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AN EVALUATION OF THE FREE AMINO ACIDS AND MINERAL CONTENTS OF SOME LOCAL EDIBLE MUSHROOMS.

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Abstract

Free amino acids and minerals (aluminum, potassium and sodium) of three edible wild mushrooms, ***Termitomyses robustus, Trichloma labayensis and Volvariella esculenta,*** *were determined. Twenty-one (21) free amino acids were found in the samples of two of the mushrooms.* ***Phenylalanine*** *was absent while glutamine was found in trace amounts in the stem of* ***T. lobayensis****. Most of the free amino acids were present in relatively high concentrations.* ***Tryptophan****, which ranged between 15.72 and 75.60mg/g predominated. Aluminum was relatively abundant in all the samples, but all the samples were very low in sodium (range 0.18 to 0.27 mg/100g).*

Introduction

The worldwide search for better and cheaper items of food, especially in the developed countries started many years ago. Since then, the increasing cost of production of animal protein for human nutrition began to reach alarmingly high levels. The conventional sources of protein and minerals were, as a result, getting beyond the reach of the low-income groups in many countries, especially, the developing countries.

The relative abundance of a variety of nutritionally good plant materials such as fruits, vegetables and more recently, mushroom, has directed major attention to the use of such items, for food. They are cheaper sources of protein, minerals and other nutritionally important health factors than animal food products. Such plant foods have been commonly consumed in many developing countries (Alofe, 1985) as part of the regular diet for decades. Fresh and actively growing specimens of some mushrooms as *Pleurotus tuber-regium* and *Tricholoma lobayensis* are sometimes kept between the cool and moist water-filled clay pots and the mud walls of thatched houses in fairly well illuminated places. In such conditions, the underground part of the fruit body continues to produce mushroom flushes of diminishing sizes and number for many months, until the food reserves have been depleted.

Today, modern technology, nutritional studies and commercial production of plant foods (Eby *et al., 1991*; Alofe *et al.,* 1996) have clearly demonstrated that such food items are of high nutritional quality, highly acceptable, and can be made readily available to the consumers.

Mushroom species that are commonly consumed in Nigeria include *Termitomyces robustus* (Beeli) Heim, *Tricholoma lobayensis* Fr. and *Volvariella esculenta* (Mass) Fries. They are not only consumed for their condiment characteristics, they are also consumed for their nutritional and medicinal properties (Oso, 1977; Alofe, 1991; Alofe *et al.*, 1996).

This study was undertaken to investigate nutritional quality of three of the most commonly consumed local mushroom species listed above.

Materials and Methods

Sample description

Termitomycetes robustus, a dark-brown capped mushroom, is called Olu-ewe' by the Nigerian Yorubas. The fruit bodies appear towards the end of the rainy season. It has a dark- brown pileus (cap) characterised by irregularly radiating light-brown lines. The stipe (stem or stalk) is long and stout. It is sought after by mushroom consumers all over the country between late July and mid-October when the mushroom normally appears. The fruit body grows symbiotically with termites, and only on termite nests.

The samples of the button and cup stages of the mushroom used in this study were collected from a cocoa and kolanut farm in September,1976. *Tricholoma lobayensis,* a pure white, fleshy and delicious mushroom, is generally believed to grow only in thick forests. The Yoruba call it 'Olu-Olosu-Meta' (meaning mushroom that lasts for 3 months) because the fruit body is believed to be subject to attack by insect and bacterial decay only after the fruit body is three months old when most of the spores would have been disseminated. Mature fruit body is used in folk medicine to cure ailments such as headaches, colds, stomach upset and fever (Alofe, 1985). Tricholoma *lobayensis* was found growing on burnt trunk and roots of Black Afra (*Terminalia superbra*), and the samples of the button and cup stages were collected from the burnt trunk of the tree in July, 1976.

Volvariella esculenta with a dark brown cap, which is streaked by irregular radially arranged light brown line is the smallest of the three mushrooms. It is also slightly fibrous, very juicy and delicious. It is never associated symbiotically with termites. Location and period of growth of *V. esculenta* have been previously described (Alofe, 1991). Samples of

the button and cup stages of the mushroom were collected from the trunks of *Sterculia tragacantha* in July, 1976. Samples of the three mushrooms were collected from various locations around Ile-Ife.

Five collections of each mushroom species were made from the same location and identified according to the methods of Zoberi (1973). The freshly collected samples were cleaned, graded, dried (60° C), pooled and homogenized as previously described by Alofe (1991) and Adewusi *et al.*, (1993). Two stages of development-the immature (button) and the mature (cup) stages-were analyzed for mineral and free amino acid contents.

Free amino acid determination

Each oven-dried sample (2g) was suspended in 50ml of 1% picric acid and homogenized (Alofe, *et al.*, 1996). The homogenized mixture was centrifuged at 3000 rpm for 10minutes and the supernatant was decanted. The pellet was rinsed with 15 ml of dionized water by manual shaking and centrifuged at 3,000 rpm after each rinse. The supernatants were combined and diluted volumetrically. The pH was adjusted using a glass electrode and drop wise addition of NHCl. Another batch of each sample (2g) was suspended in 50ml of 1% picric acid containing 6mM of norleucine as internal standard, and hydrolysed in the same manner as the unspiked mushroom samples (Eby *et al.*, 1977) Aliquots of the spiked and unspiked sample hudrolysates were analyzed for free amino acids using the method described by Benson (1976).

Mineral Determination

Oven-dried and homognized sample (2g) of each mushroom species was wet ached in20 ml of 1:5 perchloric-nitric acid mixture in a crucible covered with a watch glass. The mixture was slowly digested on an electric heater until all the sample had solubilized. The digest was evaporated to dryness and thereafter 2ml of concentrated hydrochloric acid (HCl) were added to dissolve it and the solution was again evaporated to dryness.

The residue was heated in 20 ml of 2N HCl until all the salts had dissolved (Yuan and friskell, 1959). The solution was quantitatively transferred into a 100 ml volumetric flask. The digest was diluted to volume with deionized water. A drop of chlorophenol red indicator was added to an aliquot of the digest in a centrifugetube to prevent flocculation of aluminium-aluminium complex. The content was mixed thoroughly and neutralised to the end point by dropwise addition of concentrated ammonium hydroxide(NH_4OH). The tube was then placed

in boiling water for 10 minutes and centrifuged at about 3,000 rpm for 15 minutes. The clear aluminium solution was transferred by pipetting into a 50 ml beaker. Four ml of hydroxylamine followed by 10ml of aluminium non- buffer reagent were added (Yuan and Friskell, 1959). The pH was adjusted to 3.5 using a glass electrode and dropwise addition of 2NHCl. The digest was heated to boiling, allowed to boiled gently for 5 minutes, cooled and diluted with deionized water to 50 ml. The procedure was repeated using standard aluminium solution-spiked samples of each mushroom species. Standard aluminium solution was prepared according to the method of Yuan and Friskell (1959).

To estimate potassium and sodium content of each mushroom sample, 2g of each sample was spiked with adequate amounts of potassium and sodium standard solutions that were prepared according to the method of Hansen (1973) . An unspiked sample of each mushroom species was also digested (Hansen, 1973). Aliquots of the spiked and unspiked digests were analyzed for aluminium, potassium and sodium using a Perkin-Elmer (305B) atomic absorption spectrophotometer.

Results

Distribution, composition and quantity of free amino acids

The values of the free amino acid determinations (Table1) were on fresh weight basis The recovery of the free amino acids was about 96% (range 95 to 101%). All the 21 free amino acids that were determined were present in *T. robustus* and *V.esculenta*,but phenylalanine was not found in *T.lobayensis*. Tryptophan was predominant in each mushroom species, but it was most abundant in *T. robustus*. Isoleucine, valine, threonine and serine were also relatively abundant in *T. robustus*. The free amino acid concentrations obtained for the three edible wild mushrooms are in agreement with those obtained for the cultivated mushroom, *Agaricus bisporus and some wild mushrooms* (Magioni, *et al.,1968;* Zakhary *et al., 1983).* Six of the 21 free amino acids namely methionine,phenylalanine, ornithine, glycine, proline and histidine, were limiting in V. *esculenta*, γ - amino –bensoic acid (*GABA)* and methionine were limiting in *T.robustus* while only phenylalanine was limiting in *T. lobayensis*. The concentration of each of the limiting free amino acids was below 10mg/10g. The amino acids were present in different concentrations in the pileus and stipe of each mushroom species. *T. robustus*, though very deficient in lysine and GABA, was the richest of the three mushroom species in total free amino acids.

Table 1: Free Amino Acids of three Mushrooms and their Contents[a] (mg/10g)

Amino acids	MUSHROOM SPECIES					
	Termitocytes	Robustus	Tricholoma	Iobayensis	Volvariella	esculenta
	Cap	Stem	Cap	Stem	Cap	Stem
Aspartic acid	23.2[b] 22.9[c]	0.9 0.8	7.6 7.5	8.7 8.4	10.1 9.8	2.8 2.6
Asparagine	10.6 10.6	4.4 4.2	16.3 16.2	8.5 8.3	9.7 9.4	2.6 2.3
Threonine	36.0 36.7	8.6 8.4	14.7 14.4	12.4 12.1	14.7 14.4	5.6 5.2
Serine	30.5 30.4	7.5 7.4	16.0 15.7	11.4 11.1	14.5 14.1	5.7 5.5
Glutamic acid	19.6 19.5	3.5 3.4	10.5 10.2	13.6 13.2	22.7 22.4	14.2 13.9
Glutamine	12.8 12.8	7.0 6.9	15.8 15.6	Trace Trace	29.1 28.7	17.2 16.8
Glycine	11.1 11.0	1.8 1.6	6.8 6.6	4.3 4.0	5.6 5.2	2.8 2.5
Alanine	59.3 59.2	30.5 30.5	26.6 26.5	20.7 20.5	29.5 29.2	14.5 14.0
Valine	50.4 50.0	0.5 0.5	6.7 6.4	14.8 14.5	15.5 15.2	7.2 7.0
Methionine	6.9 6.8	1.0 0.9	10.6 10.3	1.9 1.8	2.3 2.0	0.3 0.3
Isoleucine	46.0 55.9	11.4 11.1	3.9 3.7	13.6 13.3	12.7 12.4	4.9 4.7
Leucine	77.8 77.7	14.4 14.3	5.6 5.4	21.3 21.0	18.0 17.7	4.3 4.0
Tyrocine	20.7 20.7	5.2 5.2	3.9 3.7	2.0 1.7	7.9 7.6	4.2 3.9
Phenylalanine	18.8 18.6	3.7 3.6	0 0	0 0	3.3 3.1	2.0 1.7

Table 1 (Contd.): Free Amino Acids of three Mushrooms and their Contents[a] (mg/10g)

Amino acids	MUSHROOM SPECIES					
	Termitocytes	Robustus	Tricholoma	Iobayensis	Volvariella	esculenta
	Cap	Stem	Cap	Stem	Cap	Stem
Lysine	2.4	4.2	10.3	2.2	8.1	5.1
	2.2	3.9	10.0	1.9	7.7	4.8
GABA	2.9	3.4	7.1	3.2	6.7	3.6
	2.8	3.1	6.9	2.9	6.4	3.4
Tryptophan	63.3	53.3	18.9	72.8	70.2	16.1
	63.3	53.1	18.5	72.1	69.9	15.8
Ornithine	5.8	2.0	8.0	4.5	4.9	1.7
	5.7	1.8	7.7	4.3	4.6	1.4
Histidine	6.8	1.7	14.4	3.9	4.9	4.3
	6.5	1.5	14.1	3.5	4.5	3.9
Arginine	17.1	3.2	19.7	14.6	14.8	4.0
	16.9	2.9	19.4	14.3	14.5	3.7
Proline	11.8	5.0	4.4	7.8	6.3	2.4
	11.8	4.9	4.1	7.4	5.9	2.0
Total[d]	10.6	3.4	4.5	4.9	6.2	2.4
Lsd(P=0.05)	1.0	0.47	0.86	0.91	0.98	0.32

a= Values are calculated on fresh weight basis
b= Values are means of two determinations
c= Values are for button and cup stages, respectively
d= Total amounts are expressed in g/100g.
Lsd=Least significant difference

Distribution, composition and quantity of minerals

Mineral data from the spiked and unspiked mushroom samples (fresh weight basis) are presented in Tables 2 and 3, respectively. The recovery values obtained for the sample showed that 91 to 104 % of the test minerals were recovered from the spiked (Alofe, 1991) samples.

Table 2: Recovery of Minerals from Spiked Mushrooms

MINERALS	ADDED (mg)	FOUND (mg)	RECOVERED (mg)	RECOVERY (%)
Aluminium (Al)	10 –50	10.52 –91.21	9.06 – 46.05	91 – 92[a] (91)[b]
Potassium (K)	5 – 10	9.99 - 17.91	4.99 – 17.91	91 – 102 (95)
Sodium (Na)	1.17 – 1.24	1.17 – 1.24	1.17 - 1.24	100

a = Range and mean recovery % are provided, respectively
b=Average recovery %

Table 3

	MUSHROOM SPECIES					
	Termitomyces	*Robustus*	*Tricholoma*	*lobayensis*	*Volvariella*	*esculenta*
Mineral[b]	Cap	Stem	Cap	Stem	Cap	Stem
Aluminium	1.50[c]	1.46	8.74	11.32	3.06	42.96
(Al)	2.04	2.03	10.00	15.00	4.17	45.16
Potassium	3.63	3.65	7.00	4.31	5.40	4.00
(K)	3.60	3.62	7.50	4.35	5.48	4.27
Sodium (Na)	0.18	0.20	0.25	0.23	0.19	0.18
	0.21	0.21	0.27	0.23	0.19	0.19
Lsd (P = 0.05)	1.03	1.14	1.32	1.07	1.21	1.46

a = Values are calculated on fresh weight basis
b = Button and cup stages, respectively
c = Values are means of two determinations
Lsd = Least significant difference

Aluminium was predominant (2.03 to 45.16 mg/100g) in each mushroom sample with the highest level in the stipe of *V.esculenta.*

Potassium content in the mushroom sample ranges from 3.60 to 7.50mg/100g, and was highest in the pileus of *T. lobayensis.* All the samples contained very low sodium level (0.18 to 0.27mg/100g). These values are in agreement with those obtained for some other mushroom species (Seeger, 1978).

Discussion

Free amino acid content

The distribution of the 21 free amino acids among the three mushroom species and between the pileus and stripe of each species was apparently more or less similar, but different variations of free amino acids were exhibited. The concentrations of the protein amino acids of the three mushrooms have been reported to vary with species and the mushroom parts (Alofe, 1991) but not with their sizes as depicted by this study. The concentrations of the free amino acids also seem to decrease with increasing stage of development. A similar observation has been reported for *A. bisporus* by Magioni *et al* . (1968).

The three wild mushrooms are richer in free amino acids than some other food items such as tomatoes, onion, pepper, and mushrooms in many of the amino acids (Magion *et,al.,* 1968; Matsuoka *et,al.*.1981). However, *A. bisporus,* which is a cultivated mushroom, is richer in glutamic acid, proline, alanine, aspartic acid and serine than the three wild mushrooms (Oka *et,al.,*1981). The amount of free amino acids that is produced during the growth of wild-growing mushrooms or cultivated mushrooms is probably controlled by species, parts, source and availability of nitrogen (Migioni *et, al., 1968*).

Since protein (Alofe, 1991) and free amino acids are present in relatively high concentrations in the three edible wild mushrooms investigated in this study, inclusion of the mushrooms in the Nigerian diet on a regular basis could help to reduce the incidence of protein and amino acid-related diseases such as Kwashiokor in Nigerian children.

Mineral content

The concentration of aluminium was highest in the stipe of *V. esculenta.* Aluminium has been reported to be present in relatively high concentration in the mycelium of *Penicillium roqueforti,* a fungus used in the ripening process of roqueforti cheese (Shrimp and Kimsella, 1977). Although, aluminium is the third most abundant mineral in the earth's crust (Howells *et al.*, 1983), it is a generally non-toxic mineral. However, after it has combined with other elements to form various

compounds, it becomes very toxic, especially to fish (Howells *et al.*, 1983; Collier and Greenwood, 1977). Further research is, therefore, necessary to determine the probable toxicity of the aluminium component of edible mushrooms.

On the average, the mushrooms are richer in potassium than *A. bisporus* which was cultivated on ammonium sulphate-integrated compost, but they were poorer than *A. bisporus* grown in urea-integrated compost. They all had lower contents of sodium than that of *A. bisporus* cultivated on both types of compost (Magioni *et al.*, 1968).

It is a common knowledge that high dietary sodium, in some cases, aggravates hypertension. The three edible wild mushrooms investigated in this study had high potassium and very low sodium content. The high potassium and low sodium content characteristic of the mushrooms thus make them a suitable item of food that could be recommended for prevention of hypertension, which is prevalent among middle age Nigerians. Many patients with hypertension have been reported to have been treated by application of low sodium and high potassium diet (Shrine *et al* ., 1982).

Conclusion

In conclusion, the three mushrooms, *Termitomyces robustus, Tricholoma lobayensis* and *Volvariella esculenta* were found to be rich in protein free amino acids, potassium, aluminium and very low in sodium. This makes the mushrooms ideal source of protein and minerals for people, most especially hypertensive patients who are sodium-sensitive. Commercial cultivation of *T. labayensis* and *V. esculenta* is highly recommended for regular supply of the mushrooms.

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