

Amino acid composition of some Tanzanian wild mushrooms

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Abstract

Sixteen known amino acids, including the essential ones except isoleucine, were identified in the Tanzanian wild mushroom species *Boletus pruinatus* (Fr. & HÖK), *Boletinus cavipes* (Opat.) Kalchbr., *Cantharellus cibarius* (Fr.) Fr., *Inonotus* sp. cf. *obliquus* (Pers.: Fr.) Pil., *Ganoderma lucidum* (Curt.: Fr.) P. Karst, *Agaricus* sp. (L. ex Fr.), *Pleurotus sajor-caju* (Fr.) Sing., *Lactarius* sp. aff. *pseudovolemus* Heim., *Russula hiemisilvae* Buyck, and *Suillus granulatus* (L.) Kuntze using an HPLC-based amino acid analyzer. Whereas *G. lucidum* and the *Inonotus* sp. contained the least number of amino acids, *B. pruinatus* had a higher number, while *B. pruinatus* and *B. cavipes* had a higher number of essential amino acids than all other mushroom species, leucine being the most abundant in each of the mushroom species *B. pruinatus* and *B. cavipes*. These results should serve as a basis for encouraging local communities in developing countries to harness the nutritive potential of wildy occurring edible mushrooms for abating nutritional deficiencies.

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1. Introduction

Worldwide the nutritive and medicinal values of mushrooms have long been recognized (Cochran, 1978), as some of the edible mushroom species also possess pharmacological properties (Zimmerman, 2002). Mushrooms are also among the best sources of other essential nutrients, such as chromium. Previous studies (Gruen & Wong, 1982; Suzuki & Oshima, 1976; Zakhary, Abu-Bakr, El-Mahoy, & El-Tabey, 1983) have indicated that edible mushroom species are highly nutritious, their nutritional value comparing favourably with that of meat, eggs and milk. Additionally, several edible mushroom species act as sources of physiological agents for medicinal applications, possessing antitumour, cardiovascular, antiviral, antibacterial and other activities. Thus, *Ganoderma lucidum*, included in the studies we are hereby reporting, possesses potent anti-HIV activity (Chang & Mshigeni, 2001).

Despite the nutritional, medicinal, and physiological properties, almost all mushroom species growing wildy in tropical Africa, and particularly in Tanzania, have not been investigated for their constituent secondary metabolites or for their amino acid composition that would be a reliable indicator of the nutritional value. Apparently, even the biology of most of the wildy occurring Tanzanian mushroom species has not yet been sufficiently documented. For example, recently it was revealed that, out of 27 mushroom species of the genus *Lactarius* collected from various parts of Tanzania, 15 were hitherto scientifically not described (Karhula, Harkonen, Saarimaki, Verbeken, & Mwasumbi, 1998).

In Tanzania and most African countries, the use of some wild mushroom species in traditional medicines has been documented (Chang & Mshigeni, 2001; Karhula et al., 1998). Thus, in some rural areas of Tanzania, a mushroom soup is provided to mothers after childbirth in order to promote quick recovery of the mother, while other mushroom species are used as medicines for stomach and heart diseases. A few polypores, such as *Ganoderma* species, are used in the treatment of sick cows

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while some puffball mushrooms are traditionally used for wound healing in the Kilimanjaro region of Tanzania.

Although there are many edible wild mushroom species growing in Tanzania, their consumption is mainly confined to some rural communities, most of them being eaten only in the rainy season when they are abundantly available. Drying of fresh mushrooms is applied in some rural communities in order to preserve them for future use. In recent times, mushrooms have begun to assume greater importance in the diets of many urban dwellers in Tanzania and, as a consequence, the mushroom-farming business, using spores from domesticated as well as wild mushroom species, has begun to emerge in some suburbs of Dar es Salaam and other major towns (Chuwa, 1997; Mtowa, 1999). So far, few studies on the nutritive value of local edible mushrooms have appeared (Mamiro, 2002; Mshandete, 1998; Ndeky, 2002). Nevertheless, these studies have not determined the amino acid contents of wild mushroom species occurring in Tanzania. The determination of the amino acid content of these nutritional resources was conceived in order to establish a preliminary guide for assessing their relative nutritive qualities and thus provide a basis for tapping the nutritive potential of these natural resources in efforts towards abating nutritional deficiencies in developing countries. Results from these studies are discussed in this paper.

2. Materials and methods

2.1. Mushroom materials

Fruiting bodies (pileus + stipe) of mushrooms were either harvested or bought from various parts of Tanzania, including Mafinga, Mbeya, Kwamngumi forest reserve in the East Usambara Mountains, and at Ununio on the outskirts of Dar es Salaam city. The collections were done from December to May during 1999–2002; this period corresponds to the rainy season. The mushrooms were subsequently identified by Mr. L.B. Mwasumbi of the Herbarium, Department of Botany of the University of Dar es Salaam, Tanzania, and later authenticated at that Herbarium as *Boletus pruinatus* (Fr. & HÖK), *Boletinus cavipes* (Opat.) Kalchbr., *Cantharellus cibarius* (Fr.) Fr., *Inonotus* sp. cf. *obliquus* (Pers.: Fr.) Pil., *G. lucidum* (Curt.: Fr.) P. Karst, *Agaricus* sp. (L. ex Fr.), *Pleurotus sajor-caju* (Fr.) Sing., *Lactarius* sp. aff. *pseudovolemus* Heim., *Russula hiemisilvae* Buyck, and *Suillus granulatus* (L.) Kuntze.

2.2. Extraction

One gramme of each dried and pulverised mushroom species was soaked in 20 ml of distilled water for 24 h and the resulting extract was filtered and kept in the refrigerator until when required for analysis.

2.3. Amino acid analysis

The amino acid composition of each sample was determined using a high-performance liquid chromatograph (HPLC)-based amino acid analyzer (SHIMADZU, Model LC-10AT) fitted with a fluorescence detector (SHIMADZU model RF-10AXL, EX = 350 nm, Em = 450 nm) and a computer interfaced Communication Bus Module (model CBM-10A) data processing unit. A calibration chromatogram was established for 22 known amino acids (L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-cystine, L-glutamic acid, L-glutamine, glycine, L-histidine, *trans*-4-hydroxy-L-proline, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine) that were available in our laboratory. Thus, a 0.05 mmol standard solution of each of the standard amino acids was prepared by dissolving the corresponding acid in distilled water and then a mixture was constituted by mixing 1 ml of each of the 22 standard amino acid solutions and this was later used to establish the standard chromatogram. The mobile phase consisted of a 10 mM aqueous sodium phosphate (pH 6.8) solution (buffer solution A) mixed with acetonitrile, running in a gradient, starting with a mixture consisting of 5% acetonitrile in the buffer solution, and ending with acetonitrile alone. The free amino acids in the standards and in the mushroom species were automatically derivatized by reacting with *o*-phthalaldehyde under basic conditions to produce *o*-phthalaldehyde derivatives in the reaction columns of the amino acid analyser. Two derivatization reagent solutions were prepared as follows: to 10 ml of 0.01 M sodium borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) buffer solution B (pH 9.1) were added 10 ml of β -mercaptopropionic acid to make reagent solution I. Reagent solution II was prepared by mixing 10 ml of 0.01 M sodium borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) buffer solution B (pH 9.1) with 10 mg of *o*-phthalaldehyde (OPA) dissolved in 3 ml of ethanol. Solutions I and II were filtered through 0.45 mm membrane filters before use. Following derivatization, the buffer solution A (mixed in acetonitrile in a 2:1 v/v ratio), containing the derivatized amino acids, was transferred into the narrow bore HPLC system (HPLC column SRT ODSM, internal diameter = 4.6 and length = 150 mm) for separation at a temperature of 45 °C, with 10 ml injection volume and a flow volume of 1.0 ml/min.

3. Results

The percentage composition of amino acids in the analysed mushroom species is summarized in Table 1, which reveals that, a total of 16 amino acids was recorded in the mushroom species investigated in this study. The mushroom species contained different types

Table 1
Percentage composition of amino acids in some edible Tanzanian wild species of mushrooms

Scientific names of mushrooms											
Amino acids	<i>Agaricus</i> sp.	<i>Boletus pruinatus</i>	<i>Cantharellus cibarius</i>	<i>Lactarius</i> sp.	<i>Suillus</i> sp.	<i>Pleurotus sajor-caju</i>	<i>Russula hiemasilvae</i>	<i>Inonostus</i> sp.	<i>Boletinus cavipes</i>	<i>Ganoderma lucidum</i>	Frequency of appearance
Leu*	14.2	8.40	–	15.9	0.29	0.46	–	–	10.6	7.17	7
Lys*	6.26	2.59	–	0.005	–	6.33	–	–	3.43	–	5
Met*	–	1.53	9.19	2.05	0.96	–	0.09	21.4	2.31	45.6	8
Phe*	6.67	–	–	3.09	0.74	–	0.05	–	3.32	30.7	6
Thr*	0.82	5.02	7.99	11.0	–	8.56	4.05	–	7.79	7.99	8
Trp*	10.1	2.94	10.5	5.34	–	0.41	–	–	2.23	–	6
Val*	6.38	6.04	6.11	11.4	–	7.81	–	6.59	7.96	–	7
Ala	1.60	11.5	33.2	22.6	–	–	–	7.09	–	–	5
Arg	–	0.03	–	–	10.03	10.6	–	8.97	1.49	–	5
Asn	4.05	4.88	–	–	9.83	6.29	17.1	49.5	6.35	–	7
Asp	10.8	8.36	–	–	–	11.1	4.76	–	10.0	7.41	6
Gln	5.36	11.9	–	2.19	54.52	4.67	42.5	–	16.7	–	7
Glu	5.73	15.4	26.8	24.1	–	10.5	30.4	–	–	–	6
Gly	8.02	6.14	1.16	–	0.57	11.1	–	6.45	8.37	–	7
Ser	–	7.42	–	–	23.0	8.46	0.40	–	8.93	–	5
Tyr	–	3.42	–	2.30	–	3.36	–	–	4.78	1.11	5

Ala, alanine; Asp, aspartic acid; Asn, asparagine; Arg, arginine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

* Means essential amino acid.

of amino acids in varying numbers, ranging from 6 to 15. The mushroom species that contained the least number of amino acids were *G. lucidum* and the *Inonotus* sp., which had six amino acids each, while *Boletus pruinatus* contained the greatest number (15) of amino acids. Interestingly, even the essential amino acids, except isoleucine (Ile), could be identified in the studied mushroom species, with the *Lactarius* sp., *Boletus pruinatus* and *Boletinus cavipes* having more of these than the other mushroom species. For the *Lactarius* species the percentage composition of threonine (Thr), valine (Val), tryptophan (Trp), phenylalanine (Phe) and leucine (Leu) were 11.0%, 11.4%, 5.34%, 3.09% and 15.9%, respectively.

When the percentages of the essential amino acids in the three mushroom species (*Lactarius* sp., *Boletus pruinatus* and *Boletinus cavipes*) were compared, leucine (Leu) was found to be most abundant in all of them, being 15.9%, 10.6% and 8.40% in *Lactarius* sp., *Boletinus cavipes* and *Boletus pruinatus*, respectively. The second most abundant essential amino acid in the three species was valine (Val), recorded at 11.4%, 7.96% and 6.04% in *Lactarius* sp., *Boletinus cavipes* and *Boletus pruinatus*, respectively. Threonine (Thr) was the third most abundant essential amino acid in these mushrooms (11.0%, 7.79% and 5.02%, respectively). Leucine (Leu) was also the most abundant essential amino acid in the *Agaricus* sp. (14.2%). *R. hiemasilvae* registered the lowest number and amount of essential amino acids. The frequency of appearance of amino acids in various Tanzanian wild species of mushrooms ranged from 50% to 80%.

4. Discussion

These results clearly indicate the potential of wild mushroom species for their use as sources of essential amino acids. For example, of the 10 mushroom species that were investigated in this study, five were found to contain from 2 to 7 of the eight essential amino acids in different proportions. This is a 25% to 88% occurrence rate of the essential amino acids in the various mushroom species. Thus, the amino acid composition of some of the mushroom species, such as *Boletinus cavipes* (seven essential amino acids), compares favourably with the amino acid composition of *Alfalfa (Mendicago sativa)*, which is the most nutritious plant known so far, as it contains all the eight essential amino acids. Moreover, the present results corroborate well with the findings by Mashandete (1998) that the local edible mushrooms *Coprinus cinereus cinereus* (Schaell.) S. Gray, *Pleurotus flabellatus* (Berk. & Br.) Sacc. and *Volvariella volvacea* (Bull. Ex Fr.) Singer contained considerable amounts of crude protein (17–28%), carbohydrates (50–62%), crude fibre (6.6–11%), vitamin C (33–55 mg/100 g dry weight) and minerals (5.2–32 mg/100 g dry weight). The nutritional content of an *Oudemansiella* species, an edible wild Tanzanian mushroom, has been analysed (Ndekya, 2002) with emphasis on the protein, fat and vitamin contents and the protein content ranged from 6.64% to 31.70%, comparable to a *Pleurotus* and *Volvariella* species. The fat content ranged from 2.05% to 5.74% and the vitamin C content ranged from 46.8% to 58.5%, comparable to *Volvariella volvacea*. Recent studies (Mamiro, 2002) on the nutritive value of *Pleurotus*

flabellatus, harvested from differently supplemented dried and pasteurized shoots of water hyacinth, showed that the crude protein ranged from 18.7% to 39.0% and vitamin C, 23.5–42.1 mg/100 g. In addition to the protein and vitamin C contents, the crude fibre, crude fats and total carbohydrates were determined and found to range from 6.7% to 20%, 1.18–3.02% and 35–65.1%, respectively. Conceivably, these results indicate the importance of combining different species of mushrooms in a diet in order to obtain a good amount and variety of all the essential amino acids.

The preliminary results presented in this paper indicate that some edible Tanzanian wild mushroom species have a great potential for narrowing the amino acid and other nutrient supply deficits that are prevalent in many developing countries in Africa although, to date, virtually all edible mushrooms consumed in Tanzania are harvested only from the wild, as they are not yet domesticated. Therefore, in order to fully utilize these nutritional resources in abating nutritional deficiencies, intensive efforts towards the husbandry and popularization of wildy occurring Tanzanian mushroom species, particularly the more nutritious species, such as *Boletus pruinatus* and *Boletinus cavipes*, is hereby being encouraged. Furthermore, detailed analysis of the mushroom species for other nutrients, anti-nutrients and secondary metabolites with medicinal potential should be undertaken. The results from the present analysis allow a direct comparison of the amino acid content of wild edible mushrooms with those of other more common food resources.

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