Nutritional Composition and Antioxidant Activity of Mushroom By-Product *vs.* Fruiting Bodies

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I hereby declare that the work embodied in this report is the result of the original research except for the excerpts and summaries which I have just described the source.

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ABSTRACT

Nutritional Composition and Antioxidant Activity of Mushroom By-Product vs. the Fruiting Bodies

Oyster mushroom also known as *Pleurotus ostreatus* are popular due to its favourable flavour, taste and high demand among consumer. However, those mushroom farming industry cause a lot of mushroom waste produced. So in order to overcome this problem, there is a need to manipulate the waste from mushroom. The purpose of this study was to compare the nutrition composition of oyster mushroom by-product with fruiting body. Both sample had converted into powder to run proximate and antioxidant analysis. Both sample were run in triplicate in every analysis to ensure get the accurate result. The proximate analysis were determined using Gerhard Analytics, Foss Soxtec 2055, Fibertec 8000 and Ash Oven. Moisture analysis were run in manual method as daily collecting sample. Antioxidant activity were analysed using method DPPH assay. The result showed fruiting body has higher value of protein with fruiting body 27.84% compare waste 9.11%, Next was in fat with fruiting body 2.27% compare waste 1.12%. And moisture content with fruiting body 87.67% compare waste 76.67%. However, by-product also had more high value in fibre with 15.33 of waste compare 7.39% of fruiting body, ash content with 8.37% of waste compare 6.96% of fruiting body and carbohydrate content with 81.39% of waste compare 62.93% for fruiting body. Next, ascorbic acid were used as positive control in antioxidant. In antioxidant activity, fruiting body had higher of IC_{50} with fruiting body 3388.84 and by-product 3357.84. In conclusion, by-product had lower nutritional value and antioxidant activity than fruiting body.

Keyword: oyster mushroom, fruiting body, by-product, proximate analysis, antioxidant activity

ABSTRAK

Perbandingan Komposisi Pemakanan Sisa Buangan Cendawan vs Cendawan Segar

Cendawan tiram juga terkenal dikenali sebagai *Pleurotus ostreatus* kerana rasa yang menarik, dan permintaan yang tinggi dalam kalangan pengguna. Walau bagaimanapun, industry pertanian cendawan tersebut telah menyebabkan banyak sisa cendawan dihasilkan. Oleh itu, bagi mengatasi masalah ini, tindakan yang sewajarnya diperlukan untuk memanipulasikan sisa bahan buangan daripada industri cendawan. Tujuan kajian ini adalah untuk membandingkan komposisi pemakanan dalam sisa buangan cendawan tiram dengan cendawan segar. Kedua-dua sampel telah dijadikan serbuk untuk menjalankan analisis proksimat dan antioksidan dengan tiga kali ganda dalam setiap analisis untuk mendapatkan keputusan yang tepat. Analisis proksimat ditentukan menggunakan Analitis Gerhard, Foss Soxtec 2055, Fibertec 8000 dan Oven *Ash*. Untuk analisis kelembapan, kaedah manual telah dijalankan iaitu penngumpulan sampel secara harian. Untuk analisis aktiviti antioksida, menggunakan kaedah DPPH *assay*. Hasil kajian menunjukkan cendawan segar mempunyai nilai kandungan *protein* dengan cendawan segar 27.84% berbanding sisa buangan 9.11%, Seterusnya kandungan lemak yang lebih tinggi iaitu cendawan segar 2.27% berbanding sisa buangan 1.12%. Dan kandungan kelembapan yang tinggi dalam cendawan segar 87.67% berbanding sisa buangan 76.67%. Walau bagaimanapun, sisa buangan cendawan juga mempunyai nilai serat yang tinggi iaitu 15.33% berbanding cendawan segar 7.39%, kandungan mineral yang tinggi iaitu 8.37% berbanding cendawan segar 6.96% dan karbohidrat yang lebih tingg iaitu 81.39% sisa buangan berbanding 62.93% cendawan segar. Seterusnya, asid askorbik digunakan sebagai kawalan positif dalam analisis antioksida. Hasil kajian menunjukkan cendawan segar mempunyai nilai aktiviti antioksida yang lebih tinggi dalam IC_{50} berbanding sisa buangan cendawan iaitu 3388.84 dan sisa buangan 3357.84. Kesimpulannya, sisa buangan cendawan mempunyai nilai nutrien dan antioksida yang lebih rendah daripada cendawan segar.

Kata kunci: cendawan tiram, cendawan segar, sisa buangan cendawan, analisis proksimat, aktiviti antioksida

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LIST OF SYMBOL

Reference no		Page
Kg	Kilogram	7
UV	Ultra violet	13
ml	Millilitre	18
r.p.m	Revolutions per minute	19
nm	Nanometre	22
mg	Milligram	28
µg	Microgram	30

LIST OF ABBREVIATION

Reference no		Page
DPPH	1.1-diphenyl-2-picryl-hydrazil	iv
SMS	Spent mushroom substrate	2
DW	Dry weight	5
NSPs	Non starch polymers	10

CHAPTER 1

INTRODUCTION

1.1 Research Background

The word mushroom is most widely used to describe fungi belonging to the Basidiomycota and Agaricomycetes families that have a stem (Stipe), a cap (Dileus), and gills (lamellae, sing. Lamella) on the cap's underside. Mushrooms have long been used as food and medicine in many parts of the world. Mushrooms are fungi, not vegetables or fruits. It is obvious that the increasing interest in mushroom cultivation will assist in the resolution of several global issues such as protein insecurity, as well as improving people's health and well-being, provided that mushrooms are nutritious foods that are low in calories and abundant in essential minerals (Enas, Sabahelkhier, & Malaz, 2016).

The oyster mushroom is the world's third most common edible mushrooms. Oyster mushrooms are the easiest to cultivate because they can grow on a variety of substrates, However, the mushroom farming industry has raised issues of agricultural waste disposal, which can lead to leaching in the field, thus polluting the environment. To address this problem, mushroom these agricultural waste has been used as manure to

manage waste disposals (Kamthan & Tiwari, 2017). Without proper agricultural waste management, this will cause high environmental impact and industry management costs.

In this present study, caps, stipes, mushrooms that do not meet the market grade, shape, or size requirements were used as a sample to study the nutrition composition and bioactivities in comparison to the fruiting bodies. These mushroom waste could be used new goods and applications using waste as a raw material will promote circular agriculture system. The large amount of waste generated from the mushroom farming industry will lead to management issues from both an economic and environmental perspective (Antunes et al., 2020). Thus, this project aims to provide an alternative use of those mentioned agriculture waste from the mushroom farming industry, especially by identifying the nutritional content and antioxidant activities.

1.2 Problem Statement

Pleurotus ostreatus is the most commonly cultivated mushroom in the world, accounting for 90.89 % of all mushrooms cultivated in Malaysia, After *Agaricus bisporus* (Zakil, Sueb, & Isha, 2019)(Zakil et al., 2019). In general, the demand for oyster mushrooms is more than the quantity supplied by growers. Thus, there was no competition in marketing the oyster mushroom, especially in the Klang Valley area (Haimid, Hairazi, & Dardak, 2013). This agricultural wastes are high in various nutrients, making their disposal difficult to handle because excess nutrients will result in leaching if they are left in the field as compost, or will cause in environmental pollution if

incinerated. As a result, there is still a need to provide an agricultural wste management system that is both cost-efficient and environmentally friendly (Kamthan & Tiwari, 2017). Therefore, this study aims to investigate the nutritional composition and antioxidant activity of the waste (stems and non-commercial grade mushrooms) to search for their alternative application.

1.3 Objective

1. To determine the nutritional composition of mushroom by-product and fruiting bodies using proximate analysis.
2. To measure the antioxidant activity of mushroom by-products and fruiting bodies using DPPH assays.
3. To compare antioxidant activity and nutritional composition of mushroom by-product vs. the fruiting body.

1.4 Hypothesis

Ho: mushroom by-product has no nutritional composition and antioxidant compared with the fruiting body

H1: mushroom by-product has higher nutritional composition and antioxidant compared with the fruiting body

1.5 Scope of Study

Main purpose of this research was to determine the nutritional content and antioxidant activity in mushroom fruiting body and by-products. The study was focused on mushroom waste that do not meet commercial requirements such as lack of shape size and mushroom of surplus production. Those materials were obtained from the mushroom house next to male student residential college. Then, samples were subjected to proximate analysis and antioxidant assays. The analysis were conducted in the animal laboratory at UMK Jeli Campus.

1.6 Significant Study

The outcome of this study will help mushroom farmers to obtain extra income by selling those by products to the related industry such as food and cosmetics for new product developments. Furthermore, the data of nutritional composition of mushroom by-product will help to minimize waste and disposal.

CHAPTER 2

LITERATURE REVIEW

2.1 Mushroom

Mushrooms are usually noticeable as umbrella-shaped and have fruiting structures (sporophore) that are fleshy and edible fungi that belong to macro fungi species. It developed from the hyphae of fungi hidden in soil or wood to produce spores above the ground surface. Commonly, mushrooms have a stem (stipe), and gills (lamellae, sing, lamella) and a cap (pileus) physically (Enas et al., 2016). Mushroom cultivation started in France in 1650 and now more than 100 species cultivated worldwide like China, Poland, Canada, Japan, the United Kingdom and others (Samsudin & Abdullah, 2019).

However, in Malaysia mushroom cultivation started in the early 1970s and dominated by the grey oyster type. Although the Malaysian climate allows for the cultivation of a number of mushrooms, only eight species are commercially cultivated such as grey oyster (*Pleurotus florida*), red oyster *(Pleurotus flabellatus*), rice straw

(*Volvariella*), shitake *(Lentinusedones*), telinga kera (*Auricularia polytricha*), abalone *(Pleurotus cystidiosus*) and ganoderma (Mat-Amin, Harun, & Abdul-Wahab, 2014). Malaysia's mushroom industry is still new and limited, but it is rising steadily. The demand for mushrooms is rising in correlation with consumer awareness of health issues and government-sponsored advertisements (Mat-Amin et al., 2014)

2.1.1 Oyster Mushroom (*Pleurotus ostreatus)*

Oyster mushroom, is known and popular among consumers due to its favourable flavour and taste (Figure 2.1.1). To date, about 70 species of *Pleurotus spp*. have been identified, with new species being discovered on a regular basis However. The oyster mushroom is a good source of non-starchy carbohydrates, dietary fibre, and a small amount of protein and fat, as well as essential minerals, and vitamins. It is also high in Vitamin C and B complex, making it an ideal food for people suffering from hypertension, obesity, or diabetes. Oyster mushroom has been shown to have ten-fold higher niacin content than any other vegetable (Ahmed, Abdullah, & Nuruddin, 2016). Oyster mushrooms contain folic acid, which aids in the treatment of anemia and are among the few vegan sources of vitamin D and conjugated linoleic acid. Moreover, high value compound like ergothioneine that has antioxidant properties can be found in mushroom. The oyster mushroom's growth habit is also more adaptable and can endure a wider temperature range than any other mushroom species , thus leading to increase in growth of mushroom farming industry in countries with those climates, such as in Malaysia (Ahmed et al., 2016).

Figure 2.1.1: Oyster mushroom (Myronycheva, Bandura, Bisko, Gryganskyi, & Karlsson, 2017)

2.2 Mushroom By-product

A by-product is a secondary unit produced in a joint manufacturing process with little value than the primary product. In other words, it is a product that emerges unexpectedly during the manufacturing of another product (Oreopoulou & Russ, 2007)(Oreopoulou & Russ, 2007). Mushroom by-products are a waste concern, but they are also promising sources of essential compounds with nutritional and functional properties that could be used in other industries, such as food and cosmetics (Antunes et al., 2020). Along with that, by-products may have nutritional value from the compounds that could be found in their extracts, such as peptides, carotenoids, and phenolic compounds. By-products and their extracts can be effectively introduced into foods, as natural ingredients for nutraceutical and cosmeceuticals product with improved nutritional content, possible health benefits and longer lifespan (Faustino et al., 2019).

Mushroom cultivation generates a lot of waste in unusable caps, stipes, and mushrooms, along with spent mushroom substrate (SMS). Depending the size of the mushroom farm, the amount of mushroom stipes and low-quality mushrooms ranges from 5 to 20% of the total production volume. Due to the large volume and volatile degradation materials, waste disposal causes environmental concerns for producers. During mushroom cultivation, about 5 to 6 kg of waste were produced. Caps and stripes that are misshapen or do not follow manufacturer requirements are rejected and discarded causing environmental pollution (Antunes et al., 2020). Figure 2.2 shows the mushroom by-products from mushroom farming industry.

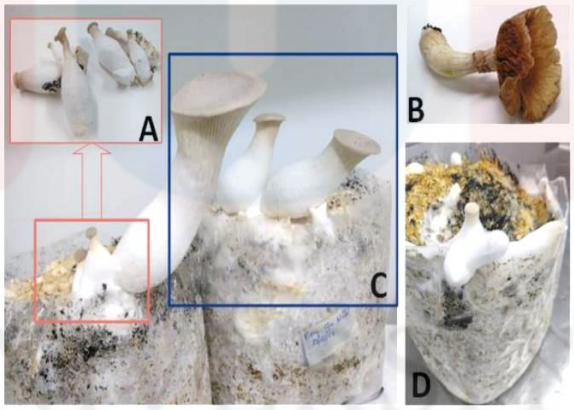


Figure 2.2: Mushroom by-product; (A) and (B) are mushroom production wastes (stipes and mushrooms that do not meet commercial shape or size requirement: (B) by-products (surplus production: 5%); (C) by-product (surplus production: less than 5%); (D) expended mushroom substrate (more that 20% of total production weight) (Antunes et al., 2020).

2.3 Nutritional Composition

Nutritional composition is the constituent in food that has effect on the human body such as carbohydrates, fats, proteins, minerals, additives, enzymes, vitamins, sugar, cholesterol, fat, and salt (Ahmed et al., 2016). In general, mushrooms can be classified into four groups: edible, poisonous, medical and magical or hallucinogenic (Enas et al., 2016).

2.4 Protein

Protein is a material with a complex structure. It can be found in mostly all living things such as meat, fish, poultry, eggs, legumes, and dairy products. Proteins have a high nutritional value since they are a necessary macromolecule that is directly involved in the body's essential chemical processes. Protein differs from one another. Muscle proteins differ from brain and liver proteins within a single organism (Felix, 2020). Proteins are converted into polypeptides or amino acids when they are hydrolysed by the protease enzyme. Amino acids are basic nutrients found in most foods, either naturally or synthetically.

Protein content is calculated using the Kjeldahl method (AOAC 1980), according to Morr et al (1985). The experiment was carried out on a dry protein substance. After

that, protein nitrogen conversion factors were determined. Kjeldahl consists of three steps: digestion, neutralization, and titration. Julian (2007) claims that samples are analysed by digesting them in the presence of high temperature, sulfuric acid, and a Kjeldahl tablet. Every nitrogen in the system is converted to ammonia, and other organic matter is converted to CO_2 and H_2O. In an acid solution, no ammonia gas is released. The nitrogen content is then calculated by titrating the ammonium borate formed with regular sulfuric or hydrochloric acid and determining the end-point of the reaction with a suitable indicator. Mushroom proteins have similar amino acid compositions to animal proteins (Enas et al., 2016).

2.5 Carbohydrate

The total carbohydrate content of mushrooms, which includes both digestible and non-digestible carbohydrates, varies by species and ranges from 35 to 70%. Mannitol and glucose, which are included in limited quantities less than 1% dry weight (DW), as well as glycogen (5–10% DW), are digestible carbohydrates present in mushrooms. Humans do not rely on mushrooms carbohydrate as a main source of nutrition. Non-digestible carbohydrates, which constitute the majority of mushroom carbohydrates, include oligosaccharides like trehalose and non-starch polysaccharides (NSPs) like chitin, b-glucans, and mannans (Wang et al., 2014)

2.6 Fibre

Dietary fibre is a form of plant-based carbohydrate that our bodies cannot digest or absorb. Fibre, on the other hand, moves across the liver, small intestine, and colon relatively undamaged and out of our bodies. Soluble and insoluble fibres are the two types of fibre. Water can dissolve soluble fibre which can be found in oats, apples, barley, and other crops. Insoluble fibre, on the other hand, aids in the flow of materials into the digestive system (Dhingra, D. 2011). Non starch polysaccharide (NSPs) are a form of dietary fibre that may have health benefits for humans. The section on cell wall polysaccharides provides insight further into these topics. Depending on their morphological type and organisms, mushrooms have a wide range of dietary fibre content. The fruiting bodies of some mushroom species have low levels of dietary fibre (e.g. 4.5% DW in *Tricholoma giganteum*), whereas others are high in fibre (e.g. 49.7% DW *in Auricularia auricula-judae*. In general, mushrooms provide dietary fibre, with 100 g of fresh mushrooms providing between 5% and 25% of the recommended dietary intake (18 g of NSP fibre/day in the UK). Mushroom dietary fibre predominantly comprises water-insoluble fibre, mainly chitin and b-glucans with low levels of water-soluble dietary fibre (less than 10%) (Wang et al., 2014).

2.7 Fat

Mushrooms have a small amount of fat. The cell walls contain some fat, which is important to handle vitamin D that mushrooms naturally produce after being exposed to sunlight. The fat present is primarily unsaturated fat, which is good for health (Enas et al., 2016).

Fats are an essential macronutrient. Dietary fat comes in a variety of shapes and sizes. It is important for bodily functions. According to Shin (2013), there are many fat identification methods available, including the automated Soxhlet process, AOAC 996.06, Folch method, and others. However, Soxhlet is the most widely used method. The sample (1g) to be tested would be placed in a thimble and loaded into Rapid automatic fat extraction system SOXTHERM using metal adaptors. In the extraction machine, beakers that had been dried and weighed in an oven at 104˚C, are put underneath each extraction thimble, and diethyl ether is applied to each of the six extraction chambers. The automated extraction program includes 90 minutes of contact with boiling diethyl ether, 90 minutes of sample rinsing, 15 minutes of recovery, and 15 minutes of evaporation/drying. The extract will be dried in an oven at 104 C and cooled in a desiccator to determine fat gravimetrically after extraction. The beakers will be weighed, and the crude fat content will be measured in percent (Shin, 2013). The term "crude fat" refers to a sample's unprocessed mixture of fat-soluble materials. The ether extract or free lipid content is another term for crude fat. Triglycerides, diglycerides, monoglycerides, phospholipids, hormones, free fatty acids, fat soluble vitamins, carotene pigments, chlorophylls, and other lipid materials can be included in the formulation.

2.8 Moisture

Moisture content, also known as water content, refers to the amount of water molecules that can be traced in foods, soil, building materials, and other products. Moisture content in any material may disrupt, alter, or affect the material's physical and chemical. Moisture content has a major influence on many aspects of food, including flavour, texture, colour, shape, and weight. It is, however, one of the origins of microbial spoilage. At a given temperature and relative humidity, the moisture sorption isotherm connects moisture content and water movement. It is recommended that only around 10% of moisture content be present, depending on the type of food (Keerthipala, 2018).

Food moisture content analysis is the foundation of food protection. On a wet basis, measured moisture content in food can be measured as a proportion by weight. Distillation, dielectric process, hydrometric, infrared spectroscopy, refractometer, chemical analysis, and oven drying are some of the methods described by Steinberg (1980) in calculating the moisture content of food. To ensure the accuracy of the readings, the analysis readings are duplicated three times. Moisture balance is a commercially used technology in the food industry. The thermogravimetric principle is used to run moisture analysers. The data will be computed by the analytical balance based on the weight loss of the sample during the drying process (Buring & Hennekens, 1990).

2.9 Ash

Ash content is the incombustible residue of a sample after being completely burned for a stated amount of time. It's also known as inorganic waste, which is the left over after combustion. The ash content of a sample can be measured using a number of analytical methods, including dry ashing, wet ashing, and low-temperature plasma dry ashing. The ash content of a sample is measured by heating it at 500–600°C in a furnace. 5g of sample is placed in a crucible to begin the procedure. After that, the crucible is set in the furnace for 12 hours, coold and dried in the desiccator until the process is complete. The weight after the crucible has cooled is measured, and ash content can be calculated (Nielsen, 1998).

2.10 Vitamin B and D

Mushrooms are high in B vitamins such as riboflavin (Vitamin B2), Pantothenic acid (Vitamin B5), and Niacin (Vitamin B3) (Vitamin B3). These B vitamins help the nervous system function properly and provide energy by breaking down enzymes of carbohydrates, fats, and proteins (Enas et al., 2016). When mushrooms are exposed to sunlight, they contain vitamin D naturally (or another source of UV light). They transform their sufficient ergosterol to ergocalciferol through the action of sunlight (vitamin D2). In Europe, wild mushrooms usually contain 2-40 microgram (mcg) vitamin D per 100g.

After being exposed to sunlight for a couple of hours in the midday sun, store-bought mushrooms can produce over 20 mcg of vitamin per 100 g (Cardwell, Bornman, James, & Black, 2018). Mushrooms contain easy-to-absorb vitamin D that helps to improve vitamin D levels (Enas et al., 2016). For thousands of years, numerous mushrooms have been used in folk medicine. Others are nutraceuticals (natural foods with the potential to improve human health and strengthen the immune system), while others can contain active nutraceuticals (compounds with medicinal properties) and are used as medications, in the form of capsules or tablets, rather than as food (Enas et al., 2016).

2.11 Antioxidant

An imbalanced metabolism and an excess of reactive oxygen species (ROS) lead to oxidative stress in humans, which causes a variety of health problems. Our oxidative homeostasis can be controlled by endogenous antioxidant defines mechanisms, as well as dietary antioxidant intake. Edible mushrooms have been described as alternative sources of antioxidant foods, as well as the mechanistic activity involved in their antioxidant properties. Mushroom chemical composition and antioxidant potential have been thoroughly studied. Edible mushrooms may be used directly in dietary supplementation to boost antioxidant defences (Kozarski et al., 2015).

2.12 Proximate Analysis Test

Proximate Analysis is a technique to determine the values of any micro or macronutrients contained in any form of sample, such as food, soil, or fuel. On the labels of final items, collected data and values are commonly expressed as nutritional facts on the product packaging. Nutritional analysis has been developed, improved, and upgraded since 1861. Nutritional labels have now become a legal requirement for almost all food products. The nutritional composition of foods will be disclosed to customers to help them make decision when purchases. Furthermore, they serve as a means of defining the necessary conditions for fair market competition among food manufacturers (Raymond, Onyango, & Onyango, 2020).

Generally, food labelling should provide and include details on the food's net calorie content, total and saturated fat, cholesterol, sodium, dietary carbohydrate fibre, calories, proteins, vitamins, calcium, and iron. Besides that, industrial regulation authorizes that nutritional labels have to display proximate value information on the five representatives of protein, fat, moisture, ash, and carbohydrates accurately (Adilin, Nor, Aliff, Fattin, & Syaswani, 2015). In conclusion, proximate analysis is significant in as food-manufacturing ensure that food products follow the specifications for relevant legislation and legal declarations, as well as the safety of finished goods when they are published to the final consumer.

2.13 Extraction Method

Extraction is a process in which the active portion of plants or animal tissues is separated from active or inert components using specific solvents and standard extraction procedures. The products obtained from the plant extraction process would be impure liquids, powders, or semi solids that can only be used for external or oral purposes (Zhang, Lin, & Ye, 2018). Modern sample processing techniques with major advantages over traditional methods for the extraction and study of medicinal plants are likely to play a key role in the overall effort to ensure the availability of high-quality herbal products to consumers around the world (Gupta, Naraniwal, & Kothari, 2012). The first step in the preparation of plant formulations is extraction. Collection of plant materials, size reduction, extraction, filtration, concentration, and finally drying and reconstitution are some of the processes or measures that may be used in the plant extraction method. The quality of the extraction will be influenced by the plant component selected, the solvent used, the extraction process, and the sample: ratio of solvents (Gupta et al., 2012).

2.14 Infusion Technique

The process of extracting chemical compounds or flavours from plant material in a solvent such as water, oil, or alcohol by allowing the material to remain suspended in the solvent over time is known as infusion. The extraction method involves boiling the plant material. The infusion process includes both solid gain and water loss, also known as osmotic dehydration. Fresh infusions are made by macerating the crude extract in cold or boiling water for a short period of time. These are dilute solutions of crude drug constituents that are easily soluble (Shi, Pan, McHugh, & Hirschberg, 2009).

CHAPTER 3

METHODOLOGY

3.1 Raw Materials

The mushrooms were harvested from mushroom houses in the campus. The samples were washed and dried in the oven at 40 °C for 1 day (Yildiz, Izli, Uylaser, & Isik, 2014). The dried samples were kept in a clean and sealed plastic bag until further used.

3.2 Sample preparation

The dried mushrooms were brought to the biology Laboratory of Agro Based Industry faculty in Universiti Malaysia Kelantan (UMK). The samples were cut and ground into powder separately (fruiting bodies vs. by-product). The samples were sieved with a U.S mesh 30' with an opening 500 µm to get a homogenous size of powder (Gooch, 2011). The amount of mushroom samples required for the study; proximate and antioxidant activities, is approximately 1 kg fresh mushrooms (fruiting bodies and by-products).

3.2 Equipment

A plastic bowl, glove and brush equipment were needed to prepare the sample of mushroom samples collection. Equipment such as sample tube, beaker, buchner funnel, fume chamber, crucible, titration flask muffle furnace, burette, sieve and electronic weighing were needed for the proximate, extraction and antioxidant test. All the equipment was provided by the FIAT's laboratory.

3.3 Determination of Proximate Analysis

3.3.1 Protein Content (dried)

The Kjeldahl method was used to determine protein content. Approximately, 1 g of sample was mix with 12 ml of concentrated sulphuric acid (H_2SO_4), as well as 2 Kjeldahl tablets, which serve as a catalyst. All of the sample tubes were put in the pre-heated block until the temperature reaches 400°C, which starts the protein digestion process. The process took about two hours to complete everything. The digestion block was switched off and allowed to cool once the samples turned clear. After that, the solution was diluted with 80 ml distilled water and 50 ml sodium hydroxide solution (40%) was added. After that, the distillation system was cleaned with distilled water and sample distillation were performed. For each sample distillation, the ammonia was collected in a beaker containing 30 ml boric acid and a few drops of methyl red indicator solution. The total nitrogen content of the distillate was determined by titration against hydrochloric acid. The calculated nitrogen content was converted to protein using a 6.25 conversion factor. This method consisted of two steps: determination of the percentage of nitrogen using a formula and determination of the percentage of protein. Using Formula 3.3.1 below.

Kjeldahl Nitrogen = $\frac{(Vs - Vb) \times N \times 14.01 \times 100}{W \times 1000}$ ..... (Formula 3.3.1)

Where;

Vs = ml of standardize acid used in titrate sample

Vb = ml of standardize acid used to titrate blank

N = Normality of standard HCL

W = Weight in g of sample or standard

Crude protein (%) = Kjeldahl Nitrogen (%) x F

F = Factors to convert nitrogen to protein

3.3.2 Fat Content (dried)

Dried powdered samples (FB and W) were extract with 30ml hexane. Five (5) g of each dried sample for 2 minutes at 2000 r.p.m. Following that, 50 ml of distilled water was added. And the sample mixture was filtered through a Buchner funnel for separately. The bottom layer was collected in a beaker, weighed and placed under the fume chamber to evaporate the hexane. The excess fat in the beaker was weighed and recorded. The following formula was used to calculate the fat content:

Fat (%) = (Weight of Dish + Fat) (g) – Weight of Dish (g) x 100…… (Formula 3.3.2)

Initial Sample Weight (g)

3.3.3 Fibre Content (dried)

The Fibertec of Dietary Fibre Analysis System (FOSS Brand), was used to determine the fibre content of each concentration of mushroom waste). It's a completely automated crude fibre analyser that's based on official method results (ISO, AOAC). The automated system uses internally preheated reagents added in a closed system. The following formula 3.3.3 was used for fibre content calculation;

Fibre (%) = (Weight of Dish + Fibre) (g) – Weight of Dish (g) x 100…. (Formula 3.3.3)

Initial Sample Weight (g)

3.3.4 Moisture Content (fresh)

Moisture content of the samples (mushroom fruiting body and by-product) were calculated using manually, where daily collection was weighed before and after

dehydration using a dehydrator machine at 40˚C for 24 hour. The following formula 3.3.4 was used for moisture content analysis;

Moisture (%) = Initial Weight (g) – Final Weight (g) x 100……. (Formula 3.3.4)

Initial Weight

3.3.5 Ash Content (dried)

The ash content of 1g of each sample (FB and W) was determined using the dry ashing process. A clean silica crucible was weighed and mushroom samples was filled in it. It was placed in a muffle furnace for 6 hours at 600°C. Then, the crucibles was cooled down in a desiccator and the ash was weighed. The total percentage of ash content was calculated using formula 3.3.5 shown below:

Ash Content (%) = (Weight of Crucible + Ash) (g) – Weight of Crucible (g) x 100

Weight of Sample (g)……..…. (Formula 3.3.5)

3.3.6 Carbohydrate Content

The carbohydrate content were calculated by subtracting the percentage of moisture, protein, fat and ash content using the formula 3.3.6 below:

Carbohydrate content =100% - [(moisture content %) + (protein content %) + (Fat content %) + (Ash content %)]…….. (Formula 3.3.6)

3.4 Infusion Extraction

50 g of sample powdered were weighed and diluted in 500 ml distilled water in a beaker before being heated with temperature 100˚C for 30 minute. After that, the sample was rested in room temperature and filtrated with vacuum filtration (Boonsong, Klaypradit, & Wilaipun, 2016). The filtered extracts were freeze died for six day.

3.5 Antioxidant Activity

Scavenging of free radicals (DPPH) 1.1-diphenyl-2-picryl-hydrazil (DPPH) was used to test sample free radical scavenging activity, with some modifications. The result

was measured using spectrophotometric at 517 nm to analyse the concentration value in µg/ml (0.05/1, 0.1/1, 0.15/1, 0.25/1, 0.5/1) and absorbance value (A). Percentage inhibition (%) was calculated using Formula 3.3.7 below. Higher free radical scavenging activity was shown by a reaction mixture with a lower absorbance. The IC_{50} value were calculated by plotting scavenging activity against different concentrations of samples extract and is characterized as the total antioxidant required to reduce the initial DPPH radical concentration by 50%. The reference substance will be ascorbic acid (Labiad, Harhar, Ghanimi, & Tabyaoui, 2017). The ascorbic acid standard was tested in the same way at different concentrations (1-6g /10ml).

Scavenging activity% = $\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$ (Formula 3.3.7)

3.6 Statistical Analysis

All data analysis was performed using GraphPad Prism 7.00. 159. All the data collected was subjected to a one-way analysis of variance (ANOVA) with ($p<0.05$) considered as significant. The results was presented as mean ± standard error (SE)

CHAPTER 4

RESULT AND DISCUSSION

4.1 Proximate analysis of fruiting body vs mushroom by-product

Samples were analysed for protein, fat, fibre, moisture and ash contents and the percentage comparison were shown as in Figure 4.1s. Overall observation showed that the fruiting bodies (FB) contain significantly higher ($p \leq 0.05$) protein, fat and ash content (Figure 4.1 A, B, and D). Meanwhile, mushroom by-products (W) has significantly higher amount of fibre, ash and carbohydrate as compared to the FB (Figure 4.1 C, E and F).

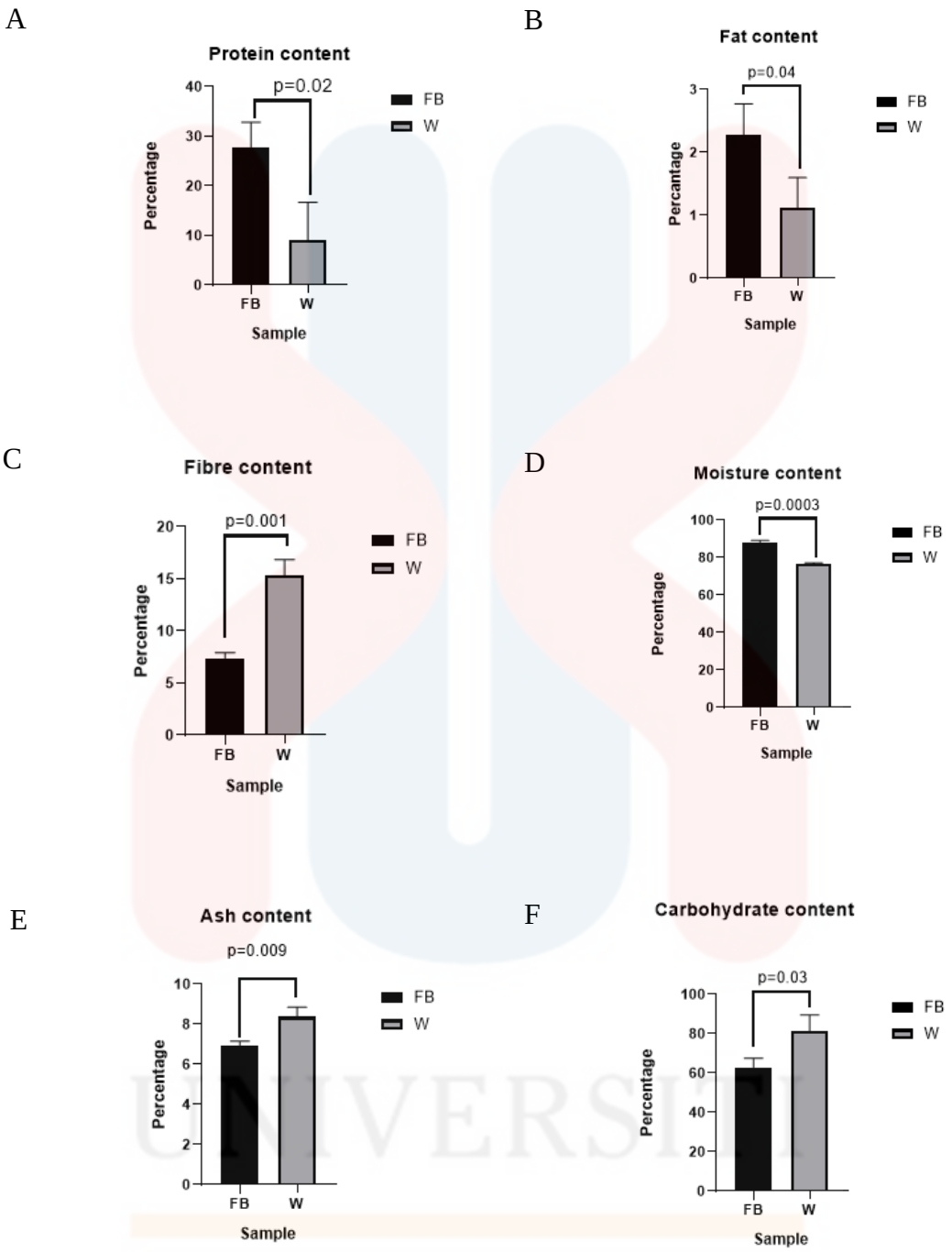


Figure 4.1: Bar chart shows the protein (A), fat (B), fibre (C), moisture (D), ash (E), and carbohydrate (F) content in the fruiting body (FB) vs. by-product waste (W)

4.2 Antioxidant Activity

The antioxidant activity was measured using DPPH assay. The methods are colormetric assays, where activities were measured based on changes on absorbance readings at specific wavelengths. The analysis required a spectrometer device for absorbance measurements (Akar, Küçük, & Doğan, 2017). The IC_{50} which is the concentration required for 50% inhibition of DPPH free radicals was calculated from the dose-response graph.

In figure 4.3, the graph of relationship between concentration of ascorbic acid and percentage of DPPH was performed. Next, the relationship between concentrations of mushroom fruiting body. Last graph show the percentage of DPPH scavenging with by-product. A lower absorbance represented a higher DPPH scavenging activity (Chirinang & Intarapichet, 2009). The statistical analysis of multiple comparison was calculated using GraphPad Prism to obtain *p*-value was 0.05. The value less than 0.05 indicated as significant (Bolstad, 2016). All of the results were obtained with a significant value below 0.05.

The antioxidant analysis of the fruiting body and by-product extracts has been expressed as IC_{50} value (g/ml). The value of $IC_{50}$was calculated using the best fitted line to the data. IC_{50} was one of the parameters used to express the result of an antioxidant analysis (Molyneux, 2004). The IC_{50} value of the extract of fruiting body and by-product was refered to the value of ascorbic acid that functioned as a positive control. Table 4.3 and 4.4 showed the results extracts were able to scavenge 50% of DPPH free radicals were 3388.84 (FB) and 3357.84 (W). This can be concluded that fruiting body has higher

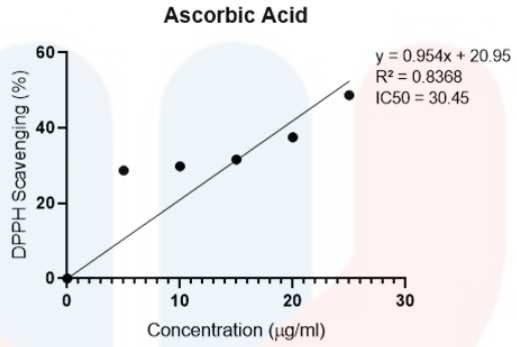
antioxidant potency compared to by-product extract. The higher the IC_{50} value, the greater the antioxidant activity, indicating that the samples can scavenge 50% of DPPH free radicals at lower concentrations. Ascorbic acid had a lower IC_{50} value than both fruiting body and by-product. As a result, ascorbic acid has a higher antioxidant potency when compared to antioxidant analyses.

The positive control used was ascorbic acid to compare with antioxidant activity of the samples. In graph A (figure 4.2) and D (figure 4.3)**,** the graph of relationship between concentration of ascorbic acid and percentage DPPH was directly performed. Next, the relationship between concentrations of mushroom fruiting body concentration in graph B (figure 4.2) and E (figure 4.3). Lastly, percentage of antioxidant scavenging with by-product in graph C (figure 4.2) and F (figure 4.3).

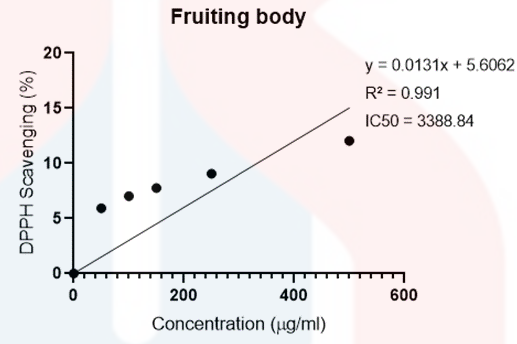
According to graph of ascorbic acid (standard curve) in A (figure 4.2) and D (figure 4.3), we can observe that concentration for inhibition, DPPH were increased from inhibition 28.7 $\pm$ 5.35 to 48.7 $\pm$ 1.6 respectively. This increasing state of inhibitions also goes towards samples of fruiting body and waste in DPPH. This showed the samples had antioxidants properties.

In first concentration, by fruiting body has 5.93 $\pm$ 0.3 compare to by-product 3.8 $\pm$ 0.26. In second concentration, the fruiting body has higher inhibition of 7.02 $\pm$ 0.4 compare fruiting body 4.68 $\pm$ 0.61. Following that, fruiting body has 7.76 $\pm$ 0.15 compare by-product with lower inhibition of 5.83 $\pm$ 0.31. In the fourth concentration, by-product has lower inhibition of 6.68 $\pm$ 0.77 compared to fruiting body 9.05 $\pm$ 0.35. In last concentration, by-product has lower inhibition of 10.24 $\pm$ 0.21 than the fruiting body 12.04 $\pm$ 0.36. The result indicated the high result of antioxidant were found in fruiting body extract.

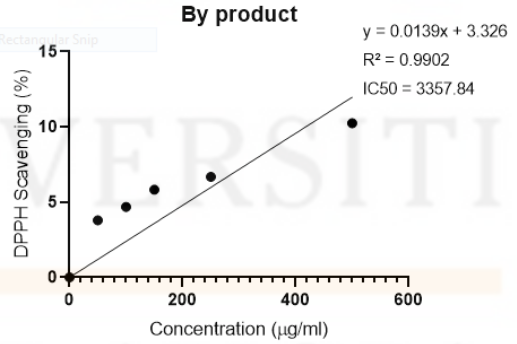
Oyster mushroom contains phenolic, L-ergotine, selenium compound, and vitamin C, white oyster mushroom (*Pleurotus ostreatus*) can act as an antioxidant. Phenolic compounds can inhibit oxidation reactions and reduce hydroxyl radicals, superoxide, and peroxide. Phenolics also influence the synthesis transcription of an endogenous antioxidant, glutathione. Because it contains antioxidant substances such as phenol, ergotine, vitamin C, selenium, and beta-carotene, 250 mg/kg BW white oyster mushroom extraction has a high antioxidant effect. The compound with the highest antioxidant activity is phenol (Egra, Kusuma, Arung, 2019).



(D)



(E)



(F)

Figure 4.3: The linear regression of relationship of DPPH with ascorbic acid (D), fruiting body (E), by-product (F)

CHAPTER 5

CONCLUSION AND RECOMMENDATION

The proximate analysis were successfully performed using Gerhard Analytics, Foss Soxtec 2055, Fibertec 8000 and Ash Oven and data were obtained automatically. Meanwhile, manual calculation method was used for moisture analysis. The results showed there significantly higher fibre, ash and carbohydrate content ($p \leq 0.05$) were observed in mushroom by-products (W). This suggestion can be utilized for new creations of dietary food and processed food product such as crackers, cereals, and sauces which are high in fibre and carbohydrate. The antioxidant activity was measured using DPPH assay, it's clearly showed the highest antioxidant activity of scavenging 50% in DPPH the highest antioxidant activity was the fruiting body with 3388.84 followed by-product with IC_{50} 3357.84 and the lowest was ascorbic acid with IC_{50} 30.45. As conclusion, which by-product has higher nutritional composition compare fruiting body was acceptance only in proximate test of fibre, ash and carbohydrate. Overall, compare of both sample, fruiting body has higher nutrition composition and antioxidant activity compare to by-product. As recommendation, the analysis can used in further study as a reference for future studies

for other mushroom type and can add replicate in order to get more accurate result compare this analysis only three replicate had run due to limitation period. Along with that, other antioxidant analysis, such as Ferric reducing ability of plasma (FRAP) and 2, 2'Azino-Bis-3-Ethylbenzoline-6-Sulfonic Acid (ABTS), should be performed to compare antioxidant activity levels in the samples.

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APPENDICES

Figure A.1: Drying of sample using dehydrator machine at 40˚C in 24 hour

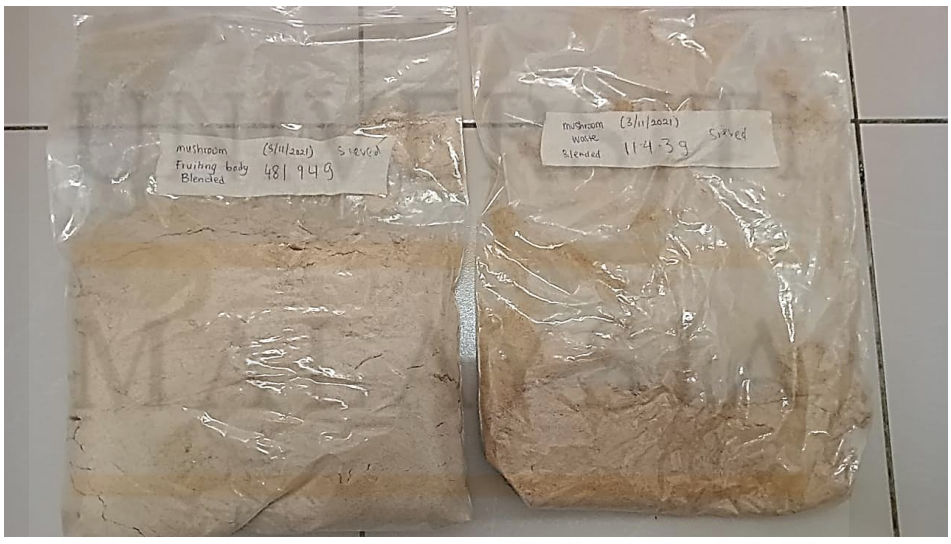

Figure A.2: Sample powder after process grind and sieve

Figure A.3: balance of sample fruiting body (left) and by-product (right) after sieve with sieve opening 500 µm

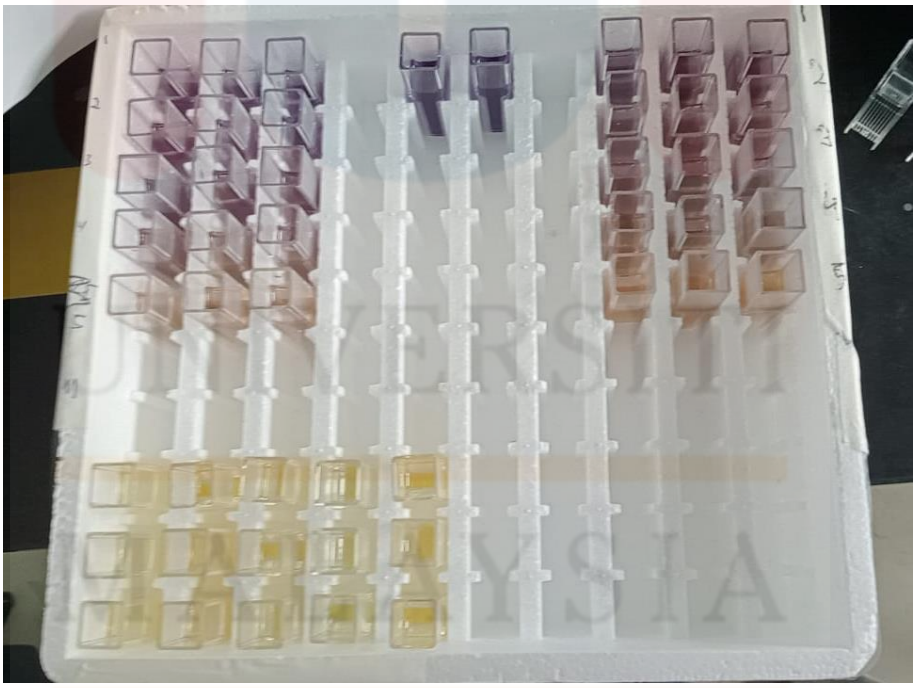

Figure A.4: preparation of DPPH assay

Figure A.5: Gerhard Analytics machine for nitrogen analysis

Figure A.6: Fibertec 8000 analytics machine for fibre analysis