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Proximate analysis and mineral constituents of *Macrolepiota dolichaula* and soils beneath its fruiting bodies

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Abstract

The nutritional quality of *Macrolepiota dolichaula* (strain MFLUCC 13-0579) grown in orchard soils was investigated. Proximate analysis showed *M. dolichaula* fruiting bodies to contain 27% protein, 38% carbohydrates, 15% fiber, 2% lipid, 10% ash and 8% moisture. The energy value was calculated as 286 KJ per 100 g dry weight of mushroom. The content of seven essential minerals in this mushroom, as well as top soils (0–5 cm) below the mushrooms were explored. In addition, the bioaccumulation potential of minerals in the fruiting bodies were calculated as a ratio between the mineral concentration in the mushrooms and the soil concentration were studied. Mineral contents were Fe (15.94 in mushrooms; 5.77 in soil), Na (5.83; 2.78), Cu (1.94; 0.64), Zn (1.69; 0.99) and Mg (0.99; 0.61), Mn (0.81; 2.21) and Ca (0.34; 14.58) for mg/100 g dry weight. The bioconcentration factors (BCF), showed that this mushroom is efficient in the absorption of Fe, Na, Zn and Mg which are low in soils (BCF > 1), while Ca, and Mn are poorly absorbed in its fruiting bodies, although the Ca and Mn contents in soils were high (BCF < 1). Based on this study, it can be concluded that *M. dolichaula* is a good dietary source of essential nutrients and minerals which are found within the acceptable limits for human consumption.

Key words – Cultivated mushroom – minerals bioaccumulation – nutritional supplements

Introduction

Macrolepiota dolichaula is consumed as a seasonal delicacy in China and Thailand (Ge et al. 2010, Kumari & Atri 2014). It is also used as a remedy for indigestion and to treat anemia by the locals in north west India (Kumari et al. 2012). In tropical regions it usually grows on fertile soils, where there has been a certain degree of disturbance, such as on pastures and roadsides (Ge et al. 2010, Kumari et al. 2012, Kumari et al. 2014).

Macrolepiota dolichaula is readily recognized due to its large, fleshy pileus covered, with squamules forming striking patterns. The taxon is a saprobe, which can grow in various culture media and agricultural wastes, which makes it a potential resource for cultivation. *Macrolepiota dolichaula* contains significant amounts of vitamins A, B_1, B_2, B_3, C and D, including thiamine, riboflavin and has laccase activity (Kumari et al. 2012). Two water soluble polysaccharides (PS-I & PS-II) have also been isolated from its fruiting body extracts (Samanta et al. 2013).

Macrolepiota species are known to bioaccumulate and recycle minerals during plant biomass degradation. Some minerals are efficiently accumulated, whereas others are excluded.

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Accumulated minerals are important as nutrients for animals and humans. This study determined the nutritional contents and essential minerals in fruiting bodies of the mushroom and in soils below the mushroom fruiting bodies. Furthermore, the mineral accumulating capacities in the fruiting bodies of this mushroom were investigated. Analytical data on nutritional and mineral contents are expected to be used as a reference for food composition and nutritional values. The mineral accumulating capacity of this mushroom could be used as a potential bioindicators of mineral contents of soils (Vetter & Siller 1997, Falandysz et al. 2008b, Falandysz & Gucia 2008, Baptista et al. 2009).

Materials & Methods

Cultivation, samples collection and preparation

Spawn of *M. dolichaula* (strain MFLUCC 13-0579) were directly inoculated in the soil in a 15 × 45 cm deep pit in orchard soils at the Mushroom Research Farm in Bandu, Chiang Rai. The mycelium was covered with soil and litter. After ten months and during the rainy season, fruiting bodies of all stages were harvested and characterized. Soil samples (0-5 cm; 15 g) beneath the fruiting bodies were also collected and air dried. The pH value of the soil samples were measured. Whole sporocarps (pileus and stipe) were dried at 40°C for 24 hours and subsequently microcharacters were studied from dry specimens, which were mounted on slides in 5% KOH and stained with Congo red. The remaining dried specimens were stored in plastic bags with collection numbers and deposited at Mae Fah Luang University (voucher specimen no. MFLUCC 13-0579) for long term herbarium storage and further study. Dried mushrooms (60g) and soils samples were powdered in a mechanical grinder, sieved through a pore size of 2 mm and stored in air tight plastic bags prior to proximate nutritional analysis and mineral testing.

Proximate nutritional analysis

The proximate nutritional analysis of fruiting body powder was carried out using the methods from the Association of the Official Analytical Chemist (AOAC 1995). Moisture content was established by drying 2g of test sample at 105ºC for 16 hours in a hot air oven (UM500, Memmret). The ash content was measured by combusting 2g of the mushroom fruiting body in an electric furnace (Eurotherm 2416CG, Lento) at 505ºC for two hours. Fiber content was determined by acid treatment and subsequent heating of 1g sample at 500ºC, in the Fibertec system M10020 Extractor (Foss Tecator). The protein content was estimated by the Kjeldahl method in which the protein content was estimated by multiplying by a conversion factor of N × 4.38 (Fujihara et al. 1995). Lipids were determined by the Soxtec 20055 Extraction unit (Foss Tecator) with petroleum ether. The total carbohydrate and energy value were calculated according to the following equations Carbohydrate (%) = 100 - (g moisture + g ash + g lipid + g protein + g fiber) (Colak et al. 2007). Energy (kJ) = 4 × (g protein) + 4.2 × (g carbohydrate) + 9.10 × (g fat) respectively (Kumari & Atri 2014). All experiments were carried out in triplicate.

Mushroom and soil sample preparation for mineral content analysis

Powdered mushroom (2g) was placed in an electric furnace (Eurotherm 2416CG, Lento) at 550 °C for four hours until becoming ash. The resulting soil (2.00g) and ash were digested with 7.5 ml concentrated nitric acid (Conc. HNO3; 65%) in quartz beakers and left for 24 hours. The digest were filtered through filter paper (Whatman No. 1) into polyethylene bottles and further diluted to 50 ml using double deionized water prior to the mineral test.

Mineral content analysis of mushrooms and soils

Ca, Mg, Zn. Fe, Cu, Mn and Na in mushrooms and soils were analyzed using an atomic absorption spectrophotometric (AAS). Stock standard solutions of each element were prepared from commercially available mineral standard solution (1000 ppm, Merck, Germany). Each standard solution prepared in different concentrations were adjusted to 50 ml with de-ionized water

and used for calibrating the AAS (Z-5000 Polarized Zeeman AAS, HITACHI, Japan) system. The mushroom and soil samples were passed through the calibrated AAS system to analyze the concentration of each mineral. All minerals were analyzed in triplicate directly using the AAS system. The detection limit was defined as the concentrations corresponding to three times the standard deviation of ten blanks. Detection limit values of element in mushrooms and soils as milligrams per 100 g (mg/100 g) in AAS were 0.989 for Ca, 0.989, for Zn, 0.998 for Cu, 0.984 for Fe, 0.998 for Mg, 0.993 for Mn, 0.995 for K, and 0.995, for Na. BFC measures the potential of fungi to absorb and accumulate minerals in the fruiting bodies of mushrooms. It was calculated as the ratio between the mineral concentration in the mushrooms and the mineral concentration of soils (Kuldo et al. 2013).

Results and Discussion

Morphology and microcharacteristics of *M. dolichaula* (strain MFLUCC-13-0579)

Young fruiting bodies were oval (Figs 1C, 1D). The cap of mature fruiting bodies were convex to plano-convex with a small round, light brown low umbo at the disc and ranged from 10–25 cm in diameter. The cap surface was covered with brownish granular squamules, which became small and sparse toward margin in mature specimens (Figs 1E, F). Lamellae were free to crowded, with short lamellulae and white when young, white to cream colored when mature. The stipe was long and sub-cylindrical, tapering upwards and slightly enlarged at the base (Fig 1E). The annulus was prominent, ascending, simple, whitish, and membranous in mature fruiting bodies (Fig 1F).

Fig 1–A. Fruiting bodies of *M. dolichaula* under banana plants B. Fruiting bodies growing at single site C–F. Fruiting bodies at different stages of development under guava trees. Scale bars: A=25 cm; B=10 cm, C, D, E, F=5 cm.

Basidiospores were covered with hyaline cap. The average size of basidiospores was 10–13.5 × 6.5–9 μm (Fig 2A). Basidia were 29–34 × 13–17 μm (Fig 2D); and cheilocystidia were hyaline, sub-cylindrical with a rounded apex, 21–34 × 12–16 μm (Fig 2 E). Squamules on the pileus were subcylindric, clampless and 6–15 μm in diameter (Fig. 2 C). Clamp connections were common at the base of basidia and cheilocystidia (Fig 2 B), but rare elsewhere. Morphological features confirmed this as *M. dolichaila* (Ge et al. 2010).

Proximate analysis

The dry weight nutritional content of *M. dolichaula* are shown in Table.1. *Macrolepiota dolichaula* is good source of protein (27%), carbohydrates (38%), fibers (15%), moisture (8%) and ash (10%). This mushroom has low lipid content (2%). The relatively low lipid contents, fibers and ash content make this mushroom ideal for consumption for those with cholesterol related ailments (Poddar et al. 2013). Therefore this mushroom can be categorized as a good edible mushroom because of its nutritional values, which are for example, higher than tropical *Laetiporus sulphureus* which has a long history of consumption especially in North America (Gilbertson & Ryvarden 1986), Japan (Imazeki et al. 1998), and Thailand (Luangharn et al. 2014).

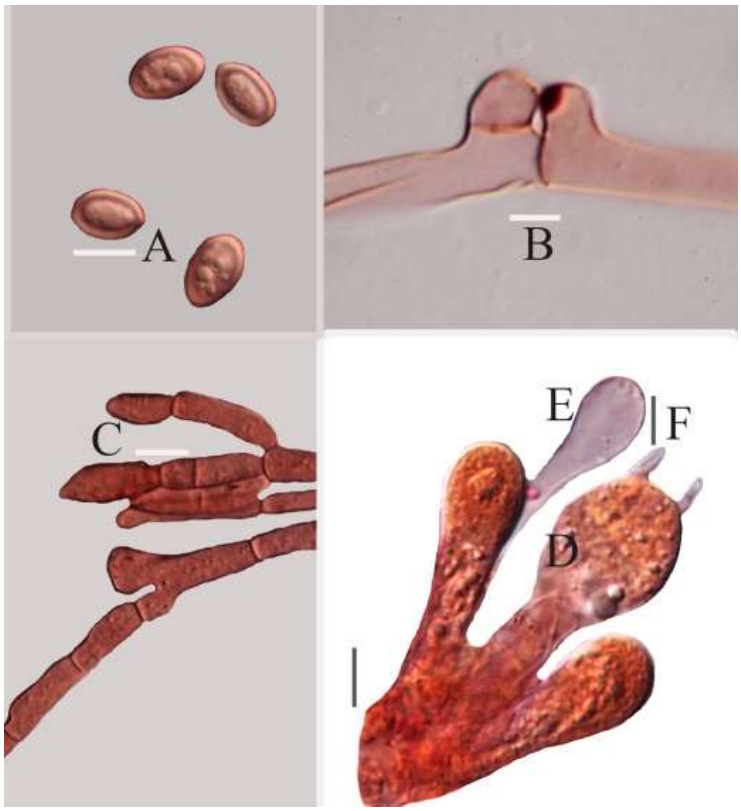

Fig 2 – Microcharacters of *M. dolichaula*. A. Basidiospore B. Clamp connection at the base of basidia C. Squamules covering pileus D. Basidia E. Cheilocystidia F. Sterigmata Scale bars: A=10 µm, B=10 µm, C=15 µm, D=10 µm, E=20 µm.

Table 1 Nutritional composition (% dried weight) and energy value (KJ/100g) of *M. dolichaula*

Nutritional composition	Amount
Ash	10.19±0.59
Fibers	15.69±0.42
Protein	27.36±0.08
Lipids	01.59±0.16
Moisture	07.60±0.12
Carbohydrates	37.57±0.08
Energy	285.60±0.07

Data shown are means and standard deviations of triplicate determinations.

Mineral content in fruiting bodies and adjacent soils

The data on mineral concentration of *M. dolichaula* and soils, and bio-concentration value are summarized in Table 2.

Table 2 Total mineral composition (mg/100 g dw) in *M. dolichaula* fruiting bodies and adjacent soils with bioconcentration values

Minerals	Mushroom	Soil	Bioconcentration values
Ca	0.34 ± 0.05	14.58 ± 0.02	0.02 ± 0.01
Zn	1.69 ± 0.42	0.99 ± 0.01	1.71 ± 0.00
Cu	1.94 ± 0.22	0.64 ± 0.01	3.03 ± 0.00
Fe	15.94 ± 6.44	5.77 ± 0.03	2.77 ± 0.00
Mg	0.99 ± 0.97	0.61 ± 0.00	1.62 ± 0.00
Mn	0.81 ± 0.25	2.21 ± 0.01	0.36 ± 0.00
Na	5.83 ± 3.29	2.78 ± 0.01	2.09 ± 0.00

Data shown are the mean from triplicate determinations; dw: dry weight

The mineral content (per mg/100 g dw) in fruiting bodies of *M. dolichaula* and adjacent soils are shown in Table 2. These data, largely agree with the observations made for a *M. procera* collected from a forest in Poland (Falandysz & Gucia 2008). *Macrolepiota dolichaula* can be considered as good dietary source of essential minerals, such as Fe, Na, Cu, Zn and Mg, which play important roles in metabolic reactions, conduction of nerve impulses, rigid bone formation and regulation of water and salt balances (Kumari & Atri 2014). The high content of iron in soils pH 6.8 is close to pork liver, which has about 12 times iron content as compared to most vegetables (Tomovic et al. 2011, Yi et al. 2014). This mushroom may therefore supplement iron in diets.

The bioconcentration factor provides an idea of a mushroom species potential for up-take of mineral (Genccelep et al. 2009). *Macrolepiota dolichaula* is efficient in accumulating Fe, Na, Zn, Cu and Mg which are poor in sample soils (median BCF >1). Ca and Mn were however, poorly accumulated even though concentrations in soils were higher than in the fruiting bodies. The data are shown in Table 2.

The Cu, Fe, Na and Mg content in the fruiting bodies are within the acceptable range for human consumption (Kumari & Atri 2014). Alonso et al. (2003) found that *Macrolepiota procera* and *Agaricus bisporus* have lower bioconcentration values for Zn as compared to the mycorrhizal mushrooms such as *Boletus edulis*, *Lactarius deliciosus*, and *Leccinum scabrum*. These variations in Zn results from various abiotic factors such as the characteristics of the soils, pH, minerals and external depositions or addition of metals on soils where the mushroom grows (Aruguete et al. 1998, Alonso et al. 2003, Colak et al. 2009). This study showed that *M. dolichaula* can accumulate minerals important for human biological systems.

Conclusion

Our study showed the proximate and mineral content of *M. dolichaula*. This mushroom is edible, cultivable and thus these data are important and can be used to indicate its nutritive quality. Because of the high accumulating potential of this mushroom for Fe, Na, Cu, Zn and Mg, fruiting bodies of *M. dolichaula* are rich in minerals and thus good for consumption by animals and humans.

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