Glu - 1.948
Ser - 3.753
Gly - 4.150
Arg - 6.041
Thr - 6.385
Ala - 6.644
Pro - 6.970
Tyr - 8.418
Val - 8.738
Met - 9.007
Ile - 9.658
Leu - 9.768
Phe - 10.353
Lys - 11.091
11.341
Auto-Scaled Chromatogram
C
AU
Minutes
Asp - 1.759
Glu - 1.954
Ser - 3.757
Gly - 4.154
Arg - 6.045
Thr - 6.387
Ala - 6.647
Pro - 6.994
Tyr - 8.412
Val - 8.750
Met - 9.018
Ile - 9.664
Leu - 9.773
Phe - 10.360
Lys - 11.096
11.348

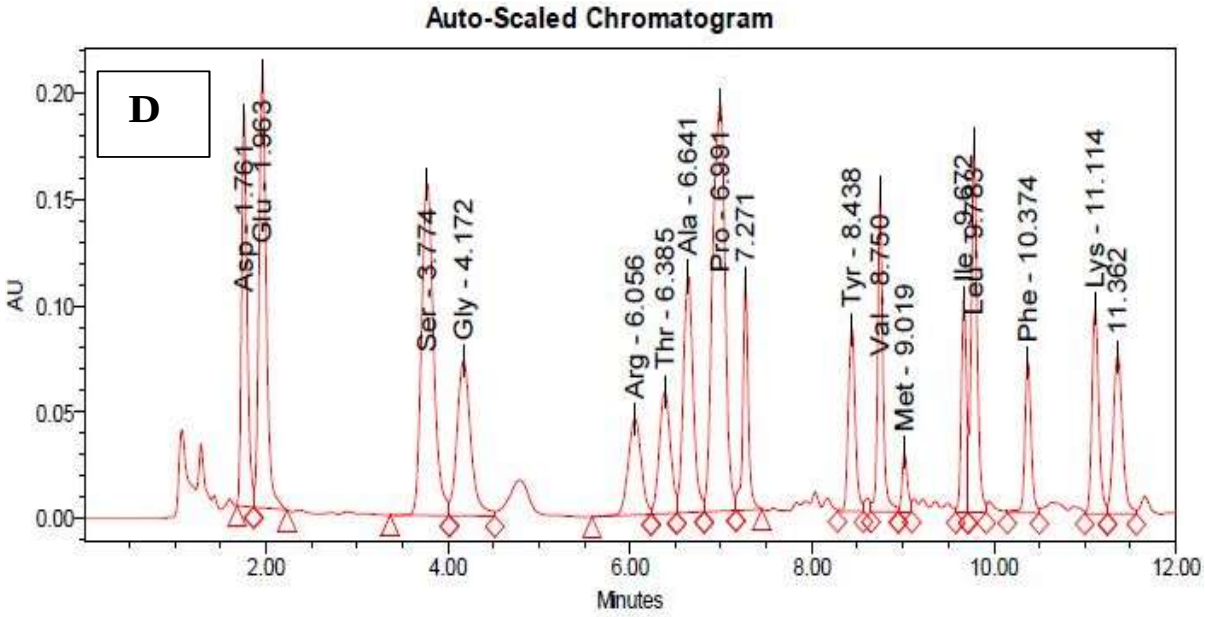


Figure 1. Peaks of amino acids, (A) *Russula alveolata*, (B) *Russula compressa*, (C) *Russula flavobrunnea var. aurantioflava* AND (D) *Russula ochrocephala* using HPLC.

biological activities of human body. They are the building blocks of the body. Besides building cells and repairing tissue, they constitute an antibody to combat bacteria and viruses; they are part of enzyme and hormonal system. Amino acids are very important for building nucleoproteins (RNA & DNA) (Imura and Okada, 1998). Eight amino acids are regarded as essential to human body: Phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine and lysine. Non synthesized amino acids are called " essential " and must be ingested by the body. The functions of the essential amino acids are numerous. They can give rise to other types of molecules. In addition, they can serve as precursors to the biosynthesis of non-essential amino acids. For example, histidine that may be converted to glutamic acid phenylalanine can be oxidized to give tyrosine. The obtained results are consistent with the assertions of Mau et al. (2002) that: "proteins that represent 4 to 35% of the dry weight of mushrooms contain many essential amino acids (from 32.9 to 48.5 g/100g protein), such as leucine, isoleucine, valine, lysine and threonine". In addition, cysteine (or sulphur containing amino acids), tyrosine (or aromatic amino acids), histidine and arginine are required by infants and growth (FAO/WHO/UNU, 2007; Sheil et al., 1986). Individual living with phenylketonuria (PKU) must have tyrosine intake in their food because the person living with PKU cannot convert the phenylalanine into tyrosine (Sheil et al., 1986).

As mentioned above, the phytochemical screening showed the presence of many phytoconstituents: Sterols and triterpenes, carotenoids, saponosides, reducing sugars and cardenolides. The sterols are lipids which help maintain the structural and functional integrity of the cell membranes in humans. They also play a role of cholesterol (Katan et al., 2003). Terpenoids are secondary metabolites synthesized by plants, marine organisms, etc. The saponosides (or saponins) are high-molecular-weight glycosides, consisting of a sugarunit(s) linked to a triterpene or a steroid aglycone. Many saponins are from plants and have detergent properties, capable of acting on the permeability of cell membranes. It would play an anti-inflammatory role with triterpenes. Carotenoids play an important role in nutrition and health. They are able to trap free radicals generated constantly by our organism (antioxidant properties) and many are provitamin A. Some of them have also anti-cancer properties. They also stimulate antibody synthesis (World Cancer Research Fund/American Institute for Cancer Research, 2007).

Finally, cardenolides or cardiotonic glycosides have the same structure as the steroidal saponins and also have detergent properties. They have a therapeutic effect: cardiotonic at very controlled dose (narrow therapeutic index), strengthens the heartbeat and reduces the side effects such as pericardial toxicity (Malcolm, 1991). With all these many nutritional virtues in these mushrooms, it can be said that they are good edible russules. Previous studies show that the nutritional value of mushrooms are based on their protein and essential amino acids content because they are good source of essential amino acids (Chang and Miles, 2004). The results showed that they are comparable to foods such as milk, beans, spinach and soybeans (Boa, 2006). Their content in vitamin C is

Table 2. Retention time (RT) and values of amino acids of different mushrooms.

S/N	Amino acids	RT (min)				Amount (g/100g)			
		R. alveolata	*R. compressa*	*R. flavobrunnea var. aurantioflava*	*R. ochrocephala*	*R. alveolata*	*R. compressa*	*R. flavobrunnea var. aurantioflava*	*R. ochrocephala*
1	Asp	1.737	1.751	1.759	1.761	1.208	1.507	1.454	1.382
2	Glu	1.937	1.948	1.954	1.963	2.000	2.268	1.928	2.202
3	Ser	3.753	3.753	3.757	3.774	1.472	1.956	1.799	1.877
4	Gly	4.146	4.150	4.154	4.172	0.652	0.811	0.828	0.697
5	His	4.789	4.789	4.789	4.789	0	0	0	0
6	Arg	6.041	6.041	6.045	6.056	0.815	1.005	0.091	0.944
7	Thr	6.380	6.385	6.387	6.385	0.601	0.777	0.743	0.671
8	Ala	6.646	6.644	6.647	6.641	0.769	0.973	0.908	0.857
9	Pro	6.993	6.970	6.994	6.991	2.073	4.082	2.918	2.081
10	Tyr	8.404	8.418	8.412	8.438	0.479	0.947	0.749	0.902
11	Val	8.741	8.738	8.750	8.750	0.752	0.975	0.925	0.832
12	Met	9.010	9.007	9.018	9.019	0.217	0.262	0.247	0.196
13	Cys	9.323	9.323	9.323	9.323	0	0	0	0
14	Ile	9.658	9.658	9.664	9.672	0.525	0.683	0.625	0.572
15	Leu	9.768	9.768	9.773	9.783	0.798	1.127	1.062	1.018
16	Phe	10.352	10.353	10.360	10.374	0.508	0.722	0.659	0.607
17	Lys	11.088	11.091	11.096	11.114	0.574	0.693	0.576	0.465

also higher than in carrot, celery and cucumber and are comparable to spinach (Chu et al., 2002). The content of riboflavin (vitamin B2) in mushrooms is generally greater than in vegetables, approaching in some cases those in eggs and cheese (Mattila et al., 2001). In addition, mushrooms are rich in fiber and minerals (Boa, 2006). Finally, although they have low fat content, their lipid profile is highly unsaturated; so mushrooms are also some sources of essential fatty acids.

Nowadays, over 80% people consider mushrooms as healthy food and do not hesitate to include them in their diet for cardiovascular health (Chu et al., 2002). In view of all these nutritional qualities of mushrooms in general and the four species of russules in particular, one can say that the four studied mushrooms are edible and their consumption needs to be popularized. But caution is needed because each species contains traces of cardenolides which, in high doses, can cause cardiac toxicity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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