Journal of Biosphere, 8: 12-20, 2019 ISSN 2278 – 3342

Comparative assessment of protein and mineral content of milky mushroom (*Calocybe indica* P & C) sporophores grown on different lignocellulosic wastes

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Abstract: Mycologists all over the world are exploring ways to make the people aware regarding the nutritive value of edible mushrooms and accelerate their transformation from consuming green to these non- green edibles. The milky mushroom (*Calocybe indica*) is a new edible species for the world mushroom growers. It was for the first time detected growing wild in West Bengal, India and is rich in protein, lipids, minerals, fiber, carbohydrate and is abundant with essential amino acids. The sporophores of *C. indica* could be considered as a very potential dietary source of proteins and essential elements such as potassium, sodium, chloride, calcium, phosphate, manganese and magnesium.

Key words: Milky mushroom, protein, mineral elements, food, lignocellulosic wastes.

1. Introduction

Presently the world population is more than 7 billion and it is expected that by 2050, the global population may reach 9 billion and during 2100 it could be 20 billion (Livi, 2012). In contrast to the increasing population, the world food supplies are growing at a slower rate than the needs, and the situation in many countries has reached almost critical point.

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Received 10.03.2019; Revised 22.04.2019; Accepted 23.04.2019; Published 01.05.2019.

The pressure of the continuously expanding populations and limited energy supply level have led many to research for new and better methods, through which more and better quality foods can be provided. In this regard, mycologists all over the world are exploring ways to make the people aware regarding the nutritive value of various edible mushrooms.

The cultivated mushrooms contain 30-50% protein on dry weight basis, which can play a constructive role in solving one of the main problems in the twentieth century, that is, the need to feed an increasing population. Apart from containing proteins, edible mushrooms are tasty, nutritious and contain carbohydrates, lipids, vitamins, minerals and fiber. Since mushroom cultivation can convert agricultural and other lignocellulosic wastes into nutritious food, they can be used as a weapon against starvation in the developing countries (Lelley, 1987). Compared with vegetables they are high in protein and have a good balance of vitamins and minerals, contain little fat and digestible carbohydrates making them suitable for low calorie diets (Mattila *et al*., 2002). The use of mushrooms

may contribute significantly in overcoming protein deficiency in the developing countries where good quality protein from animal sources are either unavailable or unacceptable for religious beliefs (Roy *et al*., 2015).

It is estimated that around 200 billion tones of organic matter are generated annually through the process of photosynthesis (Zhang, 2008). A majority of this organic matter and many agro-industrial wastes furnish large volumes of solid wastes, residues and by-products, which pose serious environmental pollution (Koopmans and Koppejan, 1997; Lal, 2005). Among the various methods, mushroom cultivation is the most economical and relatively short biological process for the biotransformation of such waste materials into protein rich food. The first report on wild occurrence of *C. indica* P & C, commonly called "Dhuth Chatt" originated from India. For several decades, people from West Bengal collected these mushrooms and sold in the local markets as its milky white colour and robust nature appealed to the consumers. In nature, milky white mushrooms are seen growing on humus rich soil in agricultural fields or along the roadside in tropical and subtropical parts of India, especially in the plains of Tamil Nadu and in Rajasthan (Purkayastha, 1984).

In view of the fact that Jammu division of J&K state (India) has subtropical conditions, which favour the cultivation of milky mushroom also called as summer mushroom, experiments were designed to find out the effect of varied agricultural wastes (viz.,wheat straw, paddy straw, maize stalk, dehulled maize cobs and sugarcane bagasse), garden wastes (viz., fallen leaves of *Lagerstroemia speciosa*, *Sterculia alata*, *Dillenia indica*, *Anthocephalous cadamba* and mixed garden litter) and forest wastes (viz., needles of *Pinus roxburghii*, leaves of *Quercus leucotrichophora*, *Ficus religiosa* and mixed forest litter) on protein and mineral contents of the sporophores of *C. indica*, CI-3 strain.

2. Material and methods

Estimation of total protein from the sporophores of C. indica. One gram of dry mushroom was taken, powdered and mixed with 10 ml of 0.1 N NaOH. The solution was boiled for 30 minutes, cooled, centrifuged, and the supernatant was collected for estimating total protein according to the method of Lowry *et. al* (1951).

Reagents

A. 2% Na_2CO_3 in 0.1 N NaOH
B. 1% Sodium potassium tartarate in H_2O
C. 0.5% $CuSO_4.5H_2O$ in H_2O
D. Reagent I: 48 ml of A, 1 ml of B and 1 ml of C
E. Reagent II: 1 part Folin- Phenol (2N. 1 part water (It was prepared by diluting it with equal volume of distilled water).
F. BSA (Bovine serum albumin) Standard – 1mg/ml

Took 0.2 ml of Bovine serum albumin (BSA) working standard in each of the 5 test tubes and made up to 1ml by using distilled water. Another test tube with 1 ml distilled water served as control. Added 4.5 ml of reagent I to these test tubes and allowed the mixture to stand at room temperature for about 10 minutes. Then added 0.5ml of reagent II to each test tube, mixed thoroughly and allowed it to stand again at room temperature for about 30 minutes. After this period, absorbance of the sample was read in a spectrophotometer (UV-1800 Schimadzu Corporation, Japan) at 660 nm and a standard graph was plotted (Fig., 1).

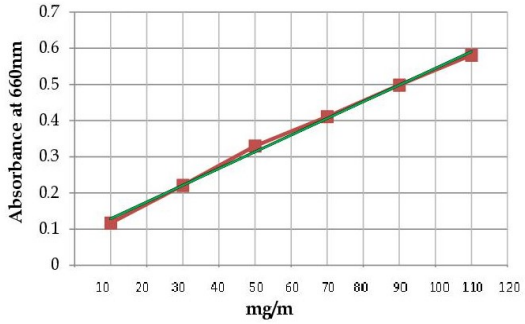

Figure 1: BSA concentration curve

Similarly, took 0.2ml of the supernatant sample and put 0.8ml of distilled water to raise its final volume to 1ml. Added 4.5ml of alkaline copper reagent (Reagent I) and allowed the mixture to stand at room temperature for about 10 minutes. Then added 0.5ml of dilute Folin-Phenol reagent with immediate mixing and allowed it to stand again at room temperature for about 30 minutes. After this period, absorbance of the sample was read in a spectrophotometer at 660 nm. 1ml of distilled water treated in a similar manner served as control. Net protein was calculated with the help of a standard graph made by using freshly prepared bovine serum albumin as standard and the values were expressed as µg of protein per ml of a sample.

Determination of mineral content. One gram powder of dry mushroom was weighed accurately into a crucible, followed by heating in a muffle furnace for about 5 to 6 hours at 600 ^{o}C. To ensure completion of ashing, the crucible was then cooled and the ash was almost white or grayish white in colour. The total ash was dissolved in 100ml ultra pure millipore water with the help of a magnetic stirrer for 15 minutes. The sample was then transferred to a 100ml polyethylene bottle. The ultra pure millipore water was purified by a Millipore (Merck, USA) water system to a specific resistance of 18 MΩcm.

Took 1ml dissolved filtered sample with the help of syringe-driven filter (Merck, USA) and raised 10ml volume by using ultra pure millipore water. The sample was then transferred to a 15ml polyethylene vial, and placed in autosampler (863 Compact Autosampler, Metrohm, Swiss) and run mobile phase in ion chromatography (887 Professional IC, Metrohm, Swiss) for analysis.

Anion Analysis (fluoride, chloride, phosphate)

Mobile phase: 3.2mM Na2CO3 + 1mM NaHCO3

Suppressor solution: 50mM H2SO4 and ultra pure water

Column: MetroSep A Supp 5 250/4.0 flow 0.7ml/min.

Cation Analysis (sodium, potassium, calcium, magnesium, manganese)

Mobile phase: 1.7mM HNO3 + 0.7mM Dipicolinic Acid or 3mM HNO3

Column: MetroSep C4 150/4.0 flow 0.7ml/min.

3. Results and discussion

Studies were conducted to assess the protein and mineral content of stipe and pileus of *C. indica* sporophores grown on different lignocellulosic wastes viz., agricultural wastes, garden wastes and forest wastes. The results obtained are presented in tables 1 and 2.

Perusal of data given in table 1 shows that the pileus of *C. indica* sporophore was usually richer in total proteins than the stipe. The total proteins of pileus varied from 9.0 to 21.5g/100g of the dry weight, whereas that of the stipe varied from 7.0 to 14.0g/100g of the dry weight depending on the age of the sporophore and the type of lignocellulosic waste used for their cultivation. Earlier, Alam *et al.* (2008) also found pileus and gills to be richer than stipe in total proteins. However, during the present investigation, the only exceptions were sporophores cultivated on dehulled maize cobs and fallen leaves of *Dillenia indica*, wherein both the stipe and pileus were detected to have same amount of total proteins (Table 1).

Highest amount of protein (21.5g/100g of dry weight) was recorded from the pileus of *C. indica* grown on mixed forest litter, followed in decreasing order from the pileus of sporophores grown on fallen leaves of *Ficus religiosa*, *Sterculia alata*, *Lagerstroemia speciosa* and *Anthocephalous cadamba*, all of which contained 20g protein per 100g of dry weight. Least amount of total protein (9g/100g of dry weight) was detected from the pileus of the sporophores grown on maize stalk and dehulled maize cobs (Table 1).

Table 1: Concentration of protein in *C. indica* sporophores grown on various lignocellulosic wastes.

Lignocellulosic wastes	Protein concentration (g/100g dry weight)	
	Pileus	Stipe
Triticum aestivum (straw)	12	9
Oryza sativa (straw)	10	7
Zea mays (stalk)	9	7
Zea mays (dehulled maize cobs)	9	9
Saccharum officinarum (sugarcane bagasse)	12	10
Anthocephalous cadamba (leaves)	20	14
Dillenia indica (leaves)	14	14
Lagerstroemia speciosa (leaves)	20	14
Sterculia alata (leaves)	20	14
Mixed garden litter	14	12
Ficus religiosa (leaves)	20	10
Pinus roxburghii (needles)	10	9
Quercus leucotrichophora (leaves)	14	12
Mixed forest litter	21.5	14

Few other researchers like Sivaprakasam *et al.* (1986), Doshi *et al.*(1988), Krishnamoorthy *et al.* (2000), Alam *et al.* (2008), Saranya *et al.* (2011), Sharma *et al.* (2013) and Sumathy *et al.* (2015) have also recorded total protein of *C.indica* sporophores to vary from 7.3 to 32.2g/100g of dry weight when grown on different substrates. Some workers have reported a higher protein content varying from 54 to 59% dry matter in few other mushrooms (Barros *et al*., 2008). Therefore, the amount of total proteins in mushrooms is not only dependent on environmental factors and stage of fruiting body maturity, but also on the part of sporophore and the mushroom species.

Similarly, from the stipes of *C. indica*, maximum total proteins (14g/100g of dry weight) were detected from the sporophores cultivated on fallen leaves of *Anthocephalous cadamba*, *Dillenia indica*, *Lagerstroemia speciosa*, *Sterculia alata* and mixed forest litter, whereas minimum amount of total proteins (7g/100g of dry weight) were detected from the stipe of the sporophores grown on maize stalk and paddy straw (Table 1). Earlier, few more researchers have also reported total proteins of mushrooms to vary with the physical and chemical differences in the growing substrates (Chang *et al*., 1981; Ragunathan and Swaminathan, 2003; Sanmee *et al*., 2003; Murugkar and Subbulakshmi, 2005). Cultivation of *C. indica* may contribute significantly in overcoming protein deficiency in the developing subtropical and tropical countries where good quality proteins from animal sources are either unavailable or unacceptable for religious beliefs (Bahl, 1985; Singh and Singh, 2002). Further, mushroom protein is even considered to have higher nutritional quality than that of plant proteins (FAO, 1991).

The concentrations of essential mineral elements detected from the dried samples of pileus and stipes of *C.indica* sporophores

grown on different lignocellulosic waste substrates are presented in ppm (part per million), that is, mg/Kg as shown in table 2. Perusal of data shows uneven distribution of elements between caps and stalks. Similar observations have been recorded by (Wang *et al*. 2015; Krishna *et al*., 2019) while studying the mineral elements of some edible species of *Boletus*. However, all the important mineral elements like calcium, phosphate, potassium, chloride, sodium and magnesium were found in appreciable amounts in both the stipes and pileus of the *C. indica* sporophores, whereas manganese and fluoride were detected only from some samples (Table 2). Of these, the dominant ones in descending order were potassium, phosphate, chloride, sodium, manganese, calcium and magnesium, which ranged from 72.3 to 540.0, 1.85 to 242.7, 4.40 to 161.4, 4.30 to 82.4, 0.05 to 45.5, 0.50 to 23.3 and 0.89 to 20.6 ppm respectively (Table 2). These results show that *C. indica* not only plays an important role in the recycling of waste substrates of plant origin but is also a very good source of food and medicine.

Table 2: Mineral content of *C. indica* sporophores grown on various lignocellulosic wastes.

Lignocellulosic wastes	Sporophore portion	Mineral content (ppm)							
		Cl	F	P	Ca	Mg	Mn	K	Na
Triticum aestivum (straw)	Pileus	11.2	–	6.48	8.80	14.1	0.31	540.0	82.4
	Stipe	17.6	–	1.85	4.03	3.79	10.7	98.7	11.0
Oryza sativa (straw)	Pileus	24.5	0.94	7.03	3.00	6.97	0.26	172.0	11.8
	Stipe	22.2	0.47	182	7.56	5.03	0.05	178.9	11.3
Zea mays (stalk)	Pileus	161.4	–	63.5	2.26	5.06	–	219.0	9.63
	Stipe	50.7	–	55.9	3.06	6.05	–	124.8	14.6
Zea mays (dehulled maize cobs)	Pileus	9.58	–	4.03	2.59	2.92	–	102.4	4.30
	Stipe	7.45	–	239.4	0.50	0.89	–	92.4	7.41
Saccharum officinarum (bagasse)	Pileus	39.6	–	21.8	3.59	3.78	–	183.1	15.7
	Stipe	11.7	–	2.52	5.01	3.85	–	76.3	18.6
Anthocephalous cadamba (leaves)	Pileus	20.0	0.10	30.6	5.63	12.9	30.8	156.3	6.58
	Stipe	9.06	2.25	134.9	2.24	3.43	–	167.5	6.70
Dillenia indica (leaves)	Pileus	20.9	–	19.2	8.63	17.8	–	330.6	8.62
	Stipe	28.5	0.03	17.8	4.88	7.87	–	125.8	9.63
Lagerstroemia speciosa (leaves)	Pileus	35.1	–	33.4	4.96	10.9	–	157.8	5.86
	Stipe	19.7	–	8.75	3.23	4.27	–	114.2	7.44
Sterculia alata (leaves)	Pileus	17.8	–	36.7	4.92	10.1	45.5	174.4	9.40
	Stipe	9.65	–	15.5	3.29	2.69	–	72.3	7.54
Mixed garden litter	Pileus	32.7	0.02	34.7	2.59	5.53	–	233.0	8.80
	Stipe	47.6	0.04	9.29	2.86	2.02	–	102.8	6.46
Ficus religiosa (leaves)	Pileus	4.40	0.62	34.2	5.45	14.9	–	227.1	13.9
	Stipe	23.0	0.52	18.3	23.3	4.60	12.6	170.4	11.7
Pinus roxburghii (needles)	Pileus	40.6	0.04	22.8	5.36	13.4	–	276.5	13.4
	Stipe	43.7	–	242.7	2.58	5.64	–	172.3	10.5
Quercus leucotrichophora (leaves)	Pileus	11.9	–	201.4	6.95	20.6	–	274.1	11.6
	Stipe	12.0	0.47	6.39	3.09	7.82	–	128.8	10.3

As depicted in table 2, presence of calcium both in the stipe (upto 23.3ppm) and pileus (upto 8.80ppm) makes the sporophores of *C. indica* as a valuable food for the formation and maintenance of bones. In addition, calcium is also required for normal function of muscles and nerves in a number of vertebrates and human beings (Wani *et al*., 2010). A number of other researchers have also detected calcium in significant amounts from other edible mushrooms, both wild and cultivated (Caglarlmak *et al* 2002; Manjunathan and Kaviyarasan, 2011; Okechukwu *et al*., 2011).

As depicted in table 2, presence of sodium and potassium in the sporophores of *C. indica* is of great importance because both these minerals are very useful in maintaining osmotic balance of the human cells. While assessing their concentration, potassium was detected in exceedingly higher amount (72.3 to 540.0 ppm) than sodium (4.30 to 82.4 ppm), which suggests that *C. indica* would be excellent in lowering blood pressure, maintaining bone health and lowering the risk of osteoporosis. Similar results, that is, high potassium and low sodium have been reported by Afiukwa *et al*. (2013) also while studying the mineral content of some edible mushroom species from Nigeria. Abundance of potassium has been commonly found in many other cultivated and wild mushrooms, which have been investigated by Mattila *et al*. (2001), La Guardia *et al.* (2005) and Lee *et al*. (2009).

Sporophores of *C. indica* were also detected to possess chloride both in the stipe and pileus and whose values ranged from 4.40 to 161.4 ppm (Table 2). Like sodium and potassium, chloride also plays a major role in balancing body cells. As a dietary element, chloride works well with potassium and sodium that helps to deliver needed nutrients to cells throughout the body. In addition, chloride is a necessary ingredient in the body's creation of hydrochloric acid, which is used to digest food in the stomach.

Investigations also revealed presence of very low amounts of fluoride (0.02 to 2.25 ppm) in some sporophores of *C. indica* (Table 2). Since public water systems include fluoride, it is most likely that during cultivation, sprinkling of supplied water for maintaining humidity may result in its incorporation via mycelium into some of the sporophores Some evidences have shown that mushrooms can absorb metal ions in high concentrations (Bystrzejewska-Piotrowska *et al.,* 2008; Gonen Tasdemir *et al.,* 2008) and the metal absorption capability appears to be species specific (Alonso *et al*., 2003). In terms of micro-nutritional value, low intake of fluoride functions by activating a number of enzymes within the human body that help with overall regulation. Similarly, low intake is also known to have positive effects towards reducing symptoms of osteoporosis and giving teeth a greater resistance to decay, plaque and weakening (Joshua, 2013). However, fluoride has cytotoxic effects if it exceeds a concentration of 80 ppm (Jeng *et al*. 1998).

Manganese and magnesium, which are indispensable in numerous biochemical pathways as important co-factors for certain enzymes were also detected from the sporophores of *C. indica*. The detected values of manganese and magnesium were quite low, ranging from 0.05 to 45.5ppm and 0.89 to 20.6ppm respectively (Table 2). Magnesium was detected from all the samples of sporophores (both stipes and pileus) cultivated on different lignocellulosic wastes. However, manganese was detected only from the stipes and pileus of sporophores cultivated on wheat and paddy straw, from the pileus of sporophores grown on fallen leaves of *Anthocephalous cadamba* and *Sterculia alata* and from the stipes of sporophores cultivated on fallen leaves of *Ficus religiosa* (Table 2). Concentration of various elements is generally assumed to be species dependent, but substrate composition is also considered to be an important factor (Kalac and Savoboda, 2000).

Phosphate, which is a vital component of bone mineralization, phospholipids in membranes, nucleotides that serve as components of DNA and RNA and phosphorylated intermediates in cellular signaling (Takeda *et al*., 2004) was detected in good amounts both from the stipes and pileus of *C. indica* cultivated on all the types of lignocellulosic wastes. The detected values of phosphate varied from 1.85 to 242.7 ppm (Table 2). It is reported earlier also as one of the dominant minerals in a number of wild and edible mushroom species (Afiukwa *et al*., 2013).

The present study, therefore shows that the large quantities of agricultural, garden and forest wastes available in Jammu Division, which are otherwise fed to the livestock or simply burnt off as they constitute a nuisance to the environment, could be effectively exploited for the cultivation of *Calocybe indica*. From nutritional point of view, sporophores of *C. indica* could be considered as a very potential dietary source of proteins and essential elements such as potassium, sodium, chloride, calcium, phosphate, manganese and magnesium. Like any other mushroom, *C. indica* can also absorb large amounts of water and minerals within a relatively short period due to the extensive mycelium overgrowing the lignocellulosic substrate.

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