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Comparative Study on the Proximate Composition and Amino Acid Levels in Chanterelle (*Cantharellus cibarius*) and Wood Ear (*Auricularia auricula*) Mushroom Samples

Arowora Kayode Adebisi[1], Moses Adondua Abah[2], Kenneth Chinekwu Ugwuoke[3], Arowora Mercy Dooshima[4], Odi Raphael Ndoma[5], Precious Chizaram Okani[6], Kimi Smith Ominisan[7], Adetoyi Moses Gbadebo[8], Aliyu Najeeb Olamilekan[9], and Asare Bernard Kwame[10]
[1,2,3,5]Federal University Wukari, Taraba State, Nigeria; [4]National Teachers Institute, Wukari Centre, Taraba State, Nigeria; [6]Michael Okpara University of Agriculture Umudike, Abia State, Nigeria; [7]Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria; [8]University of Ilorin, Kwara State, Nigeria; [9]Nile University of Nigeria, FCT, Abuja, Nigeria; [10]Fulda University of Applied Sciences, Germany
m.abah@fuwukari.edu.ng

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Abstract

Mushrooms have been consumed by mankind for millennia. They are macrofungi with a distinctive fruiting body which can be hypogeous or epigeous, large enough to be seen with unaided eyes. They are not widely consumed due to lack of information on its nutritive value. Therefore, this research was carried out to compare the proximate gross energy composition as well as amino acids levels in two selected edible mushrooms (*Cantharellus cibarius and Auricularia auricula*). Fully matured species of Chanterelle mushroom (*Cantherellus cibarius*) and Wood ear mushroom (*Auricularia auricula*) were collected from different parts of Southern and Northern Cross River; Ikom and Bekwara precisely. The collected samples were air dried for 5 days and then stored in transparent polythene bag for analysis. The dried and stored mushroom were pounded to powdered form and then subjected to amino acid analysis using isocratic HPLC and proximate analysis for proximate composition using the standard methods of Association of Official Analytical Chemist (AOAC). The results of this research showed that chanterelle mushroom has high concentration of amino acids compared to Wood ear mushroom. However, there was significant differences ($p<0.05$) in the levels of isoleucine in both mushrooms. There were no significant differences in the proximate composition and gross energy levels of both mushroom species. Some of the amino acids detected in both mushrooms were: methionine, threonine, isoleucine, leucine, phenylalanine, valine, tryptophan, histidine, arginine, aspartic acid, glycine, alanine and glutamic acid. Based on the findings of this study, the two edible mushrooms were observed to have appreciable levels of gross energy and amino acids. However, Chanterelle mushroom had higher protein composition than wood ear. Hence, it can be concluded that Chanterelle mushroom has greater nutritive value and more suitable for consumption.

Keywords: Mushroom, Amino acids, Proximate composition, *Cantharellus cibarius* and *Auricularia auricula*

INTRODUCTION

Mushrooms have long been considered as ingredient of gourmet cuisine across the globe; especially for their unique flavor. They have been valued by humankind as a culinary wonder. More than 2,000 species of mushrooms exist in nature, but around twenty-five are widely accepted as food and few are commercially cultivated (Seo, *et al*, 2003). Mushrooms are considered delicacies with high nutritional and functional value, and they are also accepted as nutraceutical foods. They are of considerable interest because of their organoleptic merit, medicinal properties, and economic significance (Seo, *et al.*, 2003; Tisdale, 2004). The most cultivated mushroom worldwide is *Agaricus bisporus*, followed by *Lentinusedodes*, *Pleurotus spp.*, and *Flammulina velutipes*. Mushroom production is on increase globally with China being the biggest producer around the world (Tisdale, 2004; Dunkwal *et al.*, 2007; Lee *et al.*, 2011).

Based on the chemical composition and benefits of mushrooms, they can be classified as poisonous and edible mushrooms. Edible mushrooms can further be categorised into wild and cultivated edible mushrooms (Waktola and Temesgen, 2020). There also exist a class of mushrooms known as medical mushrooms. However, there is not an easy distinction between edible and medical mushrooms because many of the common edible species have therapeutic properties and several used for medical purposes are also edible (Yildiz *et al*., 2002). Mushrooms have great nutritional value since they are quite rich in protein, with an important content of essential amino acids and fibre, poor fat but with excellent important fatty acids content. Moreover, edible mushrooms provide a nutritionally significant content of vitamins (B1, B2, B12, C, D, and E) (Singh *et al*., 2011; Mat *et al.,* 2013). Thus, they could be an excellent source of different nutraceuticals and might be used directly in human diet and to promote health for the synergistic effects of all the bioactive compounds present (Haimid *et al*., 2016). A large variety of mushrooms have beenutilized traditionally in many different cultures for the maintenance of health, as well as in the prevention and treatment of diseases through their immunomodulatory and antineoplastic properties. Mushrooms could also be an alternative source of new antimicrobial compounds, mainly secondary metabolites, such as terpenes, steroids, anthraquinones, benzoiccaci derivatives, and quinolones, but also of some primary metabolites like oxalic acid, peptides, and proteins (Chiroro, 2004).

With an ever-increasing demand and consumption of edible mushrooms with time, there happens to be something intriguing about it. The consumption and use of this class of mushroom comes with little or no health implication. However, there is little information on researches which had been carried out on these mushroom species (Chanterelle and Wood ear mushrooms) in Taraba state, Nigeria. Also, considering the rate at which people consumed mushroom in our community with little or no idea on their nutritional composition, efforts are needed to furnish consumers with information on the nutritional qualities of Chanterelle and Wood ear mushrooms. Hence, this project was designed to reveal some nutritional information of these edible mushrooms.

MATERIALS AND METHODS

Sample Collection and Identification

Fully matured species of Chanterelle mushroom (*Cantherellus cibarius*) and Wood ear mushroom (*Auricularia auricula*) were collected from different parts of Southern and Northern Cross River; Ikom and Bekwara precisely. The mushroom species were collected at different times of the day; morning, afternoon, and evening, by uprooting their substratum with the aid of a scalpel. The samples were then transported to the herbarium of Cross River University of Technology (CRUTECH) where they were identified.

Sample Preparation

Upon identification, the samples were air dried for 5 days and stored in transparent polythene bags that were tightened. They were then transported for analysis in Cross River University of Technology, Cross River State, Nigeria. About 100g of chanterelle and wood ear mushroom was weighed into a mortar and pounded until fine particles of the mushrooms were obtained. 240ml of distilled water was then added to the sample in a 250 ml beaker and made up to 250ml with distilled water, then allowed to settle for 4-6 minutes. The mixture was then filtered using Whatman filter paper.

Amino Acid Determination

Preparation of standard amino acid solutions

Standard solutions of the amino acids (all essential and the entire non-essential amino acids) were prepared. The solutions were prepared by adding 0.1 M HCl. The concentrations of the standard solutions were serially diluted to give 25, 20, 15 and 10nM each. They were stored in a freezer at 4°C till use. The mixed standard solution contained 25 pmol of each amino acid derivative.

Derivatization of the samples

The mixed amino acid solution or filtered sample (10 µL) was transferred to a full recovery sampler, to which 70 µL sodium hexane sulphonic acid buffers was added; the solution was vortexed briefly and then 20 µL of reconstituted buffer, and the solution was mixed by vortexing for several seconds. It was then heated on a heating block at 55°C for 10 min. Derivatives were stable at room temperature for up to 1 week.

Chromatographic conditions and procedure

Chromatographic separation of prepared samples was carried on a Buck scientific BLC10/11 – model HPLC equipped with UV 338nm detector. A C18, 2.5 x 200mm, 5um column and a mobile phase of 1:2:2 (100mM sodium phosphate, pH 7.2: Aceton nbitrile: methanol v/v/v) at a flow rate of 0.45 mL/minute and an operating temperature of 40^{o}c. Standard solutions were analysed in a similar manner. In terms of retention time, the composition of each peak was confirmed and using peak area of each amino acid the concentrations were determined in accordance with the external standard method (by extra plotting from the calibration curve, prepared by plotting a graph of peak area versus concentration of each amino acid standard solution).

The samples being air-dried and crushed into a powder with a mixer/grinder, 2.0 g of the sample were accurately weighed and quantitatively transferred into a beaker. The beaker was placed in a water bath at 40°C to melt the mass. Then, the digested sample was transferred into a 50 mL calibrated flask; the volume was made up by adding enough water to the mark and the beaker was kept in an ultrasonic bath for 30 min at 40°C. The extract was centrifuged and 30 ml of water was added to the residue, which was then sonicated for 30 min. The solution was then passed through a 0.22 µm Millipore membrane, and the filtrate was transferred to a 100 mL volumetric flask and diluted with water to make up the volume. This solution was passed through a 0.22 µm Millipore membrane, and the filtrate was used for the following experiments.

Proximate Analysis

Crude Fibre Determination

2ml of the defatted sample was weighed and dispensed into a quick fit glass. 50ml of glacial acetic acid was added to the sample and placed in a heater in a fume cupboard (digestion flask) at about 200-350 degree Celsius for 45- 60 minutes for proper digestion. After digestion, the digested sample was filtered thoroughly with an already weighed filter paper and dried in an oven at 100 degrees Celsius for 24 hours after which it was weighed and recorded. The residue was ash in a weighed crucible at 580 – 600 degree Celsius for 3– 4 hours in a furnace and was weighed and recorded (Anih *et al.,* 2023). Calculation of fiber content was as follows;

Weight of residue = weight of filter paper + residue – weight of filter paper Weight of ash = weightof ash + crucible – weight of empty crucible

Weight of crude fiber = weight of ash – weight of residue

Determination of Crude Protein

Over 2g of the chanterelle and Wood ear mushroom sample from the prepared 240g beaker was weighed into a micro Kjeldahl digestion flask and one tablet of Selenium catalyst was added. The mixture was digested on an electro thermal heater until clear solution was obtained. The flask was allowed to cool after which the solution was diluted with distilled water to 50ml and 5ml of it was transferred into the distillation apparatus, 5ml of 2% boric acid was pipetted into a 100ml conical flask (the receiver flask) and four drops of screened methyl red indicator were added. About 50% NaOH was continually added to the digested sample until the solution turned cloudy which indicated that the solution had become alkaline. Then distillation was carried out into the boric acid solution in the receiver flask with the delivery tube below the acid level. As the distillation was going on, the dark blue colour solution of the receiver flask turned blue indicating the presence of ammonia. Distillation was continued until the content of the flask was about 50ml after which the delivery of the condenser was rinsed with distilled water. The resulting solution in the conical flask was then titrated with 0.1M HCl. The crude protein (N × 6.25) of the mushroom flours was estimated by micro-Kjeldahl method (Abah *et al.,* 2024).

Ash Determination

An empty crucible was weighed and recorded. 2ml of Chantherelle and Wood ear mushroom sample from the prepared 240mls beaker were added into the crucible and cooled in a desiccator. Afterwhich, it was weighed and the new weight of the crucible plus ash was recorded (Abah *et al.,* 2024). The ash content was then calculated as follows;

Weight of ash = (weight of crucible + ash) – weight of crucible % weight of ash = ×100.

Moisture Content Determination

Moisture content was determined by weighing 2 g each of the powdered mushroom species with a silica dish which has been previously ignited and weighed, dried in an oven (Genlab MINO/30 UK) for 24 hours at 1000°C to constant weight and then cooled in a desiccator each time just before weighing (Tatah *et al.,* 2024).

Calculation: % moisture =100 x (wt of dish + powdered mushroom before drying) – wt of dish + powdered mushroom after drying) wt. of powdered mushroom

Determination of Fat

Oil was extracted from the mushroom samples sample with petroleum spirit under controlled conditions. A flask was dried in an oven at 1000°C and allowed to cool in a desiccator and weighed. 3g of the sample was weighed and ground to pass a 1 mm mesh sieve into a thimble and plunged with cotton wool. The thimble was then placed with its contents into the extractor. Extraction was done with petroleum spirit for at least 4 hours and the residue was transferred from the thimble to a small mortar, ground lightly and returned to the extracted apparatus. The mortar was washed with a small quantity of petroleum spirit and the washings were added to the flask. The extraction continued further for an hour. The thimble was removed and most of the solvent was distilled from the flask into the extractor. The flask was disconnected and placed in an oven at 1000°C for 2 hours and then allowed to cool and weighed (Anih *et al.,* 2023).

Calculation: Multiply the increase in weight by 100 and divide by the weight of the sample taken. The result gives the percentage w/w of oil in the sample

Determination of Carbohydrate

Carbohydrate content in the mushroom samples was determined using the formula presented below (Anih *et al.,* 2023):

$$\% \text{ CHO} = 100\% - (\% \text{ moisture} + \% \text{ ash} + \% \text{ protein} + \% \text{ fat} + \% \text{ cf})$$

RESULTS

Proximate Compositions of *Auricularia auricula* and *Cantharellus cibarius* Mushroom Species

Results for proximate compositions of *Auricularia auricula* and *Cantharellus cibarius* are shown in Table 1 and Figure 1 below. The energy level in both mushrooms are seen to be high; *Auricularia auricula* (45.34±0.01) and *Cantharellus cibarius* (51.22±0.01). *Cantharellus cibarius* was observed to have higher level of protein (32.55±0.00) compared to *Auricularia auricula* (28.46±0.01). The moisture content of both mushrooms was not significantly different from each other *Cantharellus cibarius* and *Auricularia auricula* had moisture content of 4.55±0.00 and 5.15±0.00 respectively. *Cantharellus cibarius* showed a significantly higher fibre content (36.34±0.01) than *Auricularia auricula* (29.21±0.01). However, *Auricularia auricula* was observed to have a significantly higher carbohydrate content (23.83±0.03) than *Cantharellus cibarius* (14.33±0.01). There was no significant difference in ash content between both mushroom species. *Cantharellus cibarius* revealed an ash content of 8.15±0.00 whereas *Auricularia auricula* gave a value of 7.20±0.00.

Table 1. Proximate composition of *Auricularia auricular* and *Cantharellus cibarius* mushroom species

Proximate Constituent (mg/100g)	**Mushroom Species**	
	Auricularia auricula	***Cantharellus cibarius***
Protein	28.46±0.01[a]	32.55±0.00 [a]
Fat	6.15±0.00 [a]	4.12±0.00 [a]
Fibre	29.21±0.01 [a]	36.34±0.01 [a]
Ash	7.20±0.00 [a]	8.15±0.00 [a]
Moisture	5.15±0.00 [a]	4.55±0.00 [a]
Carbohydrate	23.83±0.03 [a]	14.33±0.01 [a]
Energy (kcal/100g)	45.34±0.01 [a]	51.22±0.01 [a]

Result presented as mean ± standard deviation. Means within a column with the samesuperscript are not significantly different (p<0.05).

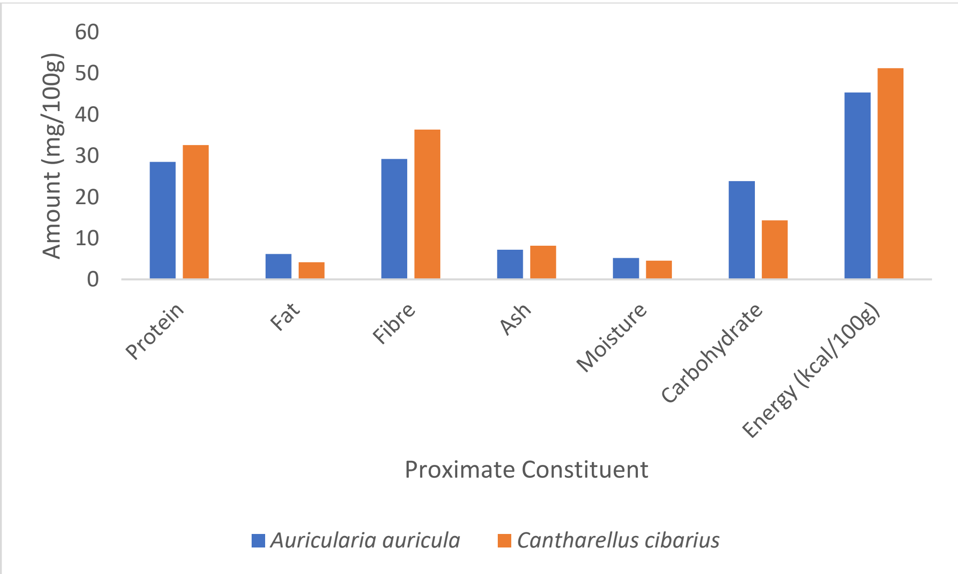

Figure 1. Proximate composition of *Auricularia auricular* and *Cantharellus cibarius* mushroom species

Amino Acids Levels in *Auricularia auricula* and *Cantharellus cibarius* Mushroom Species

The levels of both essential and non-essential amino acid levels present in the analysed mushroom samples are presented in Table 2 and Figure 2below. Of all the amino acids detected in the analysed mushroom samples, glutamic acid level was highest in *Auricularia* *auricula* and *Cantharellus cibarius*. Threonine level was the least detected in *Auricularia auricula* (1.79±0.01) and *Cantharellus cibarius* (2.18±0.01). Isoleucine (4.06±0.01) was the amino acid whose level was higher in *Auricularia auricula* compared to *Cantharellus cibarius*. Equal amount of tyrosine (6.82±0.01) was present in both species of mushroom. The following amino acid levels were higher in *Cantharellus cibarius* compared to *Auricularia auricula*: lysine (4.23±0.01), methionine (2.88±0.01), threonine (2.18±0.01), leucine (8.55±0.00), Phenylalanine (5.37±0.01), valine (4.96±0.01), tryptophan (5.29±0.01), histidine (3.86±0.01), arginine (6.32±0.01), serine (5.17±0.01), cysteine (2.98±0.01), alanine (4.22±0.00), aspartic acid (8.34±0.01), glutamic acid (8.78±0.01), glycine (5.88±0.01), and proline (3.99±0.01).

Table 2. Quantitative levels of amino acids in *Auricularia auricula* and *Cantharellus cibarius*.mushroom species

	Mushroom Species	
Amino Acid (mg/100g Protein)	***Auricularia auricula***	***Cantharellus cibarius***
Lysine	3.35±0.01	4.23±0.01
Methionine	2.76±0.00	2.88±0.01
Threonine	1.79±0.01	2.18±0.01
Isoleucine	4.06±0.01	4.04±0.01
Leucine	7.78±0.01	8.55±0.00
Phenylalanine	4.78±0.01	5.37±0.01
Valine	4.76±0.01	4.96±0.01
Tryptophan	4.71±0.01	5.29±0.01
Histidine	2.70±0.01	3.86±0.01
Arginine	5.45±0.01	6.32±0.01
Serine	4.92±0.01	5.17±0.01
Cysteine	2.90±0.01	2.98±0.01
Tyrosine	6.82±0.01	6.82±0.01
Alanine	3.83±0.01	4.22±0.00
Aspartic acid	7.83±0.01	8.34±0.01
Glutamic acid	8.62±0.01	8.78±0.01
Glycine	5.13±0.01	5.88±0.01
Proline	3.75±0.01	3.99±0.01

*Result presented as mean ± standard deviation. Means within a column with different superscriptare significantly different ($p<0.05$)

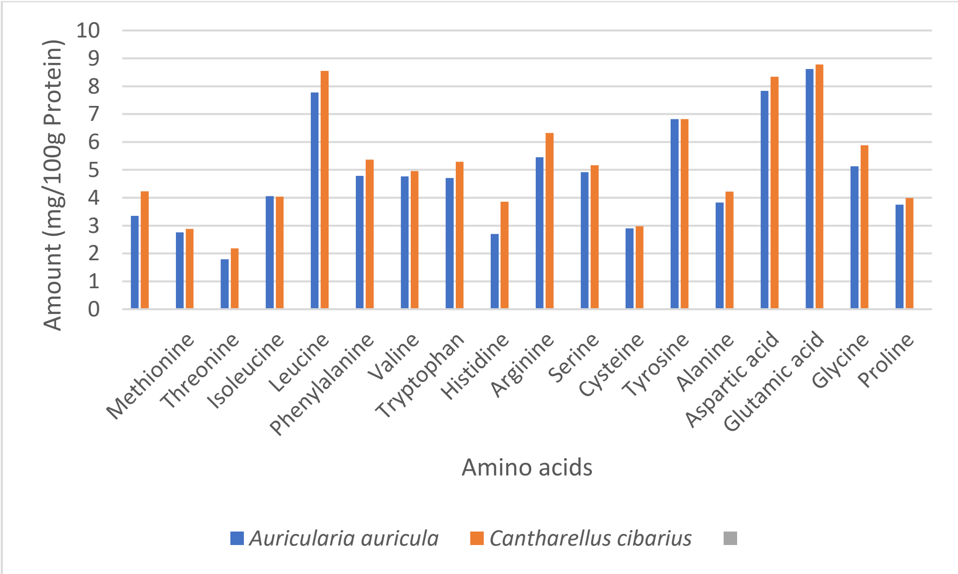

Figure 2. Quantitative levels of amino acids in *Auricularia auricula* and *Cantharellus cibarius*.mushroom species

Essential and non-essential amino acids in *Auricularia auricula* and *Cantharellus cibarius* mushroom species

Table 3 reveals the essential and non-essential amino acids that were found present in *Auricularia auricula* and *Cantharellus cibarius* mushroom species. A total of nine (9) essential amino acids were found present in the mushroom species. These amino acids are isoleucine, leucine, tryptophan, phenylalanine, histidine, methionine, valine, arginine, and histidine. A total of seven (7) non-essential amino acids were detected in both mushroom samples. The amino acids are: glycine, proline, glutamic acid, alanine, aspartic acid, cysteine, and tyrosine.

Table 3. Essential and non-essential amino acids in *Auricularia auricula* and *Cantharellus cibarius* mushroom species

Essential Amino Acids	Non-Essential Amino Acids
Isoleucine	Glycine
Leucine	Proline
Tryptophan	Glutamic acid

Phenylalanine	Alanine
Histidine	Aspartic acid
Methionine	Cysteine
Valine	Tyrosine
Arginine	
Histidine	

DISCUSSION

Mushrooms are considered delicacies with high nutritional and functional value, and they are also accepted as nutraceutical foods. Their great nutritional value is on the basis of the fact that they are quite rich in protein, with an important content of essential amino acids and fibre with excellent important fatty acids content. However, they are known to have low fat content. Edible mushrooms also provide a nutritionally significant content of vitamins (B1, B2, B12, C, D, and E) (Singh *et al*., 2011; Mat *et al.,* 2013).

The two mushroom species investigated in this study were observed to possess high protein content. However, *Cantharellus cibarius* was seen to have higher level of protein (32.55±0.00) compared to *Auricularia auricula* (28.46±0.01). The high protein content in mushrooms was also observed by Waktola and Temesgen, (2020), who worked on different species of mushrooms. The high levels of protein obtained in the mushroom species studied in this research can be beneficial in the formulation of infants and children's diet. The investigated mushrooms can also serve as alternative sources of proteins for increasing protein production to meet the growing demand due to global population growth. Mushroom-based proteins can also be used to combat food insecurity and malnutrition, as a meat substitute, as pharmaceutical agents, and as substrates to stimulate the gut microbiota to enhance human health (Chang and Wasser, 2012). Notably, mushroom proteins are future high-quality protein substitutes that are easily accessible to both wealthy and underprivileged populations.

The findings of this study revealed that both mushroom species have high fibre content. However, *Cantharellus cibarius* showed a significantly higher fibre content (36.34±0.01) than *Auricularia auricula* (29.21±0.01). The high fibre content in the mushroom species makes them suitable edibles for patients suffering from cadiovascular diseases as fibre contributes to the reduction of serum cholesterol (Guillamón *et al.,* 2010; Tatah *et al.,* 2023)). It could also be implied that *Cantharellus cibarius* would serve better in the reduction of serum cholesterol levels in patients suffering from cardiovascular diseases or those prone to it.

The results in this study revealed high carbohydrate content in the investigated mushroom species. However, *Auricularia auricula* was observed to have a significantly higher carbohydrate content (23.83±0.03) than *Cantharellus cibarius* (14.33±0.01). Carbohydrates are usually found in high proportions in edible mushrooms, including chitin, glycogen, trehalose, and mannitol; besides, they contain fiber, β-glucans, hemicelluloses, and pectic substances. Additionally, glucose, mannitol, and trehalose are abundant sugars in cultivated edible mushrooms, but fructose and sucrose are found in low amounts (Synytsya *et al.,* 2009).

Varying levels of both essential and non-essential amino acids were detected in *Auricularia auricula* and *Cantharellus cibarius* species of mushroom. Of all the amino acids detected in the analysed mushroom samples, glutamic acid level was highest in *Auricularia auricula* and *Cantharellus cibarius.* Threonine level was the least detected in *Auricularia auricula* (1.79±0.01) and *Cantharellus cibarius* (2.18±0.01). Overall, it was detected that *Cantharellus cibarius.*was richer in amino acid content compared to *Auricularia auricula.* In addition to building cells and repairing tissue, amino acids are the main construction material for antibodies to combat invading bacteria and viruses, which are an essential part of enzymes and hormones system; as the amino acids play a major role to carry oxygen to different parts of the body, an essential component of muscle activity, and the amino acid play role of neurotransmitters and raw materials to certain hormones or as an energy source. This suggestive of the fact that both mushroom species investigated in this study can exhibit the aforementioned functions upon consumption (Al- Janabi, 2000).

CONCLUSION

Based on the findings in this study, the two edible mushrooms have appreciable levels of gross energy and amino acids. However, Chanterelle mushroom showed higher protein composition than Wood ear. It can thus be concluded that Chanterelle mushroom has greater nutritional benefits compared to Wood ear mushroom.

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