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AMINO ACID AND FATTY ACID COMPOSITION OF INDIGINOUSLY CULTIVATED EDIBLE MUSHROOM *LENTINUS TUBERREGIUM* VKJM24 (HM060586)

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ABSTRACT

A total of 20 amino acids were recorded in *Lentinus tuberregium* and detected by HPLC analysis. However, the maximum amounts of aspartic acid (2.08 g), glutamic acid (1.87 g), isoleucine (1.12 g) were recorded. From these studies, it was concluded that the supplementation of this mushroom with cereal diet would help to overcome lysine deficiency. Fatty acids were recorded in *Lentinus tuberregium* and detected by gas chromatography. However, the maximum amounts of Palmitic acid (4.55%), Moroctic acid (0.43%), stearic acid (6.75%) were recorded. From these studies, it was concluded that the supplementation of this mushroom with cereal diet would help to overcome lysine deficiency. The present study proved the potential of mushrooms which can enhance the health status of an individual.

KEY WORDS: ***Lentinus tuberregium*, aminoacid, fatty acid, Gas chromatography, HPLC.**

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INTRODUCTION

Mushrooms have long been valued as delicious and nutritional food in many countries. They are appreciated for their chemical and nutritional characteristics and are considered to be a rich source of digestible proteins, the high protein content compared to vegetables and less than meats and milk. They are with rich sources of digestible proteins 10-40%, carbohydrates 3-21% and dietary fibre 3-35%[1&2],). Therefore, considerable proportions of the carbohydrate of mushrooms consist of dietary fibres which cannot easily be digested by humans and which function essentially as dietary fibre. Mushrooms contain all the essential amino acids and are limited in the sulfur containing amino acids, cysteine and methionine[3&4]. In addition, they contain major lipids, including free fatty acids, mono, di and tri glycerides, sterol esters and phospholipids. Mushrooms are excellent source of thiamine (vitamin B_1), riboflavin (vitamin B_2), nicotinic acid (vitamin B_3), biotin and ascorbic acid (vitamin C). Substantial quantities of phosphorous and potassium, less amount of calcium and iron are also found in mushrooms[5]Mushrooms contain all the essential amino acids required by an adult. - [6]reported 41.4% essential amino acids in *Podaxis pistillaris*. The total nitrogen content of dry mushrooms is contributed by protein amino acids and also revealed that crude protein is 79% compared with 100% for an ideal protein. In mushrooms, the fat content is very low as compared to carbohydrates and proteins. The fats present in mushroom fruiting bodies are dominated by unsaturated fatty acids. [7]Determined the fat content of some mushrooms as 2.04% in *Suillus granulatus*, 3.66% in *Suillus luteus* and 2.32% in *A. campestris.* Hughes (1962) [8]Observed that mushrooms are rich in linolenic acid which is an essential fatty acid. Total fat content in *A.bisporus* was reported to be 1.66 to 2.2/100 g on dry weight basis. In 100 g fresh matter of *A. bisporus* (Large) Sing and *Pleurotus ostreatus* (Jacq: Fr.) Kumm, the content of fatty compounds were found to be 0.3 and 0.4 g respectively[9] -, but on dry weight basis, it is 2 and 1.8 g respectively[10] -). [11]Worked on the fibre content of different mushrooms. Mushrooms are considered a good source of fats and minerals[12] -).

MATERIALS AND METHODS

Cultivation of mushrooms

Lentinus tuberregium was grown on paddy straw beds prepared from paddy straw soaked in water for 15 hr. The size of the paddy straw beds might vary, but the best results were achieved in beds of 1 ft2 and 9 in. in thickness. The beds were kept on a raised platform under shade. Spawns of *Lentinus tuberregium* was prepared by inoculating sterilized paddy straw in a bag; 1- month-old spawns were used for inoculating the beds. *Cajanus cajans* (red gram) powder (40 mesh) was the best source of nutrient in the beds. The beds were watered twice a day, and the mushrooms appeared 20 days after inoculation. The yield of mushrooms was about 150 to 200 g per bed[13] - Fresh mushrooms were taken and dried in a desiccator (over P205) to constant weight. Samples for analysis were prepared as described below.

Estimation of amino acid

The amino acid composition was determined by high-performance liquid chromatography (HPLC) based amino acid analyzer attached with a fluorescence detector. The standard mixed chromatograms were established such as aspartic acid, glutamic acid, isoleucine, threonine, methionine, cystine, lysine, asparagines, glycine, arginine, valine, tryptophan, tyrosine, serine, leucine, phenylanine, histidine, alanine, gulatamine and proline. The test solution was prepared by dissolving the substance which was examined in the mobile phase for obtaining a concentration of 1.0 mg/ml. For reference solution, mixed amino acids Control Reference Standard (CRS) were dissolved in the mobile phase for obtaining a concentration of 1.0 mg/ml. The column was prepared by octadecylsilyl silica gel for chromatography R (3 µm) which acts as stationary phase. The size of the column should be l = 0.10 m, Ø = 4.6 mm. The stock solutions of 20µl of test solution and standard solution of mixed standard aminoacids were prepared by dissolving in double distilled water and then the mixture was constituted by mixing 1 mL each of the 21 standard amino acid solutions and this was later used to establish the standard chromatogram. For the mobile phase, 15.2g of triethylamine R was dissolved in 800 ml of distilled water and the pH was adjusted to 3.0 with phosphoric acid R and final volume was make-up to 1000 ml with distilled water. From this 850 ml of the solution was added to a mixture of 2 volumes of propanol R and 3 volumes of acetonitrile R. The free amino acids in the standard and in *L.tuberregium* were automatically derivate by reacting with o-phthaldialdehyde under basic conditions to produce o-phthaldialdehyde derivatives in the reaction columns of the amino acid analyser. Two derivative reagent solutions were prepared as follows: 10 mL of 0.01 M sodium borate ($Na_2B_4O_7.10H_2O$) buffer solution B (pH 9.1) were added to 10 mL of b-mercaptopropionic acid to make the reagent solution I. Reagent solution II was prepared by mixing 10 mL of 0.01 M sodium borate ($Na_2B_4O_7.10H_2O$) buffer solution B (pH 9.1) with 10 mg of o-phthaldialdehyde (OPA) dissolved in 3 mL of ethanol. Solutions I and II were filtered through 0.45 mm membrane filter before use. Following derivatization, the buffer solution A (mixed in acetonitrile in a 2:1 v/v ratio), containing the derivatized amino acid 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 20 µL injection volume and a flow rate volume of 1.0-1.5 mL/min. The detection was done using spectrophotometer at 220nm and the run time was about 90 min.

Estimation of fatty acid by gas chromatography

Introduce about 0.45 g of the substance to be examined into a 10 ml volumetric flask, dissolve in hexane R containing 50 mg of butylhydroxytoluene R per litre and dilute to 10.0 ml with the same solvent. Transfer 2.0 ml of the solution into a quartz tube and evaporate the solvent with a gentle current of nitrogen R. Add 1.5 ml of a 20 g/L solution of sodium hydroxide in methanol, cover with nitrogen, cap tightly with a polytetrafluroethylene lined cap, mix and heat in a water bath for 7min. Cool, add 2 ml of borontricholoride

methanol solution, cover with nitrogen, cap tightly mix and heat in a water bath for 30 min. Cool to 40-50°C, add 1 ml of trimethylpentane, cap and vortex or shake vigrouslyfor atleast 30 seconds. Immediately add 5 ml of saturated sodium chloride solution, cover with nitrogen, cap and vortex or shake thoroughly for at least 15 seconds. Allow the upper layer to become clear and transfer to a separate tube. Shake the methanol layer once more with 1 ml of trimethylpentane and combine the trimethylpentane extracts. Wash the combined extracts with 2 quantities, each of 1 ml, of water and dry over anhydrous sodium sulphate. Prepare 2 solutions for each sample. The chromatograph consists of Ashmaco GC flame ionization detector, carrier gas as hydrogen or helium, oxygen for ignition purpose. Column BPX – 70 (50% cyanopropyl 50% methylsiloxane). Injection port 250°, detector port 280°, oven starting temperature 160° and increase by 7.0° per minute the final oven temperature is 240°.

RESULTS AND DISCUSSION

A total of 20 amino acids were recorded in *L. tuberregium* and detected by HPLC analysis. However, the maximum amounts of aspartic acid (2.08 g), glutamic acid (1.87 g), isoleucine (1.12 g) were recorded. The amino acid content varied in mushroom species[14] -). - [15]Reported the amino acid contents in *A. bisporus* and *P. ostreatus*, they contained most of the amino acids. *L. tuberregium* contained all the essential amino acids; among which, aspartic acid (2.08 g), glutamic acid (1.87 g), were the major components. The maximum level of vitamins such as niacinamide (10.65 mg/100g), folic acid (2.40 mg/100g), was recorded. The results of the present study clearly revealed that cultivation of *L. tuberregium* is simple, inexpensive and competitive to *L. edodes*. Temperature 20-25°C favored good yield of *L. tuberregium*.Mushroom as compared with fruits and vegetables is a better source of protein, containing lysine, arginine, histidine, and threonine in high concentrations. The essential amino acid composition of protein shows that mushroom is primarily deficient in phenylalanine and methionine, when compared with egg protein[16]- At the same time, when compared with the proportions of essential amino acids required for satisfactory mammalian growth, as proposed by[17] -), using tryptophan level as unity, the amino acid pattern of the mushroom protein appears to be adequate in all other amino acids, except phenylalanine and methionine. Supplementation of mushroom protein with phenylalanine and methionine would be necessary, when used as a sole source of protein in diet, to promote adequate growth. The composition of protein of this mushroom is approximately similar to that of *Agaricus campestris*[18] - except for the tryptophan content, which is higher in *Pleurotus* species. This mushroom is being utilized by people in different areas, and has been found to be nontoxic. Since mushrooms are considered as delicacies, their supplementation with a cereal diet may help to overcome lysine deficiency. Further work on the biological value and protein efficiency ratio might throw more light on the nutritive value of the protein. The present study proved the potential of mushrooms which can enhance the health status of an individual. The results for fatty acid composition, total saturated fatty acids (SFA), of the studied mushroom are shown in Table 2. In general, the major fatty acids found in the studied sample were palmitic acid (C16 30:6) and moroctic acid (C18:4 44:6), followed by stearic acid (C18 33:5). This is in agreement with the results reported for the Indian mushrooms, *Schizophyllum commune* and *Lentinus edodes*, in which linoleic (65%), palmitic (20%) and oleic (10%) acids accounted for almost the whole of the fatty acids determined[19] -. Similar observations have been made in other mushrooms[20] -. The fatty acid profile of several *Tricholoma* species was already determined and once more, for *T. portentosum* and *T. terreum*, oleic (57%) and linoleic (28%) acid were the main fatty acid constituents, while other fatty acids detected were found only in small amounts[21] -). It is known that linoleic acid is the precursor of 1-octen-3-ol, known as the alcohol of fungi, which is the principal aromatic compound in most fungi and might contribute to mushroom flavour[22] - (Fig-3 & 4) This is consistent with the observations that, in mushrooms, unsaturated fatty acids predominate over the saturated in the total fatty acid content [19,21,23] -). A rapidly expanding literature documents the importance of trans fatty acids (TFAs) in human health due to the increased risk of cardiovascular disease where they are negatively correlated with plasma HDL-cholesterol concentration and positively correlated with plasma LDL-cholesterol level[24] -. It is also important to point out that, in contrast to other fungi[19,21] -), no other fatty acids with an odd number of carbon atoms have been detected in considerable amounts.

Table 1
Aminoacid composition in fruitbody of lentinus tuberregium

PARAMETERS	AMINOACID COMPOSITION IN FRUITBODY OF LENTINUS TUBERREGIUM (100gm)
Aspartic acid	2.089 gms
Glutamic acid	1.87 gms
Isoleucine	1.121 gms
Threonine	1.087 gms
Methionine	1.076 gms
Cystine	1.044 gms
lysine	1.023 gms
Asparagine	0.997 gms
Glycine	0.987 gms
Arginine	0.9743 gms
Valine	0.7856 gms
Tryptophan	0.7044 gms
Tyrosine	0.643 gms
Serine	0.454 gms
Leucine	0.4434 gms
Phenylanine	0.404 gms
Histidine	0.344 gms
Alanine	0.221 gms
Gulatamine	0.1121 gms
Proline	In traces

Table 2
Fattyacid composition in fruitbody of lentinus tuberregium

Fatty acid	FRUITBODY
Palmitic acid	4.55%
Moroctic acid	0.43%
Stearic acid	6.75%
Oleic	5.98%
Linolenic	7.44%
Alpha linolenic	3.44%
Moroctic acid	0.112%

Figure 2
Aminoacid Standard Graph

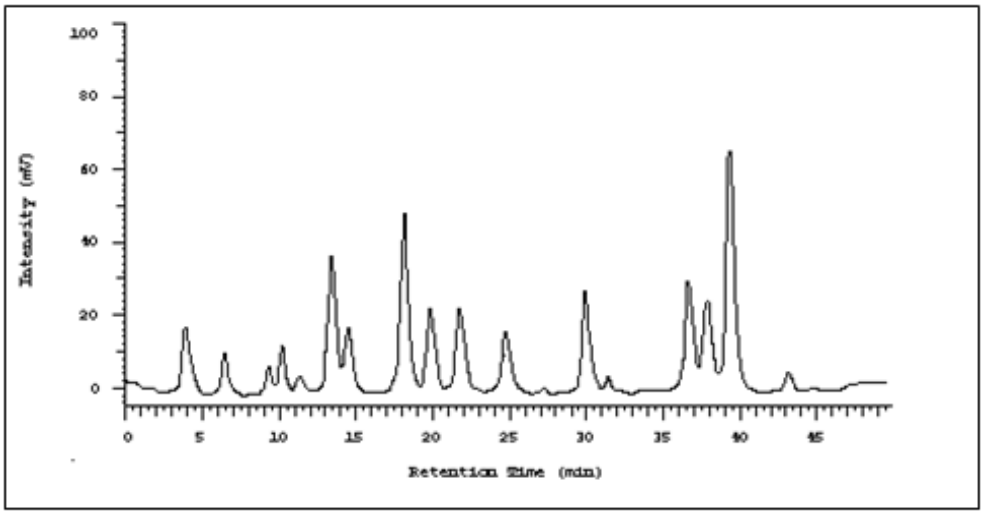


Figure 3
Fatty acid standard graph

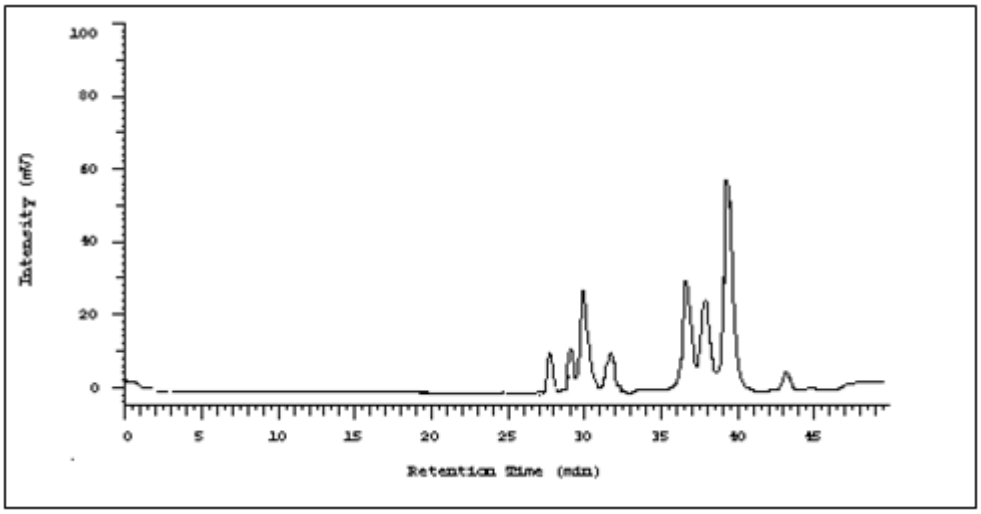
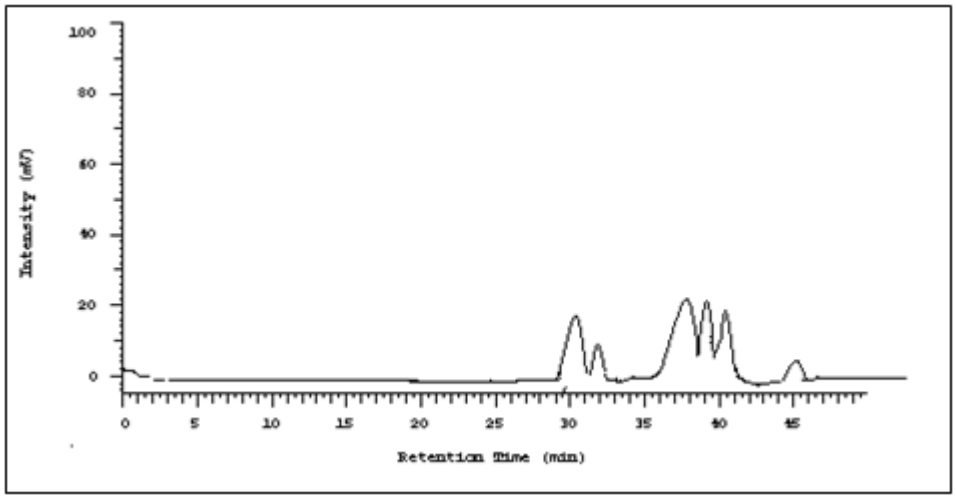


Figure 4
Fattyacid composition in Lentinus tuberregium (Fruitbody)



CONCLUSION

In conclusion, the lignocelluloses wastes at tropical regions are degraded by diversified microorganism efficiently, because the degradation or development of substrate was slower than in the temperate climate. In the present study, different agricultural wastes encouraged the fruitbody production of Lentinus tuberregium and these substrates are eco-friendly approach and needs further study to improve the bio-efficiency. Further, the mushroom studied was found to be good source of proteins, vitamins and carbohydrates with a low fat content. Therefore, the mushroom species under study resembles many of the species analyzed and reported in the literature, making them ideal components in several diets and can be regarded as healthy food. In addition, the high content of unsaturated fatty acids, particularly the essential fatty acids, linoleic acid contributes to the recommendation of mushroom in the diet of people with high blood choloestrol.

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