NUTRITIONAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF SELECTED EDIBLE WILD MUSHROOMS

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ABSTRACT

NUTRITIONAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF SELECTED EDIBLE WILD MUSHROOMS

Ten species of edible wild mushrooms that are commonly consumed by the locals were analyzed to determine their nutritional composition and antioxidant activity. Minerals content were determined by Flame Atomic and Graphite Furnace Spectrometry while vitamins were acquired by High Performance Liquid Chromatography. Fatty acid and amino acid profiles were determined by Gas Chromatography with flame ionization detector. *Lentinellus omphallodes* was found to have the highest content of protein (14% of dry weight), but low in fat (1.5%) and relatively a good source of fibre (14.8%). Potassium is the most abundant mineral in all mushroom samples analyzed, followed by magnesium and calcium. Sodium concentration was relatively low in all wild mushrooms. However, calcium content in *Pleurotus cornucopiae* is 10 times higher than the cultivated mushrooms. Overall, the trace element concentrations across all wild mushrooms were in the order Fe>Zn>Mn>Cu>Cr. The highest content of thiamin, riboflavin and ascorbic acid were found in *Hygrocybe conica* while the highest concentration for *α-tocopherol* was determined at 134.41 µg/g in *Galiella rufa*. Linoleic acid is the dominant unsaturated fatty acid in all mushrooms species in which *Pleurotus cornucopiae* contained as high as 5665.26 µg/g. *Hygrocybe conica* had a higher number of essential amino acids than all other mushroom species, while *Pleurotus sp.* and *Trametes sp.* had the least. The highest concentration of amino acids found were alanine, valine, methionine and glutamic acid. Phenolic components were abundantly found in *Schizophyllum commune* (50.5 mg GAE/g of fresh sample) as compared to *Lentinus edodes* (24 mg GAE/g of fresh sample). Methanolic extracts of *Scizophyllum commune* and *Hygrocybe conica* showed comparable DPPH radical scavenging ability (96%) to the synthetic BHA at 1 mg/ml. Besides, *Hygrocybe conica* is also a good chelator for ferrous ions with the chelating ability of 90.5% at 1 mg/ml, much higher than BHA at the same concentration. The antioxidant activity in β-carotene-linoleic acid bleaching assay were moderate for all mushrooms samples. These results demonstrate that the nutritional value of edible wild mushrooms compares favorably with cultivated mushrooms. Species with great nutritional properties would be the choice of selection for commercial cultivation and further exploration to abate micronutrient deficiency in local communities.

ABSTRAK

Sepuluh spesis cendawan liar yang biasa dimakan oleh orang tempatan telah dianalisis untuk menentukan komposisi nutrisi dan aktiviti antioksidan. Kandungan mineral telah ditentukan oleh Spektrometri Fame Atomic Absorption dan Graphite Furnace manakala vitamin ditentukan oleh HLPC. Profil asid lemak dan asid amino ditentukan oleh Gas Kromatografi dengan pengesan flame ionization. Lentinellus omphallodes telah didapati mengandungi nilai protein yang paling tinggi (14% dari berat kering), tetapi rendah dari segi lemak (1.5%) dan merupakan sumber fiber (14.8%) yang baik. Potassium merupakan mineral yang paling banyak di dalam semua sampel yang dianalisis, diikuti oleh magnesium dan kalsium. Kandungan sodium adalah rendah secara relatif di dalam semua cendawan liar. Kalsium yang terkandung dalam Pleurotus cornucopiae adalah 10 kali lebih tinggi dari cendawan tanaman. Secara keseluruhan, kandungan unsur surih di antara cendawan liar adalah di dalam turutan Fe>Zn>Mn>Cu>Cr. Hygrocybe conica mempunyai kandungan thiamin, riboflavin dan ascorbic acid yang tertinggi manakala kandungan α-tocopherol yang paling tinggi telah ditentukan pada 134.41 μg/g di dalam Galiella rufa. Asid linolenik merupakan asid lemak tidak tepu yang paling tinggi di dalam semua cendawan di mana Pleurotus cornucopiae mengandungi 566.5 mg/g. Hygrocybe conica mumpunyai bilangan asid amino perlu yang lebih tinggi dari spesis cendawan yang lain, manakala Pleurotus sp. dan Trametes sp. adalah paling rendah. Asid amino pada kepekatan yang paling tinggi di dalam semua sampel cendawan liar adalah alanine, valine, methionine dan asid glutamik. Komponen phenolic adalah kaya di dalam Schizophyllum commune (50.5 mg GAE/g dari berat segar sampel) jika dibanding dengan lentinus edodes (24 mg GAE/g dari berat segar sampel). Ekstrak methanol Schizophyllum commune dan Hygrocybe conica menunjukkan keupayaan scavenge radikal (96%) yang setanding dengan BHA pada 1 mg/ml. Selain itu, Hygrocybe conica juga merupakan chelator yang baik untuk ion ferum, iaitu 90.5% pada 1 mg/g, lebih tinggi daripada BHA pada kepekatan yang sama. Aktiviti antioxidan di dalam β-carotene-linoleic acid bleaching assay adalah sederhana pada 2.0 mg/ml. Keputusan ini menunjukkan bahawa nilai nutrisi cendawan liar adalah setanding dengan cendawan tanaman. Spesis dengan ciri nutrisi yang baik boleh dijadikan pilihan untuk penanaman secara komersial dan dikaji selanjutnya untuk menangani masalah kekurangan mikronutrien di komuniti tempatan.

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SYMBOLS

%	Percentage
cm	Centimeter
wt.	Weight
w/w	Weight per weight
ppm	Part per million (10^{-6})
g	Gram(s)
ml	Milliliter(s)
mg	Milligram
°C	Degree Celsius
RM	Ringgit Malaysia
β	Beta
γ	Gamma
α	Alpha
μ	Micro

CHAPTER 1

INTRODUCTION

A mushroom is a macrofungus with a distinctive fruiting body, which can be epigeous or hypogeous and large enough to be seen by naked eye and to be picked by hand. They are diverse in form, varying from the well-known cap and stem type to those species with shelf-like fruit bodies and those that grow flat. Despite this diversity, the fruit bodies of some closely related mushrooms could look very similar and require careful examination for accurate identification (Læssøe, 2000). Mushrooms can be roughly divided into four categories: edible mushrooms, medicinal mushrooms, poisonous mushrooms and other mushrooms (whose properties remain unknown). Certainly, this mushroom classification is not absolute because many mushrooms are not only edible but also possess tonic and medicinal qualities (Chang & Miles, 2004).

Wild edible mushrooms have been collected and consumed by people for thousands of years. The archaeological record reveals edible species associated with people living 13,000 years ago in Chile (Rojas & Mansur, 1995) but it is in China where the eating of wild mushrooms is first reliably noted, several hundred years before the birth of Christ (Aaronson, 2000). Caesar's mushroom (*Amanita caesarea*) is a reminder of an ancient tradition that still exists in many parts of Italy, embracing a diversity of edible species dominated today by truffles (*Tuber* spp.) and *porcini* (*Boletus edulis*) (Boa, 2004). China features prominently in the early and later historical record of wild edible mushrooms. The Chinese have valued for centuries many species, not only for nutrition and taste but also for their healing properties. Mushrooms such as Reishi, Maitake and Ganoderma have been used as taditional medicine to treat various diseases (Wang, 1987).

There are at least 12,000 species of fungi that can be considered as mushrooms with approximately 2,000 species showing various degrees of edibility. Even though there are well over 300 genera of edible mushrooms and related

fleshy basidiomycetes, only a few of them have been successfully cultivated. One of the reasons for this is that perhaps 75% of the field and forest mushrooms are mycorrhizal and have yet to be induced to sporulate without the presence of their host (Breene, 1990). To date, about 35 mushroom species have been cultivated commercially and of these, about 20 are cultivated on an industrial scale. The majority of these cultivated species are both edible and possess medicinal properties. Several saprophytic species have been successfully cultivated and this represents an enormous industry in many countries (Chang & Miles, 2004). Commercial markets are dominated by *Agaricus bisporus* (button mushroom), *Lentinula edodes* (shiitake) and *Pleurotus* spp. (*oyster mushroom)* and these accounts for nearly three quarters of the cultivated mushrooms grown around the world. Overall, the world production of cultivated edible was recorded as 2,961,493 metric ton in 2002 (USDA, 2006). Only about 45% of mushrooms produced are consumed in the fresh form, while the rests are processed with 5% in the dehydrated form and 50% as canned foods. Due to the short shelf life in fresh produce, mushrooms are traded in the world market mostly in the processed form (Marshall & Nair, 2009).

The acceptance of cultivated mushrooms such as shiitake, oyster mushroom and button mushroom as a delicacy are well established worldwide. These mushrooms have been used as food and food flavouring materials for centuries due to their unique and subtle flavours. They are highly appreciated for their wonderful aromas particularly prized for cooking throughout the world. However, the popularity of wild mushrooms was threatened by a few cases of poisoning by inexperienced harvesters who did not carefully examine the wild mushrooms before consumption. The threat posed by poisonous and lethal species is often overstated and over publicised by the media. Incidents of poisoning and deaths are few as compared to the regular and safe consumption of edible species, but publicity and cultural attitudes continue to fuel an intrinsic fear of wild fungi in some societies. The fears of consuming edible wild mushrooms are more commonly found in developed countries but are widely accepted in other part of the world (Boa, 2004).

The consumption of wild mushrooms is increasing in recent years especially in the developed world due to the good nutritional value of these wild species particularly as source of proteins and trace minerals (Thimmel & Kluthe, 1998). The mineral content in edible wild mushrooms is generally higher than cultivated mushrooms and vegetables (Aletor, 1995; Mattilla *et al.*, 2001; Rudawska & Leski, 2005). The qualities of protein are good, which constituted mainly as essential amino acids such as leusine, methionine, tryptophan and valine (Longvah & Deosthale, 1997; Diez & Alvarez, 2001; Agrahar-Murugkar & Suggulakshmi, 2004). However, their fat fraction is relatively small and mainly composed of polyunsaturated linoleic acid (Yilmaz *et al.*, 2005; Barros *et al.*, 2007b). Dietary fibre particularly beta glucan is also found abundantly in edible wild mushrooms which had been widely known for their functional biological properties (Manzi & Pizzoferrato, 2000).

Certain mushroom species have been shown to contain substances that could prevent or alleviate cancer, heart disease, and diseases caused by viral infections (Hobbs, 1995; Mizuno, 1995; Stamets, 2001). Edible wild mushrooms are a valuable source of biological active compounds. These bioactive compounds have been found to decrease DNA damage, reduce carcinogen concentrations and their activation, inhibit the growth of cancer cells by scavenging free radicals, stimulate the immune system, and induction of tumor cell apoptosis (Cheng, 1993; Chihara, 1970; Ooi *et al.*, 2002). The stimulation of the immune system by the beta-glucans fraction in *Grifola frondosa* protects against cold, flu, infections, as well as AIDS by inhibition of viral replication (Zhuang & Mizuno, 1999). Mushrooms contain effective substances which decrease the LDL fraction of cholesterol in blood. They also prevent the accumulation of serum triaclyglycerols, thus decreasing the risk of developing cardiovascular disease (Adachi *et al.*, 1988; Bobek, 1991; Cheng *et al.*, 2002).

Polysaccharides and polysaccharide-protein complexes with potent and unique health-enhancing properties were isolated from *Grifola frondosa* and *Lentinula edodes* (Hishida *et al.*, 1988; Mizuno, 1999). Several antitumor polysaccharides, such as hetero-glucans and their protein complexes as well as dietary fiber, lectins, and terpenoids, have been isolated from the fruiting bodies,

mycelia, and culture media of various medicinal mushrooms such as reishi (*Ganoderma lucidum*)(Lee *et al.*, 2003), turkey tail (*Trametes versicolor*) (Zjawiony, 2004), split gill (*Schizophyllum commune*) (Ochiai *et al.*, 1992), mulberry yellow polypore (*Phellinus linteus*) (Han *et al.*, 1999), and chaga or cinder conk (*Inonotus obliquus*) (Babitskaya *et al.*, 2002). Other pharmacological potential of mushrooms that had been discovered are antibacterial (Mothana *et al.*, 2000; Smania *et al.*, 2003; Smania *et al.*, 1999), antiviral (Brandt & Piraino, 2000; Mothana *et al.*, 2003; Awadh *et al.*, 2003), and antiallergic (Sano *et al.*, 2002; Koch *et al.*, 2002; Min *et al.*, 2001).

Edible wild mushrooms have been shown to possess potent antioxidant activity due to their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals (Ferreira *et al.*, 2007; Barros *et al.*, 2007; Elmastas *et al.*, 2007). They have shown to accumulate a variety of secondary metabolites including phenolic compounds, polypeptides, terpenes and steroids. Two novel prenylated phenolics, asiaticusin A and asiaticusin B, have been isolated in the fruiting bodies of an edible mushroom, *Boletimus asiaticus* (Wada *et al.*, 1996). Another mushroom, *Paxillus panuoides*, was found to contain two p-terphenyls which showed potent inhibition effects on lipid peroxidation (Yun *et al.*, 2000). These findings suggest that the edible mushrooms might be a potential source of phenolic antioxidants. The antioxidants present in dietary mushrooms are of great interest as possible protective agents to reduce the oxidative damage in human body without any chemical interference. Unlike most of the commonly used antioxidant like BHA and BHT that are suspected to be responsible for liver damage and carcinogenesis (Jayakumar *et al.*, 2007), ingestion of mushroom supplement was found to cause an acute increase in plasma antioxidant activity in human intervention study without any side effect (Wachtel-Galor *et al.*, 2004).

Malaysia is a high rainfall area and boasts some of the most diversified rain forest in this region. The high humidity level during monsoon season provides ideal atmospheric conditions for growth of many wild mushrooms. In the area where mushrooms abound, edible varieties are recognized by inspection and experience of the tribal people. Edible wild mushrooms are collected from the forest for self

consumption and to be sold at the traditional market. They are normally stir fried with meat, or used as a soup base in many delicious traditional cuisines. Mushrooms such as *Schizophyllum commune* and *Trametes* sp. are dried and rehydrated prior to cooking during off-season. They are mixed with some other local herbs to use as traditional medicine in curing ailments such as headache and cold.

About 800 million people in the world are suffering from hunger, 160 million under 5 years of age are malnourished and 12 million of them die per year (50% of the deaths are related to malnutrition) (FA0, 2006). Children under 5 years of age are accounted to 26.7% of the total population of children in the world and 182 million of them are stunted. However, it is Asia (especially South Asia) that is home to more than two thirds of the world's malnourished children compared to 25.6% in Africa and 2.3% in Latin America (WHO, 2000a). Malnutrition affects all age groups, but it is especially common among the poor and those with inadequate access to health education and to clean water and good sanitation. More than 70% of children with protein-energy malnutrition live in Asia, 26% live in Africa, and 4% in Latin America and the Caribbean (WHO, 2000b). Some 22 million pre-school children worldwide are suffering from vitamin A deficiency (Rice *et al.*, 2004). Over 30% of the world population are anaemic, many due to iron deficiency and infectious disease; 30-40% of pregnant women in Malaysia are affected by iron deficiency anaemia (WHO, 2007). Even though 180 nations attending the World Food Summit (WFS) in 1996 pledged to eradicate the undernourished problem by halving the undernourished population in 2015, but virtually no progress had been made towards the objective after 10 years (FAO, 2006).

The wide spread prevalence of malnutrition and micronutrient deficiency is a major cause of poor human health in many developing countries particularly Asia region. Integrating nutrient-rich foods such as vegetables, fruits and livestock products into diets is the most practical and sustainable way to alleviate micronutrient deficiency. Edible wild mushrooms that had explicit good micronutrient content could be a potential source to ameliorate micronutrient deficiency among the poor especially those living in rural areas. Although many

studies had been done on nutritional values and antioxidant properties of wild edible mushrooms (Aletor, 1995; Demirbas, 2000; Manzi, 2001, Elmastas *et al.*, 2007), little information is available about the edible wild fungi from Sabah. Therefore, the aims of this study were to examine the nutritional and antioxidant properties of the edible wild mushrooms commonly consumed in Sabah. The provision of such information would display the potential of these wild mushrooms to serve as a source of micronutrients for the community that suffer from such deficiency.

The specific objectives of the study are:

1. To determine the macro and micro-nutrients (minerals and vitamins) in selected edible wild mushroom

2. To determine fatty acid and amino acid profiles in selected edible wild mushroom.

3. To determine the antioxidant activity and total phenolic compounds in the edible wild mushrooms.

CHAPTER 2

LITERATURE REVIEW

2.1 Malnutrition And Micronutrient Deficiency

During the last 30 years major advances have occurred in food production, largely as a result of the adoption of 'green revolution' technology and the development of high-yielding varieties of food corps. The proportion of population in the developing world that is malnourished has decreased over the years and drop to 26.7% in 2000 (WHO, 2000a). Despite these improvements, hunger and malnutrition continue to dominate the health of the world's poorest nations. In addition to the problem of insufficient protein and energy intake, deficiencies of micronutrients such as iron, iodine, and vitamin A affect millions of people in the developing world (Lipton, 2001).

Malnutrition is usually the result of a combination of inadequate dietary intake and infection. The major forms of malnutrition include Protein-energy malnutrition (PEM) that in a serious state could lead to kwashiokor and marasmus (WHO, 2000b). Health experts have recently recognized the long-term effects of early undernutrition and inadequate infant feeding for obesity and chronic diseases, including diabetes and cardiovascular diseases (Caballero 2001; Gluckman & Hanson 2004). Chronic undernutrition causes the child to be stunted, which retard linear growth; whereas inadequate nutrition for a shorter period resulted in wasting that is reversible (Caufield *et al.*, 2004). Morbidity and mortality in child are highest among those most severely malnourished; and undernutrition is responsible for 44 to 60% of the mortality caused by measles, malaria, pneumonia, and diarrhea (Fishman *et al.*, 2004). Although the major malnutrition problems are found in developing countries, people in developed countries that are well nourished also suffer from various forms of micronutrient malnutrition. Micronutrient malnutrition is a term commonly used to refer to vitamin and mineral nutritional deficiency diseases (FAO, 1997). Health issues that often associated with micronutrient

deficiency are vitamin A deficiency blindness, iron deficiency anaemia, iodine deficiency disorder and osteoporosis.

2.1.1 Global View On Malnutrition

There are still 854 million undernourished people in the world, with 820 million in the developing country, 25 million in the transition country and 9 million in the industrialized country (Skoet & Stamoulis, 2006). Over 126 million children under age 5 in the developing world are underweight, including almost half the children in Southern Asia, 37 million in sub-Saharan Africa and 10 million in Eastern Asia (United Nation, 2004). Forty six percent of children in South Asia are stunted and about 41% of child deaths occur in sub-Saharan Africa (UNICEF, 2003).

Severe acute malnutrition contributes to one million child deaths either direct or indirect cause every year (WHO, 2007b). Protein energy malnutrition cause different magnitude of stunting, wasting and underweight in young children, with the highest prevalence in South Asia and Sub-Saharan Africa (Fishman *et al.*, 2004). Relative risks for mortality in children younger than 5 years estimate that 53% of all child deaths could be attributed to being underweight. Underweight also cause decreases in immune and non-immune host defences and as underlying causes of death that followed by infectious diseases that are the terminal associated causes (Scrimshaw & SanGiovanni, 1997; Shankar & Prasad, 1998). The fraction of disease attributable to being underweight was 61% for diarrhea, 57% for malaria, 53% for pneumonia, 45% for measles, and 53% for other infectious diseases (Ezzati *et al.*, 2003).

This global burden of malnutrition is rooted in poverty, underdevelopment and inequality but in some areas rapid population growth is an important contributing factor (Clugton & Smith, 2002). Factors that are related to poverty include inadequate food supply, limited purchasing power, poor health conditions and incomplete knowledge on nutrition (Susilowati & Karyadi, 2002). Extreme poverty remains a daily reality for more than one billion people who subsist on less than US$1 a day. Although global poverty rates are falling, led by Asia; however, millions people have sunk deep into poverty in sub-Saharan Africa, where the poor

are getting poorer (United Nation, 2005).This is due to the growing numbers of people have failed to find productive employment opportunities, agriculture has stagnated, and HIV/AIDS has taken a brutal toll on people in their most productive years, and ultimately less money spent to buy food (Skoet & Stamoulis, 2006).

2.1.2 Micronutrient Deficiency

More than two billion of people in the world are suffering from micronutrient deficiency. Iron deficiency anemia affects 70% of non pregnant women in India and almost 50 percent in Sub-Saharan Africa. Vitamin A deficiency affects the immune system of approximately 40 percent of children under five years of age living in developing countries and leads to approximately one million child deaths every year. In some countries the impact is more severe; for instance, almost 60% of preschool children in India suffered from vitamin A deficiency (UNICEF, 2005).

a. Vitamin A Deficiency

Pregnant women and preschool children in low-income countries are most vulnerable to Vitamin A deficiency (VAD). It is a common cause of preventable blindness and a risk factor for increased severity of infectious disease and mortality. One of the first symptoms of marginal VAD is night blindness. If VAD worsens, additional symptoms of xerophthalmia arise, eventually resulting in blindness. A child who becomes blind from VAD has only a 50% chance of surviving the year (Caufield *et al.*, 2005). Increased mortality is associated with VAD, most likely because of the detrimental effects on the immune system, which result in increased severity of illness (Sommer & West, 1996). VAD is responsible for almost 630,000 deaths each year worldwide due to infectious diseases; accounting for 20- 24% of the mortality from measles, diarrhea, and malaria (Rice *et al.*, 2004). Table 2.1 shows recent estimates of the prevalence of VAD in young children. South Asia has the highest prevalence of clinical VAD which accounted to 40% in children under five. Of those affected, 250,000 to 500,000 each year will lose their sight, and half of them die within a year (WHO, 2000c).