



## **Amino Acids Composition of Some Wild Edible Mushrooms from Southern Cross River State, Nigeria**

**Juliet Oluwatominiyi Oni<sup>1\*</sup>, Glory Akpan Bassey<sup>1</sup>, Mary-Ibenreh Ogaboh Agba<sup>1</sup> and Aniedi-Abasi Akpan Markson<sup>1</sup>**

<sup>1</sup>Department of Plant and Ecological Studies, University of Calabar, Cross River State, Nigeria.

### **Authors' contribution**

This work was carried out in collaboration among all authors. Author JOO designed the study, wrote the protocol, managed the literature searches, set up the experiment, managed the analysis of the study, performed the statistical analysis and wrote the first draft. Authors M-IOA and GAB contributed in setting up the experiment. Author A-AAM contributed in designing the study. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Aims:** To document the amino acid content of six wild edible mushrooms - *Lentinus squarrosulus* Mont., *Auricularia auricular-judae* (Bull.) Wettst., *Mycetinis copelandii* (Desjardin) A.W. Wilson & Desjardin, *Baeospora myosura* (Fr.) Singer, *Pleurotus ostreatus* (Jacq. ex. fr) Kummer and *Volvariella volvacea* (Bull. ex. Fr.) Singer - found in southern Cross River State, Nigeria.

**Place and duration of study:** Department of Plant and Ecological Studies, University of Calabar, Cross River State, Nigeria, between May 2018 and August 2018.

**Methodology:** The amino acids content of these mushrooms were quantitatively estimated. The samples were obtained and analyzed for amino acids on dry weight basis using standard methods.

**Results:** The amino acid analysis quantitatively estimated the alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, Isoleucine, leucine, lysine, methionine, norleucine, phenylalanine, proline, serine; threonine; tryptophan; tyrosine and valine component of the mushroom samples. All the mushrooms studied contained nine essential, five conditionally essential

\*Corresponding author: E-mail: [jooni@unical.edu.ng](mailto:jooni@unical.edu.ng);

and four non-essential amino acids with glutamic acid as the most predominant (values ranging from 6.66 to 17.26g/100g protein). Cysteine (0.30-0.48g/100g protein) and methionine (0.64-1-1.7g/100g protein) were the lowest in concentration. The concentrations of five of these essential amino acids phenylalanine (3.55 mg/100g protein), valine (3.62 mg/100g protein), threonine (3.39 mg/100g protein), tryptophan (1.58 mg/100g protein), and lysine (3.23 mg/100g protein) in *P. ostreatus* were significantly ( $P \leq 0.05$ ) higher than found in the other mushrooms. For the nonessential and conditionally essential amino acids, the concentrations of arginine (6.02 mg/100g protein), aspartic acid (6.88 mg/100g protein), cysteine (0.48 mg/100g protein), glutamine (17.26 mg/100g protein) and glycine (2.61 mg/100g protein) in *P. ostreatus* were significantly higher ( $P \leq 0.05$ ) than found in the other mushrooms.

**Conclusion:** This information reveals that mushrooms are potential sources of quality protein with substantial proportion of essential amino acids indicating they can play a significant role in the fight against malnutrition.

**Keywords:** Amino acids; edible; mushrooms; protein; wild.

## 1. INTRODUCTION

Malnutrition remains a universal problem with numerous negative impacts. It affects all geographies, age groups, people of all social and economic status and all sexes. Malnutrition is said to be responsible for more ill health and higher levels of mortality than any other cause [1]. The increasing severity of the problems of malnutrition and the slow progress on tackling all forms of it has led many to search for and prioritize nutrition-specific diets for the fight against malnutrition. Nutrition-specific diets are described as food that targets the exact nutrient requirements for the well-being of an individual or a group [1]. Mushrooms are well-balanced food reported to contain 30-50% protein on dry weight basis, which can play a significant role in the fight against malnutrition [2-8]. In addition to being tasty, edible mushrooms are nutritious and are rich in protein, carbohydrates, dietary fiber, vitamins and minerals but low in calories and fat [9-13]. Their protein is of a good quality and contains all dietary essential amino acids [14,15].

Amino acids are said to be organic compounds made of nitrogen, carbon, hydrogen and oxygen, along with a variable side chain group. They are often referred to as the building blocks of proteins and they play vital roles in the body. According to [16] they are needed for vital processes such as building of protein and synthesis of hormones, nutrient absorption and neurotransmitters. Amino acid are also said to be the main construction material for antibodies to combat invading bacteria and viruses [2]. Reports indicate that the human body needs 20 different amino acids to grow and function properly [17,18]. These 20 amino acids are categorized as essential, conditionally essential

or non-essential depending on the body's ability to synthesize them [19,14]. Histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, phenylalanine and valine are categorized as essential because they cannot be synthesized by the body and must be supplied to the body in diet [14]. They conditionally essential amino acids which include arginine, cysteine, glycine, glutamine, proline and tyrosine are considered to be essential only under specific circumstances such as illness or stress [18,20,19] while alanine, aspartic acid, asparagine, glutamic acid and serine are said to be non-essential amino acids because they can be synthesized in sufficient quantity in the body [18,16].

Mushrooms are known to be rich sources of protein. The protein in mushrooms has been reported to be good competitor with animal protein and can be used in meat products to replace animal protein [14,2]. This makes it indispensable to incorporate mushroom in tackling the problem of malnutrition. Mushrooms have also been reported to contain good quality amino acids. In a research carried out by Musieba et al. [21] eight essential amino acids and other non-essential amino acids were detected in *Pleurotus citrinopileatus*, an indigenous mushroom in Kenya. [22] reported that the mushroom, *A. brunnescens*, contains all the essential amino acids. Five different species of the genus *Lentinus* from Northern India investigated for amino acid composition by Sharma et al. [23] were found to contain aspartic acid, arginine, alanine, proline and tyrosine. Sanon et al. [24] identified seven essential and other nonessential amino acids in four species of *Russula*. Kayode et al. [25] reported on the presence of amino acids in *Pleurotus sajor-caju* grown on gmelina wood waste in Nigeria. Nine

(9) essential amino acids and other non-essential amino acid were identified in some inedible mushrooms in Nigeria by Oni et al. [26].

The present study investigates the Amino acid composition of six wild edible mushrooms – *L. squarrosulus*, *A. auricular-judae*, *M. copelandii*, *B. myosura*, *P. ostreatus* and *V. volvacea* - found in southern Cross River State, Nigeria.

## 2. MATERIAL AND METHODS

### 2.1 Collection of Mushrooms

The fresh sporocarps of *L. squarrosulus*, *A. auricular-judae*, *M. copelandii*, *B. myosura*, *P. ostreatus* and *V. volvacea* used for this study were collected from decaying woods in University of Calabar Staff Quarters during the months of June 2018 - July 2018. University of Calabar Staff Quarters in Latitude 4° 57' 32" North, Longitude 8° 19' 37" East, is located at Calabar, Southern Cross River State, Nigeria.

### 2.2 Morphological Identification of Macrofungi

The freshly harvested mushrooms were brought to the laboratory, taxonomically studied and identified morphologically. Initial identification was done based on macroscopic features according to published descriptions and manuals. Morphological characters such as color, size, texture, shape, and margin of the fruit body, other features such as odor, spore print, gill attachment, and spacing were considered as previously used by [27]. The nomenclature was based on the Index Fungorum and Mycobank.

After proper identification, the materials were air-dried under room temperature (27 °C) for ten days; grind into powder with the aid of an electronic blender in order to increase the surface area [26].



Fig. 1. Map of Cross River State showing Southern Cross River

Source: Okedu et al. [37]

### 2.3 Amino Acid Analysis of Mushrooms

The Amino acid profile of the samples was determined using a well-known standard procedure described by [28] and reported by [26]. The samples were defatted in chloroform and methanol solution in the ratio of 2:1 after 4 grams of the sample was placed in soxhlet extraction thimble and refluxed with gentle heating for 15 hours [29]. Exactly 0.2 mg of the defatted sample was weighed into glass ampoule, 7 mL of 6NHCL was added and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at  $105 \pm 5$  °C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. Tryptophan which was destroyed by 6NHCL was recovered using alkaline hydrolysis method with 4.2 m sodium hydroxide [30]. The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5 ml to acetate buffer (pH 8.0) and stored in plastic specimen bottles kept in the freezer [30].

Sixty (60) micro litre of the hydrolysate was loaded into the PTH Amino acid analyzer model 120A (USA). This was dispensed into the cartridge of the analyzer which had been designed to separate the free acidic, neutral and basic amino acids of the hydrolysate concentration of the amino acids to produce a profile [29]. The results were obtained in g/100 gm.

### 3. RESULTS AND DISCUSSION

Higher non-essential amino acid content was detected in the mushrooms (Table 1). The total amount of amino acid content was highest in *P. ostreatus* whereas the least total amount was found in *A. auricular-judae*.

Table 2 shows that all the mushroom samples analyzed contained nine essential (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine), five conditionally essential (arginine, cystine, glycine, proline and tyrosine) and four non-essential (alanine, aspartic acid, glutamic acid and serine) amino acids. The concentrations of five of these essential amino acids phenylalanine (3.55g/100g protein), valine (3.62g/100g protein), threonine (3.39g/100g protein), tryptophan (1.58g/100g protein), and lysine (3.23g/100g protein) in *P. ostreatus* were significantly ( $P \leq 0.05$ ) higher when

compared to their values in the other mushrooms. Of the remaining four essential amino acids (methionine, leucine, isoleucine and histidine) the concentrations of methionine (1.17g/100g protein) and histidine (1.34g/100g protein) in *V. volvacea* were significantly higher ( $P \leq 0.05$ ) than was recorded in other mushrooms, followed by their concentrations in *P. ostreatus* with values of 1.06g/100g protein and 1.28g/100g protein respectively. The amount of Leucine (6.01g/100g protein) and isoleucine (3.14g/100g protein) in *B. myosura* were significantly ( $P \leq 0.05$ ) higher than was found in the other mushrooms, next to this record is their amount in *P. ostreatus* (5.31g /100g protein and 3.08g/100g protein respectively).

For the nonessential and conditionally essential amino acids, the concentrations of arginine (6.02g/100g protein), aspartic acid (6.88g/100g protein), cysteine (0.48g/100g protein), glutamic acid (17.26g/100g protein) and glycine (2.61g/100g protein) in *P. ostreatus* were significantly higher ( $P \leq 0.05$ ) than their values found in the other mushrooms. Next to these were the concentrations of arginine (5.85g/100g protein) and glutamic acid (16.65g/100g protein) in *B. myosura* as well as the concentrations of aspartic acid (5.95g/100g protein) and cysteine (0.36g/100g protein) in *V. volvacea*. The values of serine (3.02g/100g protein) and tyrosine (2.92g/100g protein) in *V. volvacea* were significantly higher ( $P \leq 0.05$ ) than their values obtained in the other mushrooms. The highest value of proline (3.25g/100g protein) was found in *M. copelandii*, followed by *P. ostreatus* (3.05g/100g protein) whereas the highest value of alanine was detected in *B. myosura* (4.32g/100g protein). Norleucine was not detected in all the mushroom species analysed.

#### 3.1 Discussion

All the mushrooms studied contained essential amino acids, conditionally essential and non-essential amino acids. Glutamic acid, a non-essential amino acid, was the most predominant (values ranging from 8.33g/100g protein to 17.26g/100g protein). Cysteine, a conditionally essential (0.30-0.48g/100g protein) and methionine, an essential amino acid (0.69-1.7g/100g protein) were the lowest in concentration. Similar results have been reported by other researchers [24,21]. It was suggested that the high concentration of glutamic acid may be because of their use as precursors for the synthesis of other amino acids. Glutamic acid is

**Table 1. Total amino acid content of the different mushroom species**

Amino acid type	The amino acid content of different species (g/100g of protein)					
	<i>A. auricular-judae</i>	<i>B. myosura</i>	<i>M. copelandii</i>	<i>P. ostreatus</i>	<i>L. squarrosulus</i>	<i>V. volvacea</i>
The total amino acid content	38.48	67.71	62.78	71.13	43.85	58.69
Total essential amino acids	13.82	25.44	22.19	25.42	15.08	21.42
Total conditionally essential amino acids	8.19	13.55	13.12	14.4	9.24	12.57
Total non-essential amino acids	16.47	28.72	27.47	31.31	19.53	24.69

**Table 2. Concentration of amino acids in 6 selected mushrooms in Calabar, Southern Cross River State**

	Mushroom species						Amino acids (g /100g protein)													
	ALA	ARG	ASP	CYS	GLU	GLY	HIS*	ILU*	LEU*	LYS*	MET*	NLU	PHE*	PRO	SER	THR*	TRP*	TYR	VAL*	
<i>A. auricular-judae</i>	2.50± 0.11 <sup>f</sup>	3.61± 00.03 <sup>f</sup>	3.91± 0.06 <sup>f</sup>	0.48± 0.01 <sup>a</sup>	8.33± 0.09 <sup>e</sup>	1.28± 0.02 <sup>f</sup>	0.89± 0.01 <sup>f</sup>	1.37± 0.02 <sup>e</sup>	3.03± 0.04 <sup>f</sup>	1.43± 0.04 <sup>e</sup>	0.8± 0.04 <sup>d</sup>	0.00± 0.00	2.3± 0.01 <sup>d</sup>	1.62± 0.03 <sup>f</sup>	1.73± 0.07 <sup>e</sup>	2.00± 0.03 <sup>f</sup>	0.89± 0.03 <sup>d</sup>	1.2± 0.03 <sup>e</sup>	1.11± 0.03 <sup>f</sup>	
<i>B. myosura</i>	4.32± 0.02 <sup>a</sup>	5.85± 0.05 <sup>b</sup>	5.21± 0.1 <sup>c</sup>	0.48± 0.01 <sup>a</sup>	16.65± 1.00 <sup>a</sup>	2.52± 0.03 <sup>b</sup>	1.21± 0.02 <sup>c</sup>	3.14± 0.03 <sup>a</sup>	6.01± 0.03 <sup>a</sup>	3.02± 0.03 <sup>b</sup>	0.91± 0.02 <sup>c</sup>	0.00± 0.00	3.19± 0.05 <sup>b</sup>	2.64± 0.04 <sup>c</sup>	2.54± 0.04 <sup>b</sup>	3.22± 0.05 <sup>b</sup>	1.47± 0.03 <sup>b</sup>	2.06± 0.02 <sup>c</sup>	3.27± 0.02 <sup>b</sup>	
<i>M. copelandii</i>	3.79± 0.01 <sup>c</sup>	4.82± 0.1 <sup>c</sup>	4.84± 0.03 <sup>d</sup>	0.48± 0.00 <sup>a</sup>	15.59± 0.09 <sup>b</sup>	2.33± 0.02 <sup>d</sup>	1.15± 0.01 <sup>d</sup>	2.95± 0.04 <sup>b</sup>	4.96± 0.02 <sup>c</sup>	2.97± 0.02 <sup>bc</sup>	0.96± 0.03 <sup>c</sup>	0.00± 0.00	3.01± 0.04 <sup>c</sup>	3.25± 0.01 <sup>a</sup>	1.94± 0.02 <sup>d</sup>	3.00± 0.01 <sup>c</sup>	1.52± 0.01 <sup>ab</sup>	2.24± 0.02 <sup>b</sup>	2.98± 0.05 <sup>c</sup>	
<i>P. ostreatus</i>	4.12± 0.18 <sup>b</sup>	6.02± 0.02 <sup>a</sup>	6.88± 0.09 <sup>a</sup>	0.48± 0.01 <sup>a</sup>	17.26± 0.26 <sup>a</sup>	2.61± 0.01 <sup>a</sup>	1.28± 0.01 <sup>b</sup>	3.08± 0.04 <sup>a</sup>	5.31± 0.04 <sup>b</sup>	3.22± 0.01 <sup>a</sup>	1.06± 0.02 <sup>b</sup>	0.00± 0.00	3.55± 0.01 <sup>a</sup>	3.05± 0.04 <sup>b</sup>	2.38± 0.04 <sup>c</sup>	3.39± 0.06 <sup>a</sup>	1.58± 0.05 <sup>a</sup>	2.24± 0.07 <sup>b</sup>	3.62± 0.06 <sup>a</sup>	
<i>L. squarrosulus</i>	2.65± 0.01 <sup>e</sup>	3.78± 0.01 <sup>e</sup>	4.28± 0.05 <sup>e</sup>	0.36± 0.00 <sup>b</sup>	10.3± 0.06 <sup>d</sup>	1.42± 0.00 <sup>e</sup>	1.02± 0.03 <sup>e</sup>	1.57± 0.05 <sup>d</sup>	3.21± 0.02 <sup>e</sup>	1.59± 0.07 <sup>d</sup>	0.69± 0.05 <sup>e</sup>	0.00± 0.00	2.13± 0.05 <sup>e</sup>	2.3± 0.03 <sup>d</sup>	1.62± 0.05 <sup>f</sup>	2.27± 0.02 <sup>e</sup>	1.31± 0.03 <sup>c</sup>	1.38± 0.05 <sup>d</sup>	1.29± 0.05 <sup>e</sup>	
<i>V. volvacea</i>	2.88± 0.02 <sup>d</sup>	4.64± 0.04 <sup>d</sup>	5.95± 0.05 <sup>b</sup>	0.36± 0.01 <sup>b</sup>	13.63± 0.03 <sup>c</sup>	2.42± 0.02 <sup>c</sup>	1.34± 0.01 <sup>a</sup>	2.23± 0.02 <sup>c</sup>	4.2± 0.04 <sup>d</sup>	2.92± 0.02 <sup>c</sup>	1.17± 0.05 <sup>a</sup>	0.00± 0.00	3.19± 0.02 <sup>b</sup>	2.23± 0.03 <sup>e</sup>	3.02± 0.03 <sup>a</sup>	2.5± 0.02 <sup>d</sup>	1.47± 0.04 <sup>b</sup>	2.92± 0.01 <sup>a</sup>	2.4± 0.25 <sup>d</sup>	

Values are means of three replicate. Means followed by different letters in each column are significantly different ( $P \leq 0.05$ ) according to Duncan's Multiple Range Test (DMRT).

\*Essential amino acid

ALA=Alanine; ARG=Arginine; ASP=Aspartic acid; CYS=Cystine; GLU=Glutamic acid; GLY=Glycine; HIS=Histidine; ILU=Isoleucine; LEU=Leucine; LYS=Lysine; MET=Methionine; NLU=Norleucine; PHE=Phenylalanine; PRO=Proline; SER=Serine; THR=Threonine; TRP=Tryptophan; TYR=Tyrosine; VAL=Valine

also known to be an active participant in metabolism and synthesis of nucleotides [18,20,21,31]. It is also said to play a role in the proper functioning of the brain, nervous system and participates in the detoxification of ammonia in liver muscle and brain [32,21,31]. The high content of glutamic acid is believed to contribute to the flavoring of mushrooms [33]. Cysteine and methionine are two amino acids with high sulphur content which have been reported to be powerful antioxidant with capability to eliminate or detoxify harmful agents such as lead and other heavy metals [16,32]. Methionine is said to prevent deficiencies of hair cells, skin and nails. It protects against greasy clusters around the liver and the arteries that cause obstructions [32].

Other non-essential amino acid identified included alanine, aspartic acid and serine. Alanine values ranged from 2.50g/100g to 4.32g/100g protein. Alanine has been reported to influence transfer of nitrogen and glucose in the body, it is also believed to boost immunity producing antibodies [33,20,32]. Aspartic acid values varied from 3.91g/100g protein to 6.88g/100g protein (*P. ostreatus*). Aspartic acid is reported to be a physiological compound found in the mammalian pituitary and testis and regulates the synthesis and release of testosterone [18,20,32]. The amount of serine fluctuated between 2.27g/100g protein and 3.02g/100g protein. This amino acid is said to be necessary for muscle development, maintenance of the immune system and formation of RNA and DNA from cells [20,32].

Additional conditionally essential amino acids detected in the studied mushroom include arginine, glycine, proline and tyrosine. The values of arginine ranged between 3.61g/100g protein to 6.02g/100g protein. Arginine is said to exhibit anticancer and immune boosting properties. It is believed that it detoxifies the liver by neutralizing ammonia and reducing the toxicity of alcohol. It is considered to be the "natural Viagra" that increases blood flow in the penis and is used in the treatment of infertility in men [26,34,35]. Glycine varied with contents that are set between 1.28g/100g protein and 2.61g/100g protein. [32] reported that glycine retards muscle degeneration, improves glycogen storage and releases glucose to meet energy needs of the body. Proline varied from 1.62g/100g protein to 3.25g/100g protein. Reports indicate that proline improves the texture of skin, is used in curative treatment to avoid the

problems of cartilage, tendons and muscle of the heart [20,32].

Histidine, isoleucine, leucine, lysine, threonine, phenylalanine, tryptophan and valine were other essential amino acids found in the studied mushrooms. Histidine was in the range of 0.86g/100g protein to 1.34g/100g protein. Histidine has been shown to be an effective component in the therapy against cardiovascular diseases within physiological antioxidant role on free radicals [16,32]. Isoleucine values ranged from 1.37 g/100g of protein to 3.14g/100g of protein. Isoleucine is reported to be heavily concentrated in muscle tissue and is involved in muscle metabolism. It's also said to be important for immune function, hemoglobin production and energy regulation [16]. The amount of leucine was between 3.03 g/100g of protein and 6.01g/100g protein. Leucine is believed to interact with Isoleucine and valine to promote muscle function, skin and bone [18,20,16,35]. Phenylalanine values varied from 2.30g/100g protein to 3.55g/100g protein. Phenylalanine has been shown to be effective in the treatment of migraine, arthritis, painful menstruation, depression, obesity, schizophrenia and parkinson's disease. It is said to be precursor for the neurotransmitters tyrosine, dopamine, epinephrine and norepinephrine. [18,32]. In addition, it is recognized that phenylalanine is used by the brain to produce norepinephrine, a chemical that transmits signals between nerve cells. It is also believed to promote alertness and vitality, regulates human mood and reduces pains [18,20,32]. The result of this study indicates that the values of Theorine varied from 2.00g/100g protein to 3.39g/100g protein. Theorine is said to play a vital role in the formation of enamel, the collagen and elastin. Tryptophan which varied between 0.89g/100g to 1.58g/100g protein content is reported to play a role in reducing depression, boosting mood and improving sleep [36,35]. For Valine, the values oscillate between 1.11g/100g protein and 3.62g/100g protein. This amino acid is said to be effective in the treatment of liver and gall bladder diseases and to promote intellectual liveliness [16,33].

#### 4. CONCLUSION

The result of the amino acids profiling showed that all the mushroom samples studied contained essential (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine), conditionally essential

(arginine, cysteine, glycine, glutamine, proline and tyrosine) and nonessential (alanine, aspartic acid, serine) amino acids. Among the mushroom samples used for this study, *Pleurotus ostreatus* is outstanding in amino acids composition as it recorded higher values for 10 of the amino acids. This information shows that mushrooms are potential sources of quality protein with substantial proportion of essential amino acids indicating they can play a significant role in the fight against malnutrition.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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