International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(5): 2350-2352

Received: 14-07-2020
Accepted: 20-08-2020

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Influence of pileus size on composition of biochemical parameters in milky mushrooms

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DOI: https://doi.org/10.22271/chemi.2020.v8.i5af.10655

Abstract
Calocybe indica, known as milky white mushroom is tropical in origin and is gaining commercial importance in Telangana state due to their nutritional, antioxidant and therapeutic values. In Telangana the market for the fresh mushrooms of *Calocybe* spp. is mainly based on the size of the pileus of mushroom. Keeping this in view the present study was conducted to see the effect of the size of pileus on its biochemical composition in the milky mushroom variety *Calocybe indica.* The fresh milky mushroom sporocarps of different sizes were selected for the studies which were oven dried at 40 ^{0}C ground into fine powder and estimated for their biochemical composition. The results revealed that the protein content increased with increase in size of pileus up to 4.3cm diameter and then declined with increase in size of pileus. Lipid analysis in mushroom showed that the per cent lipid content decreased with increase in size of the pileus. While there is no specific correlation between the quantity of carbhohydrates and size of the pileus, but there is a clear cut decrease in phenol quantity with increase in pileus size.

Keywords: Pileus size, biochemical parameters, milky mushrooms

Introduction
Mushrooms are the most important omnipresent diverse group of fungi which belong to both Ascomycotina and Basidiomycotina. Of these more than 2000 species are reported to be edible throughout the world and about 283 of these are reported to be available in India. Mushrooms are valuable healthy foods, low in calories and high in proteins of non-animal origin, vitamins, iron, zinc and potassium (Ouzouni *et al*. 2009) [8]. They are a potential source of dietary fibre because fungal cell walls contain chitin, hemicelluloses, mannans and interesting bioactive components like beta glucans (Manzi and Pizzoferrato 2000) [6]. Also mushrooms contain phenolic compounds that have been found to be excellent antioxidants (Cheung *et al*. 2003) [3]. *Calocybe indica*, known as milky white mushroom is tropical in origin and is gaining commercial importance in Telangana state. The increased interest in consumption of milky mushrooms as food item depends mainly due to their nutritional, antioxidant and therapeutic values. In Telangana the market for the fresh mushrooms of *Calocybe* spp. is mainly based on the size of the pileus of mushroom. There is lot of work done on nutritional and chemical aspects of these mushrooms so far but no work on the effect of sizes of pileus on nutritional and chemical aspects has been reported because we know that sporocarp initially is enrich in proteins gradually with the increase in of the size of the pileus protein is replaced by the fibre content which is determining the market value of mushrooms in Telangana state. Keeping this in view the present study was conducted to see the effect of the pileus size on its chemical composition in the milky mushrooms.

Materials and Methods
Mushroom Cultivation
Rice straw was cut into pieces of 5 cm long. Then soaked in water for 2 to 4 hours, then cleaned and then it was sterilized in hot water for 60 minutes and air dried till it holds 70% moisture. After the sterilization, paddy straw is filled into 12x24cm polypropylene bags layer wise alternately with spawn @ 100 g per kg substrate. Small holes of 3-5mm diameter were made on bags for aeration purpose. Then it was placed into the dark room for spawn running and maintained relative humidity of 80 per cent for about 20-25days. After 25 days of incubation, after completion of spawn run the beds were made into two equal halves using

sterile knife and casing was done using sterilized red soil and vermi compost in 1:1 ratio and the beds were transferred to cropping rooms for fructification. The sporocarps of milky mushroom samples at different sizes of pileus were collected and chopped into small pieces. Later these mushroom bits were oven dried at 40 °C for 48 hrs and powdered. The air dried powder was stored in an air tight container for further use.

Mushroom extracts were analysed for the presence of the biochemicals like total amino acids, proteins, carbohydrates, fat content and phenols following the below methods

Estimation of proteins by Lowry method

Working standards were prepared by diluting in 10 ml of stock solution. 0.2,0.4,0.6,0.8,1ml of the working standard were pipetted out and the volume was made to 1 ml. 5 ml of reagent C is added to each tube by thoroughly mixing the solution. Later, 0.5 ml of reagent D is added to it by mixing well observations were recorded at the wave length of 660 nm.

Estimation of carbohydrates by Phenol Sulphuric acid method

Dried mushrooms were neutralized with sodium carbonate until effervescence ceases, make up the volume to 100 ml and centrifuge. Working standards 0, 0.2, 0.4, 0.6, 0.8, 1 ml were prepared by making upto 1 ml by adding distilled water. To these working standards reagents were added one after another and placed on water bath at 25-30ºc for 20 mins. Observations were recorded at 490 nm.

Estimation of fats by Soxhlet apparatus

The fat content in different sizes of pileus of milky mushroom was estimated by using the Soxhlet apparatus.

Estimation of phenols was done by Spectrophotometric method (Brunner, 1984)

The total phenolics were determined by using Spectrophotometric method. One gm of sample was taken to 100 ml beaker and to this 15 ml of acidified methanol was added and kept it for shaking for 30 mins. Decant into another beaker. To the residue add 15 ml of acidified methanol. This was repeated for three times and supernatant was transferred into centrifuge tubes and centrifuge @ 6000 rpm for 15 mins. Filter into 50 ml volumetric flask and make up to mark with acidified methanol. Now this extraction can be used for estimation of phenols and flavonoids.

Results

Mushroom proteins seem to be of higher nutritional value than most proteins of plant origin (Bauer Petrovska 2001) [1]. These proteins present levels of essential amino acids comparable to proteins of animal origin in relation to their good biological value (Mattila *et al*. 2002) [7]. The protein content increased with increase in size of pileus upto 4.3cm diameter and then declined with increase in size of pileus. There are all types of lipids in edible mushrooms such as free fatty acid, tri-, di-, and monoglycerides, sterols and phospholipids (Breene 1990; Hanus *et al*. 2008) [2, 4]. The per cent lipid content decreased with increase in size of the pileus. Carbohydrates are the most abundant macronutrients in mushrooms. Mushrooms accumulate a variety of secondary metabolites including phenolic compounds, polyketides, terpenes and steroids, so their consumption is recommended because phenolics have been associated with the prevention of diseases like cancer and coronary heart disease (Cheung *et al*. 2003; Kozarski *et al*. 2012) [3, 5].

It can be observed from the table that there is no specific correlation recorded between the quantity of carbhohydrates and size of the pileus and there is a clear cut decrease in phenol quantity with increase in pileus size.

Table 1: Biochemical compounds at different sizes of pileus in milky mushroom

S. No.	Diameter of Pileus (cm)	Protein (g/100g)	Amino acid (g/100g)	Lipid content (%)	Carbohydrates (g/100g)	Phenols (g/100g)
1	2.7	10.95	4.15	6.9	10.80	2.60
2	3.0	11.25	4.18	6.8	12.8 0	2.00
3	3.5	11.85	0.45	5.9	6.9 0	1.98
4	3.8	12.80	0.50	4.5	12 .0	1.84
5	4.3	12.85	0.70	4.4	15.15	1.83
6	4.5	11.25	0.50	3.7	9.65	1.82
7	4.7	10.90	0.65	3.3	10.75	1.81
8	5.0	10.30	2.18	2.0	10.10	1.78
9	6.0	10.15	0.70	4.8	13.45	1.75

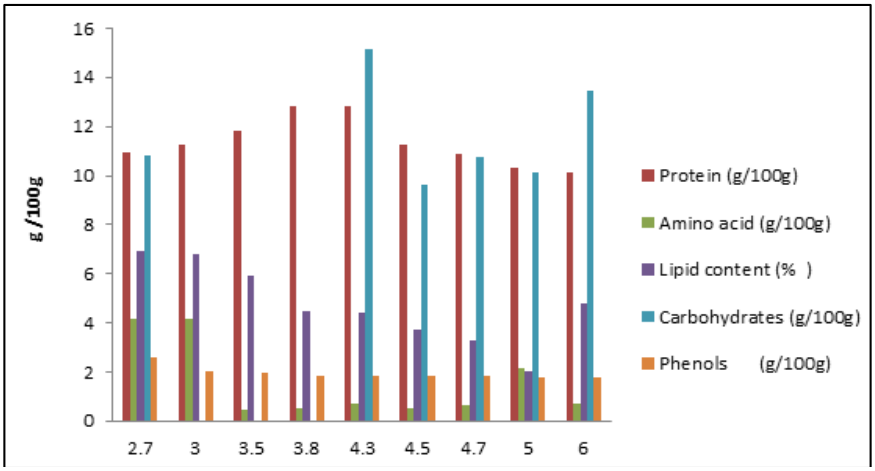

Fig 1: Shows Diameter of Pileus (cm)

Discussion

The study helps in having an apt knowledge on the effect of size of the pileus on production of biochemicals like total amino acids, proteins, carbohydrates, and phenol content maximum nutrient content the mushroom growers to decide the proper time of harvest for getting good market value.

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