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Characteristic of Oyster Mushroom Grown on Substrates Containing Paper Waste

Joko Sulistyo[1)]*, Soh Chin Quan[2)], Lee Khaa Hwee[3)]

[1)] Food Technology Program, Universitas Ciputra Surabaya, Indonesia.
[2)3)] Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Malaysia

Correspondence E-mail: joko.sulistyo@ciputra.ac.id

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Abstract

The aim of study was to determine the capacity of spawn of oyster mushroom and influence of substrates containing PW used to grow the mushroom and its properties related to nutritional value and antioxidant activity due to the use of paper waste (PW). The substrate was prepared based on 5 different formulations which were 0% (control), 20%, 30%, 40%, 50% PW. Preparation of seeds and spawn and mushroom cultivation technique was carried out manually using basic techniques of mushroom seeding and cultivation, while proximate analysis was carried out to analyze the content of fat, protein, moisture, and ash of the mushroom fruiting bodies, followed by analysis of heavy metals using AAS, and furthermore carried out extraction of bioactive compounds to analyze antioxidant activity using ABTS oxidizer. The physicochemical analysis was carried out to test the quality of the mushroom. Overall, the mushroom grown on the substrates containing 50% PW showed higher content of ash, protein, fat and crude fiber compared to the mushroom grown on other substrates with different composition of PW. However, the mushroom grown on the substrate contained 50% PW indicated considerably high content of heavy metals such as lead, (3.5%), copper (20.8%) and cadmium (4.4%), respectively, although, it showed the higher antioxidant activity (87.19%) as well compared to the mushrooms grown on the substrate containing a lower percentage of PW, thus giving a conclusion that there was probably positive correlation in between the heavy metals contained in PW with antioxidant activity.

Keywords: Antioxidant, Heavy metals, Mushroom, Proximate

INTRODUCTION

Oyster mushrooms (Pleurotus spp.) is a macro- and rapid growing fungus and has its own fruiting body. It decomposes complex organic matter to produce simple compounds as their substrate (Tirkey et al., 2017). Over the past, mushrooms have been recognized as important foods and their uses are increasingly important in human health, nutrition, and medicine. It is also rich in medicinal value by providing a wide variety of medicinal and effective features for specific life-threatening diseases, including anticancer, antibiotics, anti-inflammatory activity, immunomodulatory effects, and decreased blood fat. The attractive feature of oyster mushrooms is that they can use a wide variety of waste products and transform lignocellulosic waste into high nutritional value foods (Galappaththi et al, 2021).

Mushrooms contain high antioxidant values and can be known as therapeutic functional foods in preventing various diseases such as high blood pressure, hypercholesterolemia, and cancer (Khan et al., 2017; (Sarikurkcu et al., 2015). The mushrooms contained bioactive compounds including polyphenolic compound, and hence it showed some anti-tumor, anti-cancer, anti-bacterial, anti-fungal, anti-viral, anti-inflammatory, antioxidant, immuno-

modulatory, anti-diabetic, anti-allergic, antimitogenic, anti-hypertensive and anti-hypercholesterolemic properties (Galappaththi et al, 2021).

According to (Dubost et al., 2007), antioxidant activities of compounds are positively correlated with their total polyphenolic content. Some polysaccharides isolated from oyster mushrooms also showed considerable high antioxidant activity (Elkhateeb, 2021). The purpose of medical therapy treatment is because mushrooms produce a variety of secondary metabolites including organic acids, alkaloids, steroids, and polyphenolic compounds. The type of phenolic compounds is used to identify the main phenol in the mushrooms (Vieira et al., 2013). Edible mushrooms provide outstanding sources of valuable bioactive metabolites which demonstrate high antioxidant effects. Wild mushrooms generally produce higher antioxidant concentrations when compared to cultivated mushrooms (Mwangi et al., 2022). Antioxidants play role importantly in maintaining human health as they can remove free radicals in the body. Fortunately, the human body has its own defense system such as superoxide dismutase, glutathione enzymes and catalase to fight harmful substances and prevent cell damage. The use of additional antioxidants can be taken from our diets such as edible mushrooms (Singh et al., 2015).

Mushrooms are not like other foods which rich in protein as they can be cultivated in a variety of agricultural waste such rice and corn straw, cotton stalks and saw dust. It is due to its extensive enzyme system that can break down complex and organic compounds in agricultural waste. The selection of inappropriate substrates causes abnormal mycelia growth that causes low in yield and reduces the nutritional value. As the substrate serves as a source of nutrition and lignocellulosic materials to enhance the cultivation, development and yield of mushrooms, the selection of suitable waste including PW as a substrate is important for cultivating them to get maximum yield (Sardar et al., 2017).

PW is rich in residual lignocellulosic materials. The most effective biological method is to recycle cellulose and lignocellulosic waste. The use of this lignocellulosic waste can be used as the substrate for cultivation mushrooms due to the mushrooms being able to use a wide variety of lignocellulosic waste as they have an enzyme machine (Kulshreshtha et al., 2013). Therefore, PWs are great substrates for growing mushrooms. PW is one of the major components of municipal and industrial solid waste. This produces over 35% of the total lignocellulosic materials. Each year, million tons of PW are produced and only about 60% recycled and therefore, their remains may pollute the environment (Neelamegam et al., 2018).

The mushrooms use lignocellulolytic enzymes in a variety of applications such as bioconversion of agricultural waste to valuable products as animal feed and other purposes. The use of enzymes is beneficial in biodegradation of pollutants, xenobiotics, and industrial wastes. Some lignocellulosic waste such as corncobs, wood dust and straw have been used for the cultivation of edible mushrooms. Different substrates used in cultivation of mushrooms affect nutritional value, functional properties, organoleptic and mushroom chemical properties (Singh et al, 2015).

The present study was to determine nutritional value and functional property of mushroom grown on substrate supplemented with PW for growing of oyster mushroom.

RESEARCH METHODS

Preparation for seed and spawn

Seeds of oyster mushroom was grown on Potato Dextrose Agar (PDA) by inoculating a small piece of mushroom tissue on to the media and incubated for 5 days at 25˚C in the dark and furthermore transferred onto PDA slant (Tudses, 2016). Several subcultures were performed to obtain purified spawn. To prepare spawn, a total of 300 g of corn kernels were

submerged using tap water overnight then drained off and the kernels were added with rice bran and gypsum and the mixture was stirred thoroughly and furthermore the mixture was placed and compressed into 250 ml sterile conical flask, The flask was plug with cotton and covered with aluminum foil and then sterilized for 30 min at 121˚C. After cooling, the flask was inoculated with oyster mushroom culture. When mycelium of the mushroom has completely grown over surface of the media after incubation for 14 days at room temperature, the seed was ready to be used for inoculation of the substrate for mushroom growth (Elhami & Ansari, 2008; Fan et al., 2000).

Cultivation techniques of mushroom

As shown in Table 1, the composition of the substrate used as a treatment group is mainly based on the ratio of wastepaper and sawdust used to cultivate mushrooms (Randive, 2012). Initially, PW is cut into small pieces of about 1-2 cm. The substrate is soaked in water overnight so that it can absorb enough water. Furthermore, PW was spread over a plastic trough placed on a steep cement floor to reduce excess water so that a moisture content of less than 70% was obtained. Then, the spawn that has prepared was mixed with the substrate in the sterile plastic bag. Next, the mixed substrate was distributed into a 2-3 kg plastic bag, compressed, and sterilized by autoclaving at 121°C for 30 min. Furthermore, the prepared spawn was inoculated on the sterilized substrate for cultivation, through a mouth that has made on the top of plastic bag and plugged using sterile cotton. After the mushroom mycelia started to colonize some of the substrate in the plastic bag, they were transferred onto bamboo racks in the mushroom house, which had limited lighting and room temperature. The substrate in a plastic bag that has been overgrown with mycelia is then watered with clean water twice a day, using a water spray to maintain the moisture content in the substrate and humidity in the mushroom house.

Table 1. Formulation of media for mushroom cultivation containing paper waste (PW).

Formulation*	Saw dust (%)	Paper waste (%)	Rice bran (%)	Tap Water (ml)
F1	85	0	15	300
F2	65	20	15	300
F3	55	30	15	300
F4	45	40	15	300
F5	35	50	15	300

*) Formulation 1, 2, 3, 4, 5 (F1, F2, F3, F4, F5)

Proximate Analysis

The oyster mushrooms were dried using a dryer (60˚C) until they reached a stable weight, then the mushroom samples were ground to a fine powder using a blender and stored in the refrigerator at 4˚C. Analysis of fat, protein, crude fiber, water content, and ash content was carried out using the AOAC method.

Fat Analysis

A dry extraction method has been used to determine the fat. It consists of extracting a dry sample using several organic solvents. Crude fat is determined by extraction using ether and a Soxhlet apparatus. About 1 g of dry sample was wrapped in sample paper and placed in a grease-free beaker glass, then put into an extraction tube. The beaker glass was added with petroleum ether, then weighed, cleaned, dried, and furthermore installed into the apparatus. The heater was turned on to start the extraction process, until the ether was evaporated completely,

and then the glassware was removed before the final inhalation. The extract was transferred to a clean glass cup and washed with ether, then evaporated in a water bath. Furthermore, the glass cup was heat in the oven at high temperature (105˚C) for two hours and cooled in a desiccator. Crude fat was determined according to the formula reported (Gul & Safdar, 2009).

Protein Analysis

The reagents used were hydrochloric acid (HCl), sulfuric acid (H2SO4), and natrium hydroxide (NaOH), while digestion mixture consist of kalium sulfate (K2SO4) and copper sulfate (CuSO4), boric acid solution (H3BO3) and methyl red (C15H15N3O2). The protein content was determined by the Kjeldahl method. Dried sample of mushroom (c.a. one gram) was taken from the digestion bottle, then concentrated sulfuric acid and reaction mixture (K2SO4:CuSO4, 8:1) were added. The flask was swirled to ensure the contents were thoroughly mixed. Furthermore, the flask was placed on a heater to start the digestion until the mixture became clear blue green within 2 h. The digest was cooled and transferred into volumetric flask and added distilled water until it reached the marked volume. Digestive distillation was carried out using a Kjeldahl apparatus. About 10 ml of the digested material is put into the distillation tube, then ten ml of natrium hydroxide gradually added into the tube. The distillation was continued for a few minutes, and the resulting NH3 was collected as NH4OH in a conical flask containing H3BO3 solution with a few drops of methyl red (C15H15N3O2) indicator. During distillation, a yellow color appears due to the existence of NH4OH. The distillation results are then titrated with a standard solution of hydrochloric acid until the distillate solution is pink. An empty sample is also run through all the steps as above. The percentage of protein content is calculated based on the Gul and Safdar formula (2009).

Coarse Fiber Analysis

Samples of free moisture and crude fibers extracted from ether and made from cellulose were initially hydrolyzed with sulfuric acid and subsequently with potassium hydroxide solution. The unhydrolyzed residue collected after hydrolysis has been annealed and the weight loss after annealing was known as crude fiber. The reagents used were sulfuric acid solution, potassium hydroxide solution in distilled water and acetone. The sample was weighed and transferred to a porous crucible. After heated sulfuric acid solution was added, and a drop of foam suppressor was added to each column. When it starts to boil, turn down the heat and leave it for half an hour. The collected fiber was then rinsed with distilled water three times to convince that the acid was completely removed.

Moisture and Ash Content

Test portions containing about 2 g of dry matter were dried to constant weight at 95˚C - 100˚C under pressure ≤ 100mm Hg for 5h. Loss on drying (LOD) is reported as an estimate of moisture content (AOAC, 2000). For ash determination, a clean empty ceramic bowl was placed in a muffle furnace at high temperature (600ºC) for one hour then cooled in a desiccator. The sample was ashes by burning using a blowpipe. Then, the crucible was placed in a muffle furnace (550ºC) for 3h. Total oxidation of all organic matter in the sample was indicated by the appearance of grayish-white ash powder. After furnacing the ash was closed, the bowl was cooled and weighed. The percentage of ash was then calculated.

Analysis of Heavy Metal

Two grams of mushroom sample in ash was placed onto oven at 60˚C for 3h prior to be dissolved into organic solvents. Approximately 0.5 g of cold ash was digested with a mixture of 10 ml of HCl, HNO3, and HClO4 by heating for 2 h. The digested mixture was evaporated to 5ml using a rotary evaporator. Then, it is made up to 10 ml of 2M HNO3 and 30ml of distilled

water was added and stored in a 100 ml beaker glass. Sample was analyzed to determine the lead (Pb), copper (Cu) and cadmium (Cd) using an atomic absorption spectrophotometer (AAS). Approximately 2 ml of the digested sample was taken and diluted to 50 ml. The sample was irradiated at 324.75 AAS wavelength to detect Cu concentration. Approximately 2 ml of digested sample was taken and diluted to 100ml. The presence of Pb and Cd were detected by AAS at absorbance of 217 nm and 228 nm and using a suitable lamp. The content of Pb and Cd were calculated with the formula of Cu in ppm (Chittaragi & Naika, 2014).

Extraction of Polyphenol Compounds

Dried mushroom samples (10 g) were extracted using 50% ethanol. The extraction process is carried out using an ultrasonic bath at a temperature of 45˚C for 40 min. The volume of the liquid extract obtained after filtration was measured and the extraction solvent was removed from the rotary evaporator under vacuum. After that, the sample is dried at a temperature of 60˚C until it gets a constant mass. The dried extract was placed in a glass bottle and stored at 4˚C to avoid oxidative damage (Mujic et al., 2010).

Analysis of Antioxidant Activity using ABTS

A reagent of 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic Acid) (ABTS) was prepared by reacting five ml of ABTS stock solution (7 mM) with 88 microliters of potassium persulfate solution (140 mM) and reacted at room temperature room in the dark for more than 12 hours. ABTS reagent is diluted with distilled water to provide absorbance between 700-750 nm for measurement of 20 µl of sample solution reacted with 180 µl of ABTS reagent, then reacted in the dark room at room temperature for half an hour, then the absorbance was measured at a wavelength of 734 nm with a UV-VIS spectrophotometer, with the positive control being ascorbic acid (Tachalerdmanee et al., 2016).

Statistical Analysis

The analysis was carried out in duplicate (n = 2) and used one-way ANOVA analysis and Tukey's test with 95% confidence to determine the existence of significant differences ($p<0.05$) for the attributes between the mushroom samples tested. Significant differences were determined at the 95% confidence level and presented as mean ± standard deviation (M ± SD) (Mohd Rashidi & Yang, 2016)

RESULTS AND DISCUSSION

Cultivation of Oyster Mushroom

At first, the mycelia colonized the substrate for growth very slowly, thus the growth of the starter culture took 2-3 weeks. Fig. 2-4 showed the growth performance of mushrooms in week 3 to 5. Oyster mushroom grew fastest on a substrate containing 0% and 20% PW (PW), followed by substrates containing 30% and 40% PW, and finally spawned on a substrate containing 50% PW. Substrates containing 0% and 20% PW spawned oyster mushrooms in the fourth week, while substrates containing 30% and 40% PW spawned mushrooms at the beginning of the fifth week and substrates containing 50% PW spawned mushrooms at the week 5 to 6.

Fig. 1. Substrate conditions of mushroom on the first (A) and fourth weeks (B) incubation.

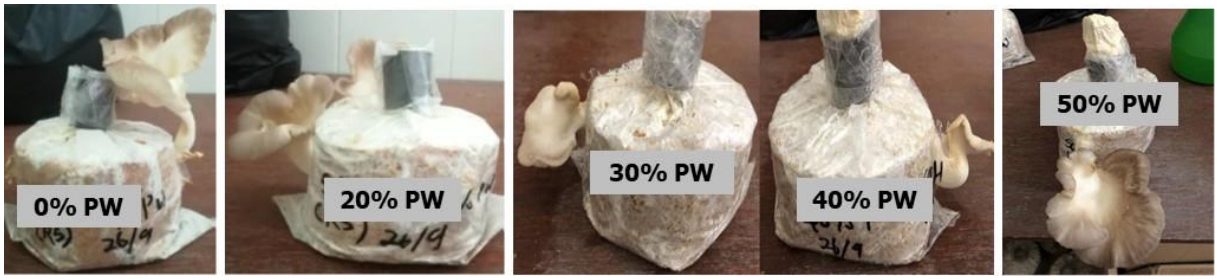

Fig. 2. Mushroom spawn performance on different substrate containing PW on fifth week.

Proximate Analysis

Proximate analysis was carried out on mushrooms grown in substrates containing 0%, 20%, 30%, 40% and 50% PW to determine the content of moisture, ash, protein, coarse fiber, and fat. Table 2 showed the proximate mean values of the mushrooms grown on different percentages of PW. The moisture content of substrate containing 0% and 20% PW did not show a significant difference ($p>0.05$), while the moisture content of substrate contained 30% PW showed a significant difference ($p<0.05$) compared to the other substrate with PW. The increase in moisture content of substrate grown by mushroom with containing higher PW might be due to the capability of PW in absorbing more water compared to lower content of PW in the substrates, since sawdust absorbed limited water compared to the PW (Ogundele et al., 2017). The moisture content of the substrate containing 40% and 50% PW showed no significant difference ($p>0.05$). This is probably because the percentage of PW was not much different on the two substrates.

Table 2. Proximate analysis of oyster mushroom grown in different media containing PW.

Composition	**PW (0%)**	**PW (20%)**	**PW (30%)**	**PW (40%)**	**PW (50%)**
Moisture	90.1 ± 0.04^{a}	89.9 ± 0.02^{ab}	90.0 ± 0.02^{c}	88.7 ± 0.01^{d}	90.0 ± 0.02^{bc}
Ashes	1.2 ± 0.02^{a}	1.2 ± 0.01^{a}	1.1 ± 0.01^{a}	1.1 ± 0.02^{a}	1.1 ± 0.01^{a}
Crude fiber	1.6 ± 0.01^{c}	1.8 ± 0.01^{b}	1.9 ± 0.02^{b}	1.6 ± 0.01^{c}	2.3 ± 0.01^{a}
Protein	4.0 ± 0.31^{a}	4.1 ± 0.25^{a}	4.2 ± 0.12^{a}	4.1 ± 0.20^{a}	4.3 ± 0.18^{a}
Fat	1.2 ± 0.03^{a}	1.2 ± 0.02^{a}	1.2 ± 0.03^{a}	1.2 ± 0.03^{a}	1.2 ± 0.02^{a}

Mean values that have the same symbol in the same row do not have a significant difference ($p>0.05$).

Substrate moisture content for the growth of oyster mushrooms grown on substrates containing PW is supported by previous research according to Bhattacharjya et al., (2015) that oyster mushrooms require a substrate with a moisture percentage of around 88.5-90.2%, considering the water content in the mushrooms affects the quality of the mushrooms as an ingredient. The high-water content of the substrate also supports the growth of microorganisms. In general, the more water, the greater the opportunity for the growth of various types of microorganisms. Reduction of moisture content through drying reduces the susceptibility to attack by microorganisms but has a profound effect on the texture and color and taste of the mushrooms.

The content of protein of the oyster mushrooms grown on the substrates containing different percentage of PW did not show significant difference ($p>0.05$) from each other. The results of this study were the same as those of other studies which showed no significant difference ($p>0.05$) in mushrooms using blank paper or printed paper as the substrate (Fernandes et al., 2015). Wheat bran supplementation has the potential to increase protein accumulation in the mushroom compared to rice bran and corn bran (Wang et al., 2001). In this study we used rice bran as a supplement substrate hence the cultivated mushroom showed a high protein content.

Previous study reported that as much as 50% of PW increased the crude fiber content of mold, thus showing a significant difference ($p<0.05$) compared to other PWs. Differences in crude fiber content are associated with variations in substrate composition and types of mushrooms used (Michael et al., 2011). Other studies have shown that the crude fiber content in mushrooms was approximately 12.9% and 10.4% for fresh and dried samples of mushroom respectively (Tolera & Abera, 2017). It has been reported that the mushroom has 11.4 – 20.5% crude fiber when cultivated on various agricultural wastes. Fiber is an important nutrient that consists of a healthy diet and can prevent reducing cholesterol (Machado et al., 2016).

Heavy Metal Content

Analysis of heavy metal content was carried out on mushrooms grown in substrates containing 0%, 20%, 30%, 40% and 50% PW to determine the content of lead, copper, and cadmium. Table 3 showed the mean value of heavy metal content in oyster mushrooms grown in different percentages of PW. Heavy metals are dangerous because they tend to bioaccumulate, the process of increasing the concentration of a chemical in the organism's biology over time compared to the concentration of the chemical in the environment. They will also absorb through the food chain and accumulate in the organism at any time. Heavy metals cannot be metabolized biologically, removed, or broken down into harmless products. Mushrooms absorb heavy metals from the substrate through extensive mycelium (Quarcoo, 2013).

The results showed that the level of heavy metals in different substrates did not affect mycelial growth. Substrate characteristics such as pH, redox potential, organic matter content, substrate mineralogy, competition with other metal ions and solution composition affect the absorption and accumulation of metals in mushrooms (Meguid & Bably, 2015). Unlike plants, mushrooms can accumulate high concentrations of trace metals such as lead, cadmium, iron, copper, manganese, zinc, chromium, nickel, aluminum, and mercury in the fruit body (Sithole et al., 2017). Heavy metals penetrate from the substrate during growth to the fruit body. The higher the PW content in the substrate, the higher the concentration of heavy metals contained in the dried mushroom fruiting bodies. In addition, PW consists of organic materials as well as printing inks, binders, and additives (coatings, fillers, surfactants, and preservatives). Printing ink is an inorganic pigment that contains heavy metals dispersed in the binder component and is a resin solution in mineral or oil (Kopiński & Kwiatkowska-Marks, 2012).

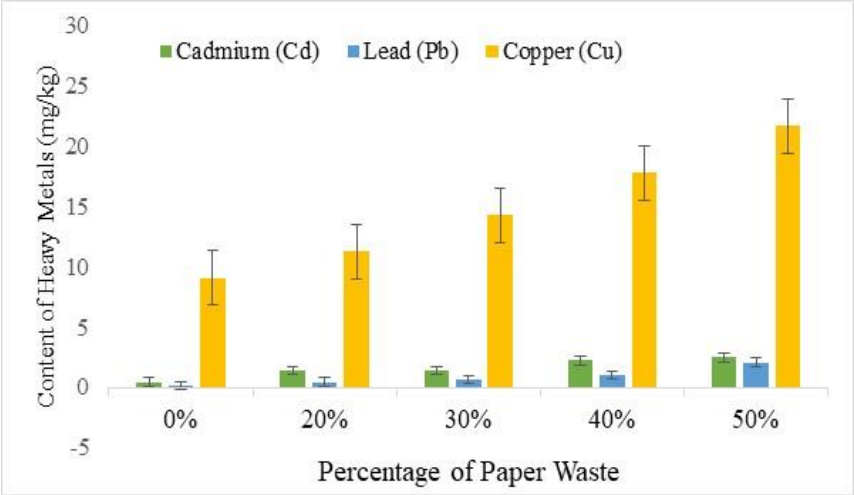

Fig. 3. Heavy metal content of mushrooms grown on different percentages of PW.

Antioxidant activity

Antioxidant activity analysis was carried out on mushrooms grown on substrate containing 0%, 20%, 30%, 40% and 50% PW. Fig. 4 showed the value of antioxidant activity in the mushrooms growing in different percentages of PW. The antioxidant activity in mushrooms grown on substrate containing 0% PW and 20% PW, as well as on substrate containing 30% PW and 40% PW, respectively did not show a significant difference ($p<0.05$), while antioxidant activity in mushroom grown on substrate containing 50% PW showed significant differences compared to each other sample of mushrooms.

Fig. 4 showed that the antioxidant activity increased in mushrooms grown on substrate containing from 0% PW to 50% PW. The reduction of antioxidant activity is related to the loss of antioxidants such as total phenols, flavonoids, L-ascorbic acid, and carotenoids (Selvakumar & Sankar, 2015). The antioxidant activity in this study showed a value of 75.33% to 87.56% in mushrooms. The antioxidant activity in this study showed a range value of antioxidant activity of 75.33% to 87.56%. Differences in mycelial wet weight resulted in low and different phenolic content in the mushrooms. This may be due to the variation in their growth conditions and where the mushrooms are grown (Iwalokun et al., 2007).

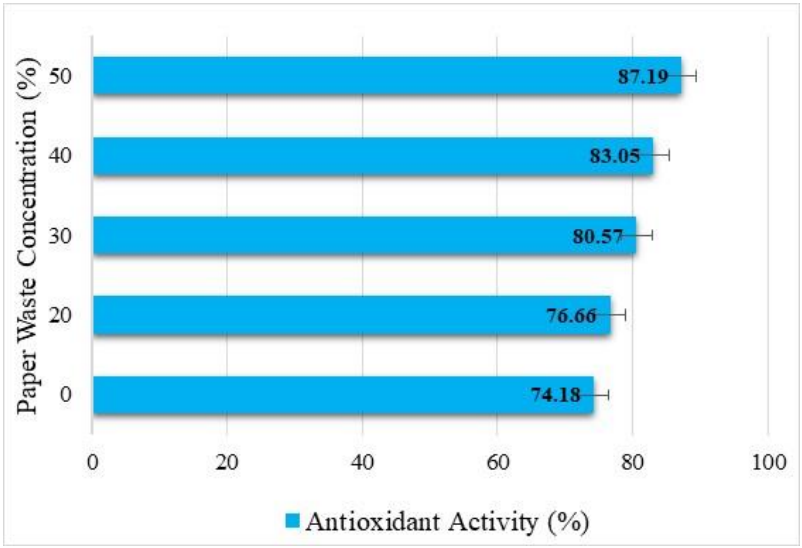

Fig. 4. Antioxidant activity of oyster mushrooms grown on different percentages of PW.

Biorefinery Production of Poly-3-hydroxybutyrate

Phenolic compounds are the main component identified. Phenolic compounds could trap metals, inhibit lipoxygenase, and trap free radicals. Phenolic compounds are important plant constituents because they can trap free radicals due to their hydroxyl group. Phenolic compounds may contribute to antioxidant properties. In addition, phenolic compounds are also associated with antioxidant activity and play an important role in stabilizing lipid peroxidation. The ABTS radical scavenging assay is widely used to determine the antioxidant activity of lipophilic compounds. The ABTS assay is an excellent tool to determine antioxidant activity that donates hydrogen and breaks prooxidant chains (Sudha et al., 2012).

CONCLUSION

Oyster mushrooms grown on substrates containing PW showed high moisture content. While ash, protein, and crude fiber content of mushroom grown on substrates containing 50% PW showed the highest among of the mushrooms. The mushroom grown on 40% PW has high fat content compared to the mushroom grown on other substrates. The mushroom grown on 50% PW shown a higher content of heavy metals including lead, copper and cadmium compared to other substrates containing PW. Antioxidant activity increased in the mushrooms derived from the substrates containing 0% to 50% PW and the substrate containing 50% PW was the best substrate for growing oyster mushrooms since it exhibited higher content of antioxidant properties.

Our suggestion here, though the use of PW may help to reduce environmental problems, PW contains high heavy metals concentration including lead, copper, and cadmium. The ink in the wastepaper needs to be removed to reduce the heavy metal content. Wastepaper needs to be processed before being used as a substrate for growing mushrooms. High costs may be required to process the paper until it is safe to use. Therefore, alternative methods need to be studied to solve the problem, among them by soaking the PW using an enzyme solution prior to use as formula of substrate for growing mushroom.

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