International Food Research Journal 24(5): 1998-2008 (October 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

# Effects of substrates and drying methods on antioxidant compound and antioxidant activity of fruiting body extracts of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*)

[1,3]Hoa, H. T., [2]Wang, C. H., [3]Tam, N. V. and [2*]Wang, C. L.

[1]*Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan*
[2]*Department of Plant Industry, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan*
[3]*Thai Nguyen University of Agriculture and Forestry, Thai Nguyen, Viet Nam*

**Article history**

*Received: 4 September 2016*
*Received in revised form: 29 September 2016*
*Accepted: 30 September 2016*

**Keywords**

*Antioxidant activity*
*Oyster mushroom*
*Substrate*
*Total phenolic contents*

**Abstract**

The study was conducted to compare the effect of different substrate formulas and different fruiting body drying methods on the total phenolic contents (TPC) and total flavonoid contents (TFC), as well as the antioxidant activity of oyster mushroom *Pleurotus ostreatus* (PO) and *Pleurotus cystidiosus* (PC). Seven substrate formulas were used: 100% sawdust (SD), 100% sugarcane bagasse (SB), 50% SD+50% SB, 80% SD+20% SB, 100% corncob (CC), 50% SD+50% CC, 80% SD+20% CC. Oven-drying and freeze-drying methods were investigated. The antioxidant potential was determined using 1,1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, reducing power and chelating ability. The results indicated that the increasing SD ratio in substrate formulas reduced TPC and TFC which in turn decreased antioxidant activity of mushroom PO and PC extracts. In substrates containing higher contents of CC and SB (100% CC, 50% CC, 100% SB, and 50% SB), higher values of TPC, TFC, as well as high efficiency of DPPH radical scavenging ability, reducing power and chelating ability were obtained. These results suggest that CC and SB can be used to partially or entirely replace SD in substrate formulation for oyster mushroom cultivation which will improved its antioxidative capacity. With freeze-drying method, PO and PC showed better efficiency in TPC as well as antioxidant activities in comparison with oven-drying method. PO and PC might be used as potential source of natural antioxidants for food supplements as well as in the development of nutraceuticals.



## Introduction

Oyster mushroom belongs to the family of Pleurotaceae and includes 40 species (Neelam and Singh, 2013). Oyster mushroom, the nutritionally gifted fungi, now ranks second most cultivated mushrooms in the world because of their low cost, easy to grow on a large of agro wastes, high biological efficiency and high nutritional value (Mane *et al*., 2007; Sánchez, 2010). Oyster mushrooms have great nutritional value since they are rich in protein, with all essential amino acids, mineral, fiber, and low in lipid (Purkayastha and Nayak, 1981; Eva *et al*., 2013). Moreover, oyster mushrooms are considered as the most potential edible mushroom with high therapeutic values. Secondary metabolites are compounds that are used as food and medicine to protect against illness and to maintain human health. Oyster mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polysaccharides, polypeptides, and steroids. Oyster mushrooms such as *Pleurotus ostreatus* (PO) and *Pleurotus cystidiosus* (PC) have been found to possess antioxidant activity, which is well correlated to their total phenolic contents (TPC) and total flavonoid contents (TFC) (Yang *et al*., 2002; Li *et al*., 2007).

Oxidation is essential to many living organisms for the production of energy to fuel in biological processes. Free radicals are produced in normal and or pathological cell metabolism. The uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases. Phenolic compounds are one of antioxidant components that defend our body against free radicals. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells (Baillie *et al*., 2009). The major antioxidant compounds found in mushrooms are phenolic acids

*Corresponding author.
Email: *lelia@mail.npust.edu.tw*

and flavonoids (Ferreira *et al*., 2009).

There are limited data in the literature concerning the antioxidant properties of oyster mushroom grown in different substrates and comparison among drying methods of fruiting bodies. Hence, the aim of our study is to determine the TPC and TFC as well as the antioxidant activity of PO and PC fruiting bodies grown in different substrate formulas and dried by different drying methods.

## Materials and Methods

*Mushroom material and spawn preparation*

Oyster mushrooms PC (strain AG 2041) and PO (strain AG 2042) obtained from Plant Physiology and Value Added Microorganisms Laboratory (Department of Plant Industry, National Pingtung University of Science and Technology (NPUST), Taiwan) were grown in potato sucrose agar (PSA) (1000 mL boiled extract of 200 g potato, 20 g agar powder, 20 g sucrose) at 28°C for regular subculture and maintained on PSA at 4°C. Spawns were prepared in 850 mL polypropylene plastic bottles filled with 600 g acacia sawdust supplemented with 9% rice bran, 1% sugar, 1% calcium carbonate, 0.03% ammonium chloride, 0.03% magnesium sulfate, and 0.03% mono-potassium phosphate (w/w) and 60~65% water content, and then sterilized at 121°C for 5 hr. These materials were bought from local market, Pingtung county, Taiwan. After cooling at room temperature, 10~20 mycelium discs (diameter 1 cm) of each oyster mushroom were inoculated into each bottle of sterilized spawn. The spawn was incubated at 28°C until the substrate was fully colonized.

*Substrate preparation, mushroom cultivation and harvest*

In order to determine suitable substrates and suitable ratios for antioxidant compounds and activity of PO and PC, seven substrate formulas: 100% sawdust (SD), 100% sugarcance bagasse (SB), 50% SD+50% SB, 80% SD+20% SB, 100% corncob (CC), 50% SD+50% CC, 80% SD+20% CC (w/w) were investigated. After mixing materials with the above proportion, Fifteen kg of each substrate formula were supplemented with 9% rice bran, 1% sugar, 1% calcium carbonate, 0.03% ammonium chloride, 0.03% magnesium sulfate, and 0.03% mono-potassium phosphate. Water content of the final mixture was adjusted to about 65%. Each substrate formula after supplementing nutrients and distilled water was filled into 10 × 23 cm polyethylene plastic bags (1 kg/bag) and sterilized in an autoclave at 121°C for 5 hr. Twenty-four culture bags were used for each substrate formula. After substrates were cooled to room temperature, they were inoculated with the 2 g spawn per bag and incubated at 28°C and 60 ~70% relative humidity under dark condition. After the surface of substrates was entirely covered and filled with mycelium, then the substrates were moved to a cultivation room in which temperature was maintained at 24°C and kept at relative humidity ≥ 90%. Mushroom fruiting bodies were harvested from each of the culture bags when the in-rolled margins of the mushroom caps began to flatten. The oyster mushrooms PO and PC were cultivated during around 3 months.

*Sample preparation and extraction*

Fresh fruiting bodies of two oyster mushrooms PO and PC were dried by oven-drying method at 40°C (Memmert DIN 40050 IP 20) and freeze-drying method at -40°C (Freeze dryer FD-series, Taiwan) for 48 hr. The samples were grinded to powder with a grinder (Yuqi, DM-6, Taiwan), and stored at -20°C. Five grams of dried mushroom samples were extracted by using reflux extraction method (ECB-6D Hipoint, Taiwan) with 100 mL of methanol at 74°C for 2 hr. The extracts were filtered through the filter paper (Whatman No. 2 filter paper, Japan). The residues were then extracted with additional 100 mL of methanol. The pooled extracts were then evaporated using a vacuum concentrator (Heidolph, Laborta 4000, Germany) at 40°C to dryness. The dried extracts obtained were re-dissolved in methanol to a concentration of 100 mg/mL and stored at 4°C prior to analyses of antioxidant attributes.

*Determination of total phenolic contents (TPC)*

The TPC were analyzed by the Folin-Ciocalteu's colorimetric method using gallic acid (Sigma-Chemical Co., USA) as a standard (Sato *et al.*, 1996). Sample extract (100 mg/mL methanol) was diluted with methanol at rate 1:9 to get sample extract of 10 mg/ml concentration in methanol. Each 0.1 mL of sample extract with concentration of 10 mg/mL in methanol was mixed with 2 mL Folin-Ciocalteu's phenol reagent (Sigma-Aldirch Inc., USA). The mixture was incubated in room temperature for 5 min. After that, 1.8 mL of 20% sodium carbonate ($Na_2CO_3$, Nihon Shiyaku, Japan) were added, the mixture was centrifuged (Centrifuge, Type H-100B3, Japan) at 3000 rpm for 10 min. After that, the mixture was kept for 90 min at room temperature. The absorbance of each reaction mixture was measured at 735 nm by a spectrophotometer (Hitachi U-2800, Japan). Gallic acid (Sigma Chemical Co., USA) was used as a standard for the calibration curve. The TPC

were obtained by interpolation from linear regression analysis in mg gallic acid equivalents (mg GAE)/g dry extract. All tests were performed in triplicate.

*Determination of total flavonoid contents (TFC)*

The TFC were determined according to the method of Jia *et al.* (1999). Each sample extract (0.2 mL) with concentration of 100 mg/mL in methanol was mixed with 1.5 mL of distilled water. Then 0.1 mL of 10% aluminium nitrate [$Al(NO)_3$] (Johnson Matthey Co., USA) and 0.1 mL of 1M potassium acetate (CH3COOK) (Baker Co., USA) was added with the solution. After 40 min at room temperature, the absorbance of mixture was measured at 415 nm. Quercetin (Sigma Chemical Co., USA) was used as a standard for the calibration curve. The TFC were obtained by interpolation from linear regression analysis in mg quercetin equivalents (QE)/g dry extract. All tests were performed in triplicate

*Scavenging ability on 1,1- diphenyl-2-picrylhydrazyl (DPPH) radical*

Sample extract of 10 mg/mL concentration was diluted with methanol at rates of 1:19; 1:9; 1:3; 1:1; 3:1 to get methanol extract solution of 0.5, 1, 2.5, 5, and 7.5 mg/mL concentrations, respectively. Each sample extract (4 mL) of different concentrations (0.5, 1, 2.5, 5, 7.5, and 10 mg/mL) in methanol was mixed with 1 mL of methanolic solution containing DPPH (Sigma Chemical Co., USA) resulting in a final concentration of 0.001 M DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark condition, and the absorbance was measured at 517nm against a blank (Shimada *et al*., 1992). The blank sample was also prepared as above without any extract, and methanol was used for the baseline correlation. The scavenging ability on DPPH radicals were calculated as the inhibition percentage according to the formula:

Scavenging ability (%) = (1- [Absorbance $_{(Sample)}$/ Absorbance $_{(Blank)}$]) x 100

*Chelating ability on ferrous ions*

Chelating ability was determined according to the method of Dinis *et al.* (1994). Each sample extract (1 mL) of different concentrations (0.5, 1, 2.5, 5, 7.5, and 10 mg/mL) in methanol was mixed with 3.7 mL of methanol and 0.1 mL of 2 mM ferrous chloride ($FeCl_2.4H_2O$) (Baker Co., USA). The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine (Sigma-Aldirch Co., India). After 10 min at room temperature, the absorbance of mixture was determined at 562 nm against a blank. The chelating ability on ferrous ions was calculated according to the formula:

Chelating ability (%) = (1- [Absorbance $_{(Sample)}$/ Absorbance $_{(Blank)}$]) x 100

*Reducing power on hydroxyl ions*

The reducing power was determined according to the method of Oyaizu (1986). Each sample extract (2.5 mL) of different concentrations (0.5, 1, 2.5, 5, 7.5, and 10 mg/mL) in methanol was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) (Nihon Shiyaku, Japan) and 2.5 mL of 10 mg/mL potassium ferricyanide (Riedel-de Haen, Sigma-Aldirch Co., India), and the mixture was incubated at 50°C water bath for 20 min. Then, 2.5 mL of 100 mg/mL tricholoacetic acid (Riedel-de Haen, Sigma-Aldirch Co., India) was added to the reaction mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (5 mL) was mixed with distilled water (5 mL) and 1 mL of 1 mg/mL ferric chloride (Showa Chemical Co., Japan), and the absorbance is measured at 700 nm. A higher absorbance of mixture indicated a higher reducing power.

*Experimental design and data analysis*

The experiments were conducted in Department of Plant Industry, NPUST in Taiwan during autumn-winter season (July to December, 2014). The experiment was arranged in a randomized complete block design with three replications and eight culture bags per replication. One-way analysis of variance (ANOVA) was conducted with Duncan's multiple range tests to compare the mean significant differences ($p \leq 0.05$) among treatments by using computer software SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA).

## Results and Discussion

*Total phenolic contents (TPC)*

Evaluating the amount of TPC as well as the identification of the main phenolics in mushrooms, have great importance in their nutritional and functional characterization. As shown in Table 1, PO and PC extracts grown in seven substrate formulas were rich in TPC with significant difference ($p \leq 0.05$) between freeze- and oven-drying. In oven-drying, TPC of PO extracts grown in different substrates varied from 23.71 ± 0.50 to 31.67 ± 1.11 mg GAE/g extract. The highest value was achieved at substrate containing 100% CC (31.67±1.11 mg GAE/g extract), followed by substrates 50% CC

Table 1. The TPC and TFC of PO and PC cultivated with seven substrate formulas and dried by different methods

| Substrate formula | TPC (mg GAE/g extract) | | TFC (mg QE/g extract) | |
|---|---|---|---|---|
| | Oven-drying | Freeze-drying | Oven-drying | Freeze-drying |
| **PO** | | | | |
| 100% SD | 24.28±0.64 $^{dA}$ | 25.71±0.63 $^{cA}$ | 5.25± 0.15 $^{cA}$ | 5.65±0.17 $^{cA}$ |
| 100% SB | 28.19±1.06 $^{bB}$ | 30.26±0.94 $^{bA}$ | 6.11±0.24 $^{bB}$ | 6.97±0.23 $^{abA}$ |
| 50% SD + 50% SB | 27.92±0.39 $^{bcA}$ | 28.40±0.45 $^{bcA}$ | 6.01±0.29 $^{bA}$ | 6.93±0.31 $^{abA}$ |
| 80% SD + 20% SB | 23.71±0.50 $^{dB}$ | 25.72±0.71 $^{cA}$ | 5.24±0.25 $^{cA}$ | 5.71±0.26 $^{cA}$ |
| 100% CC | 31.67±1.11 $^{aB}$ | 33.90±1.13 $^{aA}$ | 6.80±0.08 $^{aB}$ | 7.60±0.19 $^{aA}$ |
| 50% SD + 50% CC | 28.36±0.65 $^{bA}$ | 30.30±1.34 $^{bA}$ | 6.04±0.03 $^{bA}$ | 6.86±0.22 $^{abA}$ |
| 80% SD + 20% CC | 25.39±1.44 $^{cdB}$ | 27.65±0.59 $^{bcA}$ | 5.78±0.08 $^{bcA}$ | 6.48±0.29 $^{bA}$ |
| **PC** | | | | |
| 100% SD | 30.00±0.57 $^{dA}$ | 31.63±2.18 $^{cA}$ | 6.45±0.24 $^{dA}$ | 6.67±0.11 $^{dA}$ |
| 100% SB | 35.83±0.83 $^{abA}$ | 37.41±0.63 $^{abA}$ | 7.38±0.22 $^{bcA}$ | 7.66±0.11 $^{bA}$ |
| 50% SD + 50% SB | 33.70±0.39 $^{cB}$ | 36.29±0.85 $^{abA}$ | 7.00±0.22 $^{cA}$ | 7.36±0.18 $^{bcA}$ |
| 80% SD + 20% SB | 33.27±1.06 $^{cA}$ | 32.77±1.93 $^{bcA}$ | 7.00±0.03 $^{cA}$ | 7.10±0.13 $^{cA}$ |
| 100% CC | 37.53±0.28 $^{aB}$ | 40.02±0.8 $^{aA}$ | 7.84±0.02 $^{aB}$ | 8.12±0.06 $^{aA}$ |
| 50% SD + 50% CC | 36.58±0.51 $^{aA}$ | 37.29±0.94 $^{abA}$ | 7.45±0.14 $^{abA}$ | 7.61±0.21 $^{bA}$ |
| 80% SD + 20% CC | 33.97±1.13 $^{bcA}$ | 34.17±1.34 $^{bcA}$ | 7.26±0.06 $^{bcA}$ | 7.40±0.14 $^{bcA}$ |

Each value is expressed as means ± standard error of three replicates. Means within the same capital letters followed by the same row of each item are not significantly different ($p \leq 0.05$). Means within the same column of each oyster mushroom followed by the same small letters are not significantly different ($p \leq 0.05$). PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; SD, sawdust; SB, sugarcane bagasse; CC, corncob; TPC, total phenolic contents; TFC, total flavonoid contents

(28.36 ± 0.65 mg GAE/g extract), 100% SB (28.19 ± 1.06 mg GAE/g extract), and 50% SB (27.92 ± 0.03 mg GAE/g extract). Substrate 100% SD, 20% SB, and 20% CC gave the lowest values of TPC (24.28 ~ 25.39 mg GAE/g extract). On the contrary, for freeze-drying, the TPC of PO extracts ranged from 25.71 ± 0.63 to 33.90 ± 1.13 mg GAE/g extract. Substrate 100% CC also gave the highest TPC (33.90 ± 1.13 mg GAE/g extract). Substrate containing 100% CC, 100% SB, and 50% CC were significantly different from substrate 100% SD in terms of TPC. The TPC of PC were higher than those of PO which ranged from 30.00 ± 0.57 to 37.53 ± 0.28 mg GAE/g extract (oven-drying) and from 31.63 ± 2.18 to 40.02 ± 0.81 mg GAE/g extract (freeze-drying).. Substrates containing 100% CC, 50% CC, and 100% SB gave the highest values of TPC (37.53 ± 0.28, 36.58 ± 0.51, and 35.83 ± 0.83 mg GEA/g extract, respectively) (oven-drying) and (40.02 ± 0.81, 37.29 ± 0.94, and 37.41 ± 0.63 mg GAE/g extract, respectively) (freeze-drying). The TPC results in the present study were almost similar to some previous reports. According to Kim *et al.* (2009), the difference in color of oyster mushrooms (dark-grey, pink, and yellow) showed the difference in TPC (21.2, 30.1, and 39.3 mg GAE/g extract, respectively). Parihar *et al.* (2015) indicated that TPC of PO was 24.01 mg GAE/g extract (methanolic extract). In another research, TPC of PO water and ethanol extracts were 30.93 and 42.47 mg GAE/g extract (Chirinang and Intarapichet, 2009). However, the TPC results in the present study were somehow lower than those of other reports. TPC of ethanolic, methanolic, hot water, and cold water extracts of PO collected from Romanian Markets were 70.2, 67.6, 57.2, and 58.8 mg GAE/g extract, respectively) (Vamanu, 2013). Jayakumar *et al.* (2009) also indicated that TPC of PO ethanolic extract was 54.9 mg GAE/g extract. It is also noteworthy that the TPC in the present study were much higher when compared to results of yet another reports. The TPC in methanolic extract of PC was 10.2 mg GAE/g extract (Yang *et al*., 2002), while the ethanolic extract of *P. florida* and PO was 6.7 and 7.1 mg GAE/g extract respectively (Tsai *et al*., 2009). The TPC of shiitake and other species of oyster mushroom were found between 6 and 15 mg/g dry weight and depending on the species of mushroom chosen (Yang *et al.*, 2002).

The changes in the TPC of PO and PC fruiting body grown in different substrate formulas are explained by the difference in lignin composition of the substrates. SD contained high amount of lignin (27.1%) (Ragauskas, 2006), while CC and SB contained lower amount of lignin (15% and 20%, respectively) (Sain, 2015). Therefore, substrates containing higher contents of CC and SB (100% CC, 50% CC, 100% SB, and 50% SB) had lower lignin content than those of 100% SD, 20% CC and 20% SB hence resulted in higher values of TPC. The presence of lignin in substrates reduces the number of some biological active molecules which is directly linked to a decreased biological activity (Stefan *et al.*, 2015). Biological activity of mushrooms also depended on how easily the substrate is decomposed by the mycelia and the quality of nutrients assimilated by the mushrooms. Our results are similar to the findings of Paz *et al.* (2012), Oyetayo and Ariyo (2013), and Stefan *et al*. (2015).

TPC of PO and PC extracts in the present study not only depended on substrate formulas and mushroom species, but also on the drying methods. In comparison to oven-drying method, freeze-drying method of PO yielded the higher TPC (except for PO grown in substrates 100% SD, 50% CC, and 50% SB) (Table 1). PC grown in substrate 100% CC and 50% SB also achieved significantly higher values of TPC in freeze-drying (Table 1). PC grown in other substrate formulas had no significant difference between the two drying methods (Table 1). In previous research, freeze dried Shiitake showed the highest values of TPC and antioxidant capacity when compared with microwave-, oven-, and sun-drying methods (Zhang *et al.*, 2009). In other research, when the temperature was increased from 60 to 80°C, the TPC of PO decreased from 11.8 to 5.7 mg GAE/g extract due to initial enzymatic degradation of antioxidant compounds where the slow heat transfer in oven-drying resulted inefficient denaturation of the enzyme involved (Bhattacharya *et al*., 2014). Besides, at 50°C, mushrooms also risk losing protein and taste (Celen *et al.*, 2010). In the present study, the temperature for oven-drying method (40°C) was not too high hence not much change in TPC when compared to freeze-drying method. On the contrary, oven heating at 50°C has been shown to rapidly inactivate polyphenol oxidase present in plants and mushrooms. However, some of their initial activities may have occurred earlier and caused some polyphenols to be degraded which was the reason for the decrease in TPC in oven-drying with high temperature (Zhang *et a*l., 2009). With regard to freeze-drying, there was less effect on tissue structures since it had no thermal degradation. Furthermore, freeze-drying is known to have high extraction efficiency because ice crystals formed within the sample matrix can rupture the cell structure which allows exit of cellular components and access of solvent and consequently better extraction (Asami *et al.*, 2003).

*Total flavonoid contents (TFC)*

Flavonoids are important for human health due to their high pharmacological activities as radical scavengers. Flavonoids can be classified as anthocyanidins, flavanols, flavones, flavanones and flavonols, which occur naturally in foods of plant origin, and possess a number of beneficial effects on human health, such as antioxidant, anti-inflammatory, antiviral, and anticarcinogenic activities (Yao *et al.*, 2004). In this study, PO and PC yielded high TFC when grown in all substrate formulas (Table 1). The TFC ranged from 5.25 ± 0.15 to 7.60 ± 0.19 mg QE/g extract (PO) and 6.45 ± 0.24 to 8.12 ± 0.06 mg QE/g extract (PC). In general, when using CC and SB at low rate (20%) to replace SD in the substrate formulas, the TFC showed no significant difference. However, when CC and SB were increased (50% and 100%) to replace SD in substrate formulas, the TFC significantly increased. In our previous study, substrates CC and SB showed higher yield and nutritional composition in PO and PC when compared with those grown on substrate SD (Ha *et al*., 2015). In the present study, substrate CC (100%) not only gave the highest values of TPC but also gave the highest values of TFC in PO under oven-drying and freeze drying methods (6.80 ± 0.08 and 7.60 ± 0.19 mg QE/g extract, respectively). Substrate CC (100%) also gave the highest TFC values in PC under oven-drying and freeze-drying methods (7.84 ± 0.02 and 8.12 ± 0.06 mg QE/g extract, respectively). These results suggest that CC can be used for PO and PC cultivation with high yield, high TPC, and high TFC. The TFC values in the present study highly depended on the mushroom species, and the substrate formulas used, but not on the drying methods (except for PO grown in substrates 100% CC and 100% SB, and PC grown in 100% CC). The reason might be due to the oven-drying method at medium temperature (40°C). The changes in TFC which were influenced by substrates can also be explained by the different quantity of lignin much in the same way it influenced the TPC. Stefan *et al.* (2015) stated that the maximum usage of beech shavings (high lignin content) in substrate of PO yielded 19.45 μg QE/mL extract, whereas, the minimum usage of beech shavings in substrate yielded 64.50 μg QE/mL extract.

Table 2. The inhibition concentration at 50% (IC¬50) values of DPPH radical and reducing power from PO and PC cultivated with seven substrate formulas and dried by different methods.

| Substrate formula | $IC_{50}$ value of DPPH radical (mg/mL) | | $IC_{50}$ value of reducing power (mg/mL) | |
|---|---|---|---|---|
| | Oven-drying | Freeze-drying | Oven-drying | Freeze-drying |
| **PO** | | | | |
| 100% SD | 5.87 $^{aA}$ | 4.76 $^{aB}$ | 4.74 $^{aA}$ | 4.30 $^{aA}$ |
| 100% SB | 4.78 $^{dA}$ | 4.12 $^{dB}$ | 4.00 $^{dA}$ | 3.40 $^{bB}$ |
| 50% SD + 50% SB | 4.84 $^{dA}$ | 4.39 $^{cA}$ | 4.03 $^{dA}$ | 3.57 $^{bA}$ |
| 80% SD + 20% SB | 5.92 $^{aA}$ | 4.64 $^{abA}$ | 4.41 $^{bA}$ | 4.39 $^{aA}$ |
| 100% CC | 4.44 $^{eA}$ | 3.87 $^{eB}$ | 3.55 $^{eA}$ | 2.95 $^{cB}$ |
| 50% SD + 50% CC | 5.06 $^{cA}$ | 4.28 $^{cB}$ | 4.15 $^{cA}$ | 4.15 $^{aA}$ |
| 80% SD + 20% CC | 5.34 $^{bA}$ | 4.51 $^{bB}$ | 4.42 $^{bA}$ | 4.21 $^{aA}$ |
| **PC** | | | | |
| 100% SD | 5.67 $^{aA}$ | 5.58 $^{aA}$ | 3.34 $^{aA}$ | 3.18 $^{aA}$ |
| 100% SB | 4.21 $^{dA}$ | 3.40 $^{eB}$ | 2.81 $^{cA}$ | 2.15 $^{cB}$ |
| 50% SD + 50% SB | 4.90 $^{bA}$ | 3.74 $^{dB}$ | 2.99 $^{bcA}$ | 2.94 $^{bA}$ |
| 80% SD + 20% SB | 4.91 $^{bA}$ | 4.73 $^{bA}$ | 3.21 $^{abA}$ | 2.94 $^{bA}$ |
| 100% CC | 4.02 $^{eA}$ | 3.07 $^{fB}$ | 2.40 $^{dA}$ | 2.10 $^{cA}$ |
| 50% SD + 50% CC | 4.03 $^{eA}$ | 3.77 $^{dB}$ | 2.93 $^{bcA}$ | 2.19 $^{cB}$ |
| 80% SD + 20% CC | 4.30 $^{cA}$ | 4.32 $^{cA}$ | 3.10 $^{abcA}$ | 2.95 $^{bA}$ |

Means within the same capital letters followed by the same row of each item are not significantly different ($p \leq 0.05$). Means within the same column of each oyster mushroom followed by the same small letters are not significantly different ($p \leq 0.05$). $IC_{50}$ values, the inhibition concentration at which the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals were scavenged by 50%, the absorbance was 0.5 for reducing power; $IC_{50}$ values were obtained by interpolation from linear regression analysis. PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; SD, sawdust; SB, sugarcane bagasse; CC, corncob.

*Scavenging ability on DPPH radicals*

DPPH is a stable free radical used for determining the electron-donating capacity (Brand-Williams *et al*., 1995). The scavenging ability on DPPH radicals by PO and PC grown in different substrates and with different drying method are showed in Figure 1 and Table 2. Based on the results, PO and PC extracts showed positive antioxidant activity. Different concentrations of each sample from different substrate had different scavenging ability. The scavenging activities of radical were directly proportional to the concentration of the sample extracts. At 10 mg/mL concentration, PO and PC showed the highest values of scavenging ability on DPPH free radical with the range of 80.42-91.43% (PO-oven-drying), 88.79-93% (PO-freeze-drying), 83.78-91.02% (PC-oven-drying), and 86.16-92.44% (PC-freeze-drying). In terms of $IC_{50}$ (concentration of extract able to inhibit 50% of radical solution of PO), $IC_{50}$ ¬values varied from 4.44 - 5.92 mg/mL (oven-drying) and 3.87 - 4.76 mg/mL (freeze-drying). The $IC_{50}$ values of PC ranged from 4.02 - 5.67 mg/mL (oven-drying) and3.07 - 5.58 mg/mL (freeze-drying). Fruiting body extracts of both PO and PC grown in substrate 100% CC showed the highest values of DPPH free radical scavenging ability with the lowest values of $IC_{50}$ (Table 1). This fact might be due to PO and PC having the highest values of TPC as well as TFC which contributed to relatively strong antioxidant activity. The lowest value of DPPH radical scavenging ability by PC was recorded at substrate 100% SD. On the contrary, substrates containing 100% SD and 20% SB showed the lowest DPPH radical scavenging ability by PO extracts. Freeze-dried PO and PC grown in different substrates showed significantly higher DPPH radical scavenging activity than oven-dried extracts except PO grown in substrates 50% SB and 20% SB, and PC grown in substrates 100% SD, 20% CC and 20% SB. Bhattacharya *et al*. (2014) reported that the IC50 of DPPH radical gradually increased and the antioxidant activity decreased with rise in temperature from 60°C to 80°C during drying process of PO fruiting body. PO and PC in our study had high efficiency of scavenging ability on DPPH free radical because they were very rich in TPC (Table 1). The results were similar to previous research which reported that the methanolic extract of PO at 6.4 mg/ml scavenged DPPH radical by 81.8%, whereas scavenging effects of extracts from other commercial mushrooms were 42.9 to 69.9% (Mau *et al*., 2002). The ethanolic extracts of P. citrinopileatus mycelium and fruiting bodies showed high scavenging of DPPH (92.8% at

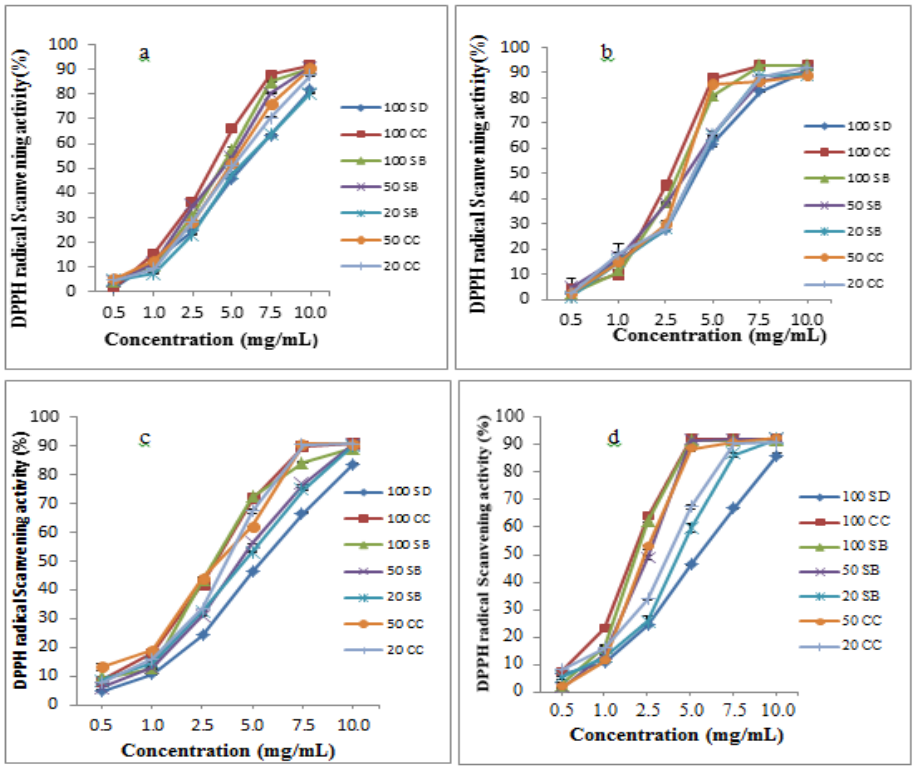


Figure 1. DPPH radical scavenging activity of methanolic extracts from PO-oven-drying (a), PO-freeze-drying (b), PC-oven-drying (c), PC-freeze-drying (d), Each value is expressed mean ± standard error (n=3). PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; SD, sawdust; SB, sugarcane bagasse; CC, corncob; DPPH, 1,1- diphenyl-2-picrylhydrazyl.

20 mg/mL, and 94% at 5 mg/mL) (Lee *et al*., 2007). In other studies, the $IC_{50}$ of water extracts of *P. sajor caju* fruiting bodies were in the range 9.0 - 10.4 mg/mL (Finimundy *et al*., 2013), while the $IC_{50}$ of ethanolic extracts of PO and *P. ferulae* fruiting bodies were less than 14 mg/mL.

*Reducing power*

Reducing power of a compound may serve as a significant indication of its potential activity (Oyaizu, 1986). The presence of reducers (i.e. antioxidants) causes the reduction of $Fe^{3+}$/Ferricyanide complex to ferrous form. The yellow color of the test solution changes to various shades of green and blue, which depends on the reducing power of each compound. The reducing power of oyster mushroom extracts was due to their hydrogen-donating ability that stabilizes the corresponding molecules by accepting hydrogen ions from the extracts and terminating the radical chains (Shahidi and Wanasundara, 1992). Thus, the reducing capacity of a compound will in turn serve as a significant indicator of its antioxidant potential as reported by Meir *et al.* (1995). The reducing power of PO and PC fruiting body extracts cultivated with different substrate formulas and dried by oven-drying method and freeze-drying method was shown in Figure 2 and Table 2. The reducing power of PO and PC extracts increased with the concentrations. At 10 mg/mL concentration, the reducing power of PO extracts ranged from 0.89 ± 0.033 to 1.20 ± 0.037(oven-drying), and 0.92 ± 0.012 to 1.22 ± 0.016 (freeze-drying) while PC extracts ranged from 1.00 ± 0.046 to 1.28 ± 0.014 (oven-drying), and 1.08 ± 0.101 to 1.45 ± 0.027 (freeze-drying). The significantly highest reducing power inhibition was identified in extract of PO grown in the substrate 100% CC with the $IC_{50}$ values (concentration at 0.5 of absorbance) of 3.55 mg/mL (oven-drying), and 2.95 mg/mL (freeze-drying). In case of PC (oven-dried), substrate 100% CC also indicated the most effective with the lowest value of $IC_{50}$ (2.40 mg/mL). On the contrary, in freeze-drying method, PC fruiting body extract grown in substrate 100% CC showed the lowest value of $IC_{50}$ (2.10 mg/mL) and was not significantly different with $IC_{50}$ values of fruiting body extracts grown in substrates 100% SB (2.15 mg/mL), and 50% CC (2.19 mg/mL). In general, reducing power of freeze-dried mushroom extracts did not showed significant difference with oven-dried mushroom extracts, except the extract of PC grown in substrates 100% SB, and 50% CC and extract of PO grown in substrates 100% CC and

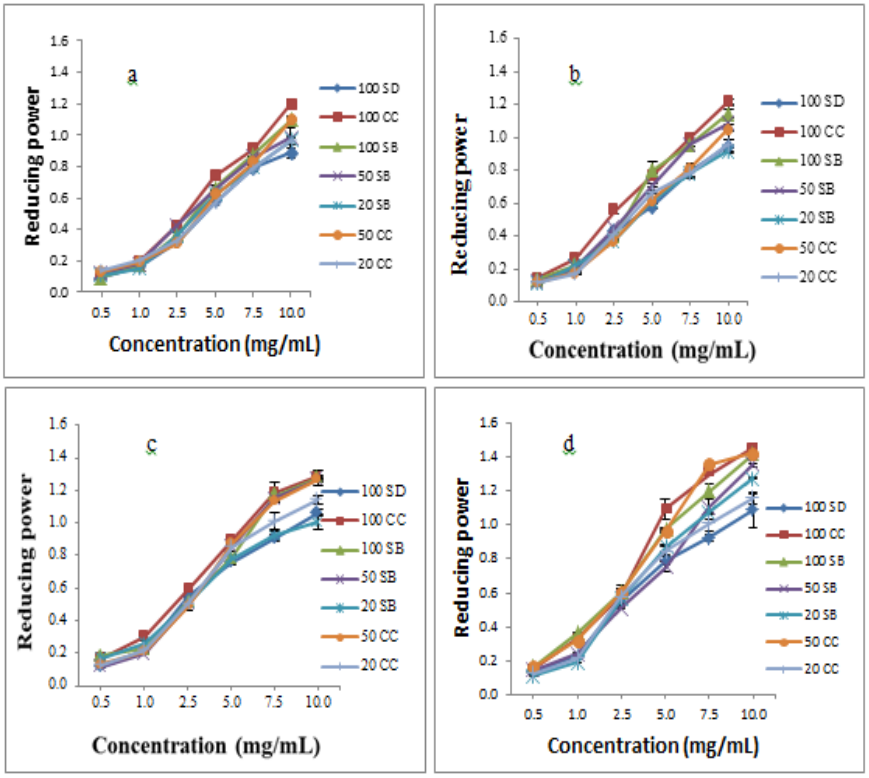


Figure 2. Reducing power of methanolic extracts from PO-oven-drying (a), PO-freeze-drying (b), PC-oven-drying (c), PC-freeze-drying (d), Each value is expressed mean ± standard error (n=3). PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; SD, sawdust; SB, sugarcane bagasse; CC, corncob

100% SB. According to previous researchers, the reducing power of mushroom extracts depended on some factors such as mushroom species/strain (Mau *et al*., 2002), extraction methods and solvent types (Vamanu, 2013). In our study, the substrates used for mushroom cultivation have also been found to significantly affect to reducing power on ferric ions of PO and PC due to its effect on the TPC and TFC of the fruiting body extracts which play important role in antioxidant activities.

According to Shimada *et al.* (1992), the reducing power might be due to their hydrogen-donating ability, and certain mushrooms contain higher amount of reductone, which could react with free radicals to stabilize and terminate radical chain reactions. Huang (2000) stated that the methanolic extract from *A. camphorata* (Chang-chih) showed a strong reducing power of 0.96-0.97 at 10 mg/mL concentration, whereas Brazilian mushrooms showed a reducing power of 0.86 at 10 mg/mL. Methanolic extracts from other mushrooms as *G. lucidum* (antler Ling-chih), *G. tsugae* (Sung-shan-ling-chih) showed an excellent reducing power of 1.25 and 1.26 at 2 mg/mL, respectively (Lin, 1999). Whereas, Chao (2001) reported that methanolic extract from *T. fuciformis* (silver ears) was 0.32 at 5 mg/mL. Methanolic extracts of PC and PO in earlier work exhibited excellent reducing powers of 1.00 and 1.19 at 10 mg/mL (Mau *et al*., 2002). Jose and Janardhanan (2000) and Jayakumar *et al*. (2007) also reported that fruiting body extracts of PO and *P. florida* showed excellent reducing power on ferric ions, and potent scavenging of hydroxyl radicals.

*Chelating effect on ferrous ions*

Chelating effect of extracts from PO and PC fruiting bodies grown in different substrates on ferrous ions increased with concentration from 0.5 mg/mL to 10 mg/mL (Figure 3). Chelating effects of oyster mushroom extracts were more than 80% at 5 mg/mL for both oven-and freeze-drying. At 10 mg/mL, the chelating abilities of PO for both oven and freeze-drying achieved the highest values which ranged from 95.13 to 97.76% and 96.41 to 99.78%, respectively. PC also yielded the highest values at 10 mg/mL with the range of 90.43 to 98.58% (oven-drying) and 91.10 to 99.85% (freeze-drying). PC grown in substrate 50% CC had the highest value in chelating ability on ferrous ions, and no significant difference with those grown in substrates 100% CC, 20% CC, 100% SB, and 50% SB (freeze-dried) at 5, 7.5, and 10 mg/mL concentrations. The highest values of chelating ability of methanolic extracts of PC (oven-dried) were also achieved at substrate 50%

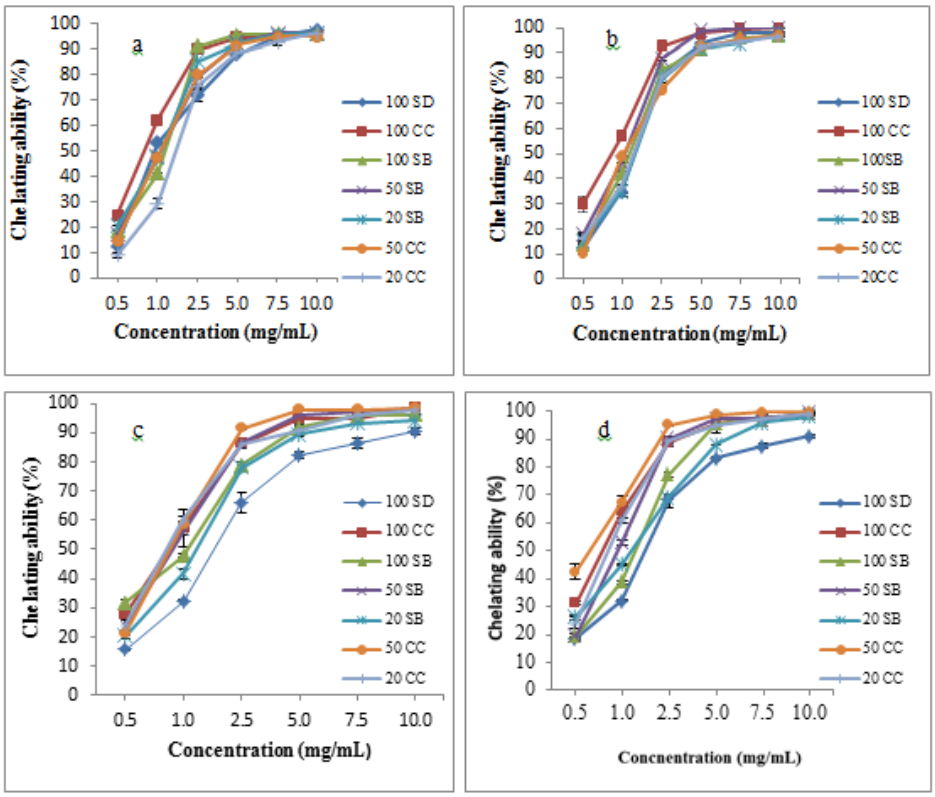


Figure 3. The chelating ability on ferrous ions of methanol extracts from PO-oven-drying (a), PO-freeze-drying (b), PC-oven-drying (c), PC-freeze-drying (d), Each value is expressed mean ± standard error (n=3). PO, *Pleurotus ostreatus*; PC, *Pleurotus cytidiosus*; SD, sawdust; SB, sugarcane bagasse; CC, corncob.

CC. However, there were no significant differences with those grown in substrates 100% CC, 50% SB, and 20% CC at 1, 2.5, 7.5, and 10 mg/mL concentration. The lowest effective chelating ability on ferrous ions was recorded at substrate 100% SD in both oven- and freeze-drying (Figure 3).

In PO, the methanolic extracts of fruiting bodies grown in substrate 100% CC had the most effective chelating ability on ferrous ions in both oven- and freeze-drying (Figure 3). Methanolic extracts of fruiting body grown in other substrates were not significantly different in ferrous ion chelating activity. In comparison between oven- and freeze-drying methods, there was no significant difference in chelating ability between PO and PC. The chelating ability of PO and PC in our study was lower than that indicated by Alam *et al*. (2010) (75-85% at 1 mg/mL concentration) but much higher than that of *G. tsugae*, and *A. cylindracea* hot water extract (39.5- 42.6% and 45.8%, respectively) at 20 mg/mL (Mau *et al*., 2005; Tsai *et al*., 2006), and also ethanolic extract of *P. eryngii* and *P. florida* which showed 22.6% and 93.3%, respectively at 50 mg/mL (Wong *et al.*, 2013). In our study, the high ferrous ion chelating ability of methanolic extracts of PO and PC fruiting bodies was beneficial. This was due to ferrous ions being the most effective pro-oxidations in food systems. Stefan *et al.* (2015) stated that the PO extracts obtained from cultivation on *V. pannonica* straws and on the substrate with a majority of beech shavings had a similar activity of approximately 79%. Besides, proteins and polysaccharides have also been implicated in metal-chelating abilities (Rainha *et al*., 2011). Previously, metal chelating power of phenolics was indicated by the chemical structures and the availability of properly oriented function groups, with six-membered ring complexes exhibited stronger chelating potential than five-membered ring structures (Thompson *et al*., 1976).

## Conclusion

The results showed that substrates containing higher contents of CC and SB (100% CC, 50% CC, 100% SB, and 50% SB) had low lignin which resulted in higher values of TPC and TFC, as well as high efficiency of DPPH radical scavenging ability, reducing power, and chelating ability. Whereas, substrate 100% SD yielded lower TPC and TFC which resulted in a decreased antioxidant activity of PO and PC extracts. These results suggested that CC and SB can be used to replace some parts or all SD in substrate formulas for oyster mushroom cultivation which will also improve antioxidant component and

activities of mushroom extracts. With freeze-drying method, PO and PC extracts showed improvement in the TPC as well as antioxidant activities in comparison with oven drying method.

## References


Alam, N., Yoon, K. N., Lee K. R., Shin P. G., Cheong J. C., Yoo Y. B., Shim, J. M., Lee, M. W., Lee, U. Y. and Lee, T. S. 2010. Antioxidant activities and tyrosinase inhibitory effects of different extracts from Pleurotus ostreatus fruiting bodies. Mycobiology 38(4): 295-301.

Asami, D.K., Hong Y. J., Barrett, D.M. and Mitchell, A. E. 2003. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultrual practices. Journal of Agricultural and Food Chemistry 51: 1237-1241.

Baillie, J. K., Thompson A. A. R., Irving, J. B., Bates, M. G. D., Sutherland, A. I., Macnee, W., Maxwell, S. R. and Webb, D. J. 2009. Oral antioxidant supplimentation does no prevent acute mountain sickness: double blind, randomized placebo-controlled trial. International Journal of Medicine 102(3): 342-348.

Bhattacharya, M., Srivastav, P. P. and Mishra, H. N. 2014. Optimization of microwave-convective drying of oyster mushrooms *(Pleurotus ostreatus)* using response- surface methodology. International Food Research Journal 21(1): 1575-1581.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT- Food Science and Technology 28(1), 25-30.

Celen, S., Kahveci, K. Akyol, U. and Haksever, A. 2010. Drying behaviour of cultured mushrooms. Journal of Food Processing Preservation 34(1): 27-42.

Chao, G. R. 2001. Antioxidant properties and polisaccharide compositton analysis of ear mushrooms. Taichung, Taiwan: National Chung-Hsing University, MSc thesis.

Chirinang, P. and Intarapichet, K. O. 2009. Amino acids and antioxidant properties of the oyster mushrooms, *Pleurotus ostreatus* and *Pleurotus sajor-caju*. Science Asia 35: 326-331.

Dinis, T. C. P., Madeira V. M. C. and Almeida, L. M. 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-Aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of Biochemistry and Biophysics 315(1): 161-169.

Eva, S., Rohaľová L. and Hedvigy, M. 2013. Semi-solid fermentation of *Pleurotus ostreatus*. Journal of Microbiology, Biotechnology and Food Sciences 2(1): 1950-1958.

Finimundy, T. C., Gambato, G., Fontana R., Camassola, M., Salvador, M., Moura, S., Hess, J., Henriques, J. A. P., Dillon, A. J. P. and Roesch-Ely, M. 2013. Aqueous extracts of *Lentinula edodes* and *Plerotus sajor-caju* exhibit high antioxidant capacity and promising in vitro antitumor activity. Nutrition Research 33(1): 76-84.

Hoa, H. T., Wang, C. L. and Wang, C. H. 2015. The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). Mycobiology 43(4): 423-434.

Huang, L. C. 2000. Antioxidant properties and polysaccharide compositton analysis of *Antrodia camphorata* and *Agaricus blazei*. Taichung, Taiwan: National Chung-Hsing University, MSc thesis.

Jayakumar, T., Thomas, P. A. and Geraldine P. 2009. In vitro antioxidant activities of an ethanolic extract of the oyster mushroom, *Pleurotus ostreatus.* Innovative Food Science and Emerging Technologies 10(2): 228-234.

Jayakumar, T., Thomas, P. A. and Geraldine, P. 2007. Protective effect of an extract of the oyster mushroom, *Pleurotus ostreatus*, on antioxidants of major organs of aged rats. Experimental Gerontology 42(3): 183-191.

Jia, Z., Tang, M. and Wu J. 1999. The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64: 555-559.

Jose, N. and Janardhanan, K. K. 2000. Antioxidant and antitumor activity of *Pleurotus florida*. Current Science 79(7): 941-943.

Kim, J. H., Kim, S. J., Park, H. R., Choi, J. I., Ju, Y. C., Nam, K. C., Kim, S. J. and Lee, S. C. 2009. The different antioxidant and anticancer activities depending on the color of oyster mushrooms. Journal of Medicinal Plants Research 3(12): 1016-1020.

Lee, Y. L., Huang, G. W., Liang, Z. C. and Mau, J. L. 2007. Antioxidant properties of three extracts from *Pleurotus citrinopileatus*. Lebensmittel Wissenschaft and Technologie 40: 823–833.

Li, L., Ng, T. B., Song, M., Yuan, F., Liu, Z. K., Wang, C. L., Jiang, Y., Fu, M. and Liu, F. 2007. A polysaccharide-peptide complex from abalone mushroom *(Pleurotus abalonus)* fruiting bodies increases activities and gene expression of antioxidant enzymes and reduces lipid peroxidation in senescence-accelerated mice. Applied Microbiology and Biotechnology 75(4): 863-869.

Lin, H. C. 1999. Evaluation of taste quality and antioxidant properties of edible and medicinal mushrooms. Taichung, Taiwan: National Chung-Hsing University, MSc thesis.

Mane, V.P., Patil, S.S., Syed, A.A. and Baig, M. M. V. 2007. Bioconversion of low quality lignocellulosic agricultural waste into edible protein by Pleurotus sajor-caju (Fr.) Singer. Journal of Zhejiang Univesity of Science 8: 745-751.

Mau, J. L., Lin, H. C. and Song, S. F. 2002. Antioxidant properties of several specialty mushrooms. Food Research International 35: 519-526.

Mau, J. L., TSai, S. Y., Tseng, Y. H. and Huang, S. J. 2005. Antioxidant properties of hot water extracts from *Gonoderma tsugae* Murrill. LWT- Food Science and

Technology 38: 589-597.

Meir, S., Kanner, J., Akiri, B. and Philosoph-Hadas, S. 1995. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. Journal of Agricultural and Food Chemistry 43: 1813-1819.

Neelam, S. and Singh, S. 2013. Comparative studies on antioxidant capacity of ethanol extracts of *Pleurotus florida* and *Pleurotus ostreatus*. Annals of Biological Research 4(4): 77-82.

Oyaizu, M. 1986. Studies on products of browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. The Japanese Journal of Nutrition and Dietetics 44(6): 307-315.

Oyetayo, V. O. and Ariyo, O. O. 2013. Antimicrobial and antioxidant properties of *Pleurotus ostreastus* (Jacq: Fries) cultivation on different tropical woody substrates. Journal of Waster conversion, Bioproducts and Biotechnology 1(2): 28-32.

Parihar, S., Virani, K. D., Pithawala, E. A., Shukla, M. D., Lahiri, S. K., Jain, N.K. and Modi, H. A. 2015. Phytochemical screening, total phenolic content, antibacterial and antioxidant activity of will edible mushroom Pleurotus ostreatus. International Research Journal of Pharmacy 6(1) 65-69.

Paz, M. F., Breywe, C. A., Longhi, R. F. and Oviedo, M. S. 2012. Determining the basic composition and total phenolic compounds of *Pleurotus sajor-caju* cultivated in three different substrates by solid state bioprocess. Journal of Biotechnology and Biodiversity 3(2): 11-14.

Purkayastha, R. P. and Nayak, D. 1981. Analysis of protein patterns of an edible mushroom by gel electrophoresis and its amino acid composition. Journal of Food Science and Technology 18: 89-91.

Ragauskas, A. 2006. Short review: The chemistry and pulping of Acacia IPST - GATECH - Georgia Institute of Technology: P12.

Rainha, N., Lima, E., Baptista, J. P. and Rodrigues, C. 2011. Antioxidant properties, total phenolic, total carotenoid and chlorophyll content of annatomical parts of Hyperium foliosum. Journal of Medicine Plants Research 5(10): 1930-1940.

Sain, M and Omar, F. 2015. Lignin in polymer composites. 1st ed. New York: Elsevier Science Publishing Co Inc.

Sánchez, C. 2010. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. Applied Microbiology and Biotechnology 85(5): 1321-1337.

Sato, M., Ramarathnam, N., Suzuki, Y., Ohkubo, T., Takeuchi, M. and Ochi, H. 1996. Varietial differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. Journal of Agricultural and Food Chemistry 44(1): 37-41.

Shahidi, F. and Wanasundara, P. K. 1992. Phenolic antioxidants. Critical. Reviews in Food Science and Nutrition. 32(1) 67- 103.

Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry 40(6): 945-948.

Stefan, R. I., Vamanu, E. and Angelescu, C. 2015. Antioxidant Activity of crude methanolic extracts from Pleurotus ostreatus. Research Journal of Phytochemistry 9(1): 25-32.

Thompson, M., Williams, C. R. and Elliot, G. E. 1976. Stability of flavonoid complexes of copper (II) and flavonoid antioxidant activity. Analytica chimica acta 85(2): 375-381.

Tsai, S. Y., Huang, S. J., Lo, S. H., Wu, T.P., Lian, P.Y. and Mau, J. L. 2009. Flavour componants and antioxidant properties of several cultivated mushrooms. Food Chemistry 113(2): 578-584.

Tsai, S. Y., Huang, S. J. and Mau, J. L. 2006. Antioxidant properties of hot water extracts from Agrocybe cylindracea. Food Chemistry 98(4): 670-677.

Vamanu, E. 2013. Antioxidant properties and chemical compositions of various extracts of the edible commercial mushroom, *Pleurotus ostreatus*, in Romanian Markets. Revista De Chimie 64(1): 49-54.

Wong, F. C., Chai, T. T., Tan, S. L. and Yong A. L. 2013. Evaluation of bioactivities and phenolic content of selected edible mushrooms in Malaysia. Tropical Journal of Pharm Research 12(6): 1011-1016.

Yang, J. H., Lin, H. C. and Mau, J. L. 2002. Antioxidant properties of several commercial mushrooms. Food Chemistry 77(2) 229-235.

Yao, L. H., Jiang, Y. M., Shi, J., Tomas-baeberan, F. A., Datta N. and Singanusong, R. 2004. Flavonoids in food and their health benefits. Plant Foods for Human Nutrition 59(3): 113-122.

Zhang, Z., Guoying, L. V., Huiyuan, P. A., Yong Zhi, W.U. and Leifa F. A. 2009. Effects of different drying methods and extraction condition on antioxidant properties of Shiitake *(Lentinus edodes)*. Food Science and Technology Research 15(5): 547-552.