



Nutrient contents and antioxidant properties of *Pleurotus* spp. cultivated on substrate fortified with Selenium

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Abstract

This study investigated the proximate, mineral, amino acid and antioxidant properties of *Pleurotus* spp. fortified with selenium. The proximate analysis showed that *Pleurotus* mushrooms harvested from substrate fortified with selenium are rich in protein with values of 16.35 % and 18.21 for *P. pulmonarius* and *P. ostreatus*, respectively. Low fat content of 0.39 to 0.40 % was obtained in all cultivated mushrooms. Calcium have the highest value of 20.94 mg/100g in fortified *Pleurotus ostreatus*. Essential and non-essential amino acids were present in cultivated *Pleurotus* spp. with the highest values of glutamic acid ranged from 10.61 to 12.27 mg/100 g. Ethanolic extract from selenium fortified *Pleurotus* spp. exhibited higher and better antioxidant effect (91.58 %) when compared ($p < 0.05$) to the non-selenium fortified *Pleurotus* spp. (75.5 %). Selenium-biofortification of *Pleurotus* spp. resulted in a considerable increase of its antioxidant properties. Therefore, selenium enriched *Pleurotus* species could serve as a rich source of natural antioxidant food for the enhancement of the body against oxidative damage.

Key words – Edible mushroom – amino acids – free radicals – mineral-enrichment – nutraceuticals

Introduction

Mushrooms are able to synthesize a great amount of secondary metabolites that possess antioxidant activities (Ling et al. 2017). The genus *Pleurotus*, a well-known mushroom belongs to Basidiomycota, order Agaricales, and the family *Tricholomataceae* (Chang & Miles 2004). The genus *Pleurotus* is an important edible mushroom known for its high content of protein as well as low fat content (Krishnamoorthy & Sankaran 2014). Ling et al. (2017) showed that the mycelia of 10 *Pleurotus* species with 41 strains of different origin can assimilate selenium (Se) and can therefore present as an excellent dietary source of antioxidant agents. Selenium is classified as a metalloid and, is an essential trace element for animals and humans. Consequently, the importance of dietary selenium in human health has received considerable attention in the last several years (De Silva et al. 2012).

Dietary selenium has been recognized as an antioxidant, and the deficiency of this element has been associated with numerous chronic degenerative diseases, including multiple types of cancer, cardiomyopathy and endemic osteoarthropathy (Fernandes et al. 2015). This element has many physiological functions, but is most often recognized for its role as a cofactor for the enzyme glutathione peroxidase, which is responsible for the removal of free radicals that reduce oxidative

damage in cells (Facchini et al. 2014). In several regions of the world, the content of selenium in the general diet has been estimated to be insufficient to maintain the proper level of activity of protective seleno-enzymes (Mishra et al. 2013). The specific type of selenium that is ingested is an important factor for the determination of toxicity, nutritional importance and metabolic factors (Facchini et al. 2014).

Oxidative stress is a well-known phenomenon caused by an imbalance between formation and removal of reactive micro-molecules such as reactive oxygen species (ROS) (Bhatia et al. 2014). The term ROS may refer to both the oxygen centered radicals and non-radical derivatives of O₂ such as hydrogen peroxide, singlet oxygen, and hypochlorous acid (Huang et al. 2015). These reactive molecules may be produced in all mammalian cells in response to exogenous stimuli through normal cellular metabolism or activation of membrane-bound enzymes (Li & Shah 2015). Oxidative stress has the potential to damage cells and may eventually lead to cancer, cardiovascular diseases, diabetes, aging, and a vast number of other illnesses and disorders (Mishra et al. 2013). The human body's defense system responds to oxidative stress generally through scavenging or lessening ROS formation using endogenous and/or diet-derived molecules (Mitra et al. 2013). However, the antioxidant defense system needs to be assisted by consumption of antioxidant-containing supplements. The increasing carcinogenic risks associated with consuming synthetic antioxidants, has caused interest to replace synthetic antioxidants by natural sources of antioxidants, namely vegetables, herbs, fruits and mushrooms (Ren et al. 2015, Sharareh et al. 2016).

Pleurotus mushrooms are rich in vitamin and selenium content which are the important natural antioxidants in biological systems (Vamanu 2012). Free radical scavenging and Nitric Oxide Synthase (NOS) activation properties of water soluble crude polysaccharide from *P. ostreatus* showed superior antioxidant property, which might be due to presence of β -glucan (Saha et al. 2012). Oyster mushrooms are now widely used as ingredient in dietary supplements to maintain health and prevent diseases, due to their higher free radical scavenging activities (Yang et al. 2002). Keeping in view the health benefits of mushrooms and selenium, this study was conducted to determine the effects of selenium-fortification on the proximate, mineral, amino acid contents and antioxidant activities of *P. ostreatus* and *P. pulmonarius*.

Materials & Methods

Collection of corn cobs

The corn cobs used as substrates for this research work were procured at the King's market, Akure, Ondo State.

Source of *Pleurotus* spp. Spawn

Spawns of *Pleurotus ostreatus* and *Pleurotus pulmonarius* were obtained from Federal Institute of Industrial Research (FIRO), Oshodi Lagos, Nigeria.

Substrate Preparation

The substrates (corn cobs) were sundried and broken into smaller pieces using mortar and pestle. It was further pulverized into fine powdered using a mill machine (5657 HAAN 1 TYPE ZM1, Retsch GmbH, Haan, Germany). The substrate (corn cobs) was moistened with water to a concentration of 60%. One thousand grams (1000 g) of the substrate was packed into polypropylene bags and sealed with paper with the aid of polyvinyl rings. The bags were then autoclaved for 2 hours at 121 °C

Mushroom Preparation

The substrates were allowed to cool down and then inoculated with 30 g of *Pleurotus* spawn. Sodium selenite (50 mg/Kg) was injected into each substrate. A control treatment with no sodium selenite was also prepared. The bags were then kept in spawn room with 75% relative humidity

until formation of primordial. The bags were then uncapped and transferred to the fruiting room to allow the normal development of the fruitbodies.

Proximate and mineral analysis of cultivated *Pleurotus* spp.

The proximate analysis of the cultivated *Pleurotus* spp. were carried out according to the method of AOAC (2005). The harvested mushrooms were dried at 26 °C for 6 days before proximate analysis was carried out. The minerals of the mushroom were analyzed using the samples obtained by ashing at 550 °C and dissolving in HCl (25 mL) and 5 % lanthanum chloride (2 mL), boiled, filtered and make it up to standard volume with deionized water (AOAC 2005). Mn, Cu, Co, Zn, Fe, Mg, and Ca were determined with a Buck Atomic Absorption Spectrometer (Buck Scientific, Model 200A/200, Inc. East Norwalk, Connecticut, U.S.A).

Determination of amino acid content of cultivated *Pleurotus* spp.

The amino acid profile of *Pleurotus* spp. was determined using the method described by Benitez (1989). Four stages were involved in the determination of the amino acid content of *Pleurotus* spp. Five grams (5 g) of the samples were dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into Applied Biosystems PTH Amino Acid Analyzer.

Preparation of mushroom extracts from cultivated mushrooms

The mushroom samples were cut into small pieces and air-dried. The dried sample was ground into fine powder. Mushroom sample (250 g) was soaked in 1000 ml of ethanol and kept for three days with occasional shaking. Each portion was then filtered using Whatman filter paper No 1. The filtrates were concentrated and the extracts (residues) were stored at 4 °C in a sterile container for future use.

DPPH free radical scavenging activity of extracts from harvested *Pleurotus* spp.

The scavenging ability of extracts of the cultivated *Pleurotus* spp. on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals was determined according to the method of Tihwua et al. (2012). Each extract (0.01-0.14 mg/ml) was mixed with 1 ml of ethanolic extract containing DPPH radicals (0.4 mM) (A1). The mixture was shaken vigorously and left to stand for 30 min in the dark. The absorbance was measured at 515 nm. Absorbance of the controls solution containing only DPPH (A0) and the sample extract solution without DPPH (As) was also measured at 515 nm. The percentage of DPPH radical scavenging activity of each extract was determined within the range of dose response and was calculated as:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A0 - (A1 - As)}{A0} \times 100$$

Where

A0 = Absorbance of the control solution containing only DPPH

A1 = Absorbance in the presence of mushroom extract in DPPH solution

As = The absorbance of the sample extract solution without DPPH

Hydroxyl radical scavenging ability of extracts from harvested *Pleurotus* spp.

The determination of scavenging effect on hydroxyl radical was carried out as described in Oyetayo et al. (2009). The reaction mixture in a final volume of 1.0 ml, containing 0.4 ml of 20 mmol/ml sodium phosphate buffer (pH 7.4), 0.1 ml of 0.125–2 mg/ml extracts, 0.1 ml of 60 nmol/L deoxyribose, 0.1 ml of 10 mmol/L hydrogen peroxide, 0.1 ml of 1 mmol/L ferric chloride, 0.1 ml of 1.04 mmol/L EDTA and 0.1 ml of 2 mmol/L ascorbic acid was incubated at 37 °C for 1 hour. Solutions of FeCl₂ and ascorbic acid were made up immediately before use in de-ionised water. The reaction was stopped by adding 1 ml of 17 mmol/L thiobarbituric acid (TBA) and 1 ml of 17

mmol/L trichloroacetic acid (TCA). The mixture was boiled for 15 min, cooled in ice and then the absorbance measured at 532 nm using a UNICO 2100 spectrophotometer (As). BHT was used as positive control while distilled water in place of extracts or BHT was used as blank (Ab) and the sample solution without adding deoxyribose as sample blank (Asb).

$$\text{Scavenging activity (\%)} = \frac{Ab - (As - Asb)}{Ab} \times 100$$

Where Ab, As and Asb are the absorbance at 532 nm of the blank, the extract or BHT and the sample blank without deoxyribose, respectively.

Ferric Reducing Antioxidant Power (FRAP) of extracts from harvested *Pleurotus* spp.

Ferric reducing antioxidant power (FRAP) assay was done according to the method of Dong et al. (2014). The oxidant in the FRAP assay was prepared by mixing 2,4,6-tri(2-pyridyl)-s-triazine (10 mM in 40 mM HCl, 2.5 ml), acetate buffer (0.3 M pH 3.6, 25 ml), and 2.5 ml of FeCl₃·6H₂O (20 mM). The combination prepared by mixing all these reagents is known as FRAP reagent. An amount of 1.8 ml of freshly prepared FRAP reagent, 100 ml of ethanolic extract and 100 ml of distilled water was added to the test tube. The tubes were then incubated at 37 °C for 30 minutes and absorbance was taken at 595 nm in spectrophotometer (Perkin Elmer UV/VIS).

Statistical analysis

Data obtained were analyzed using analysis of variance (ANOVA). Means were separated using Duncan's New Multiple Range Test at 95% confidence level using Statistical Packages for the Social Sciences (SPSS) version 17.0. Differences between means were considered significant at P ≤ 0.05.

Results

The proximate composition of the cultivated *Pleurotus* spp. in percentage is presented in Table 1. Higher protein contents of 16.35% and 18.21 % were found in *Pleurotus pulmonarius* and *Pleurotus ostreatus* fortified with selenium. The carbohydrate content in cultivated *Pleurotus* spp. (non-fortified and fortified with selenium) are not significantly different (p < 0.05) from each other with their values ranging from 53.84 to 58.10 %. Low fat content of 0.39 to 0.40% was obtained in cultivated mushrooms. Table 2 shows the mineral composition (mg/100 g) of cultivated *Pleurotus* species. The results revealed that calcium (1.78 to 20.94 mg/100 g) was the highest occurring mineral in all mushroom samples, while nickel (0.01 to 0.03 mg/100 g) was the least occurring mineral and no lead content observed. The amino acid composition (mg/100 g) of the cultivated *Pleurotus* species were recorded in Table 3. Glutamic acid had the highest values ranging from 10.61 to 12.27. The presence of essential and non-essential amino acids was obtained in both fortified and non-fortified *Pleurotus* species. Fig. 1 shows the DPPH scavenging effects of extracts of the cultivated *Pleurotus* spp. Extracts from selenium fortified *Pleurotus ostreatus* (POS) exhibited the greatest DPPH scavenging effects (45.09-97.92 %) at concentrations of 50 µg/ml and 250 µg/ml, respectively, while extracts of non-selenium fortified *Pleurotus ostreatus* (PON) had the least scavenging effects on DPPH radicals (27.81-74.45 %) at concentrations of 50 µg/ml and 250 µg/ml respectively. Fig. 2 shows the hydroxyl ion scavenging effects of the cultivated *Pleurotus* spp. Selenium fortified *Pleurotus* spp. (POS and PPS) exhibited moderate and concentration dependent hydroxyl radical scavenging ability than non-selenium fortified *Pleurotus* spp. selenium fortified *Pleurotus ostreatus* (POS) and fortified *Pleurotus pulmonarius* (PPS) had hydroxyl ion scavenging ability of 91.58% at 250 µg/ml and 88.45% at 250 µg/ml, respectively. Non-selenium fortified *Pleurotus ostreatus* (PON) and non-selenium fortified *Pleurotus pulmonarius* (PPN) at a concentration of 250 µg/ml had hydroxyl ion scavenging effects of 75.50% selenium and 69.89% respectively. Fig. 3 shows that ferric reductive capacity of the extracts is

concentration dependent. Selenium enriched *Pleurotus* species exhibited a better ($p \leq 0.05$) reducing power than non-enriched *Pleurotus* species. The POS, PPS, PON and PPN exhibited highest reducing power at 1.68, 1.62, 1.52 and 1.48 respectively at 250 $\mu\text{g/ml}$ concentration.

Discussion

The protein contents of *Pleurotus* spp. were found to be higher in selenium enriched *Pleurotus* spp. Turlo et al. (2010) reported 213.9 mg/100g from selenium enriched *Lentinus edodes*. Similarly, Akyuz et al. (2010) reported relatively higher protein content of 17 to 42 g per 100 g dried fruitbodies of *P. ostreatus*. The researchers also stated that the content of protein in *P. ostreatus* vary according to strains, substrate-mineral enrichment, physical and chemical differences in growth medium, composition of the substrate, size of the pileus, and harvest time. Carbohydrate content in *Pleurotus* spp. constitutes the major component of mushroom dry matter, usually about 50-60% (Kalac 2012). Synytsya et al. (2010) reported the composition of these polysaccharides in mushroom fruit bodies vary with the strains, ranging from 37 to 48 g/100 g dry fruit bodies, which were lower than the result of this study.

Table 1 Proximate composition (%) of cultivated *Pleurotus* spp.

Parameters	PPN	PPS	POS	PON
Moisture	9.03 \pm 0.03 ^b	8.56 \pm 0.01 ^a	9.77 \pm 0.00 ^b	9.02 \pm 0.00 ^b
Ash	6.20 \pm 0.03 ^b	8.21 \pm 0.59 ^{bc}	5.12 \pm 0.00 ^a	5.93 \pm 0.03 ^a
Protein	10.31 \pm 0.00 ^a	16.35 \pm 0.01 ^b	18.21 \pm 0.00 ^c	10.69 \pm 0.00 ^a
Crude fibre	19.70 \pm 0.00 ^c	11.82 \pm 0.06 ^b	10.40 \pm 0.02 ^a	20.12 \pm 0.02 ^c
Fat	0.40 \pm 0.00 ^a	0.39 \pm 0.01 ^a	0.40 \pm 0.00 ^a	0.40 \pm 0.00 ^a
Carbohydrates	54.36 \pm 0.01 ^a	54.67 \pm 0.00 ^a	58.10 \pm 0.00 ^a	53.84 \pm 0.00 ^a

Values carrying the same alphabets in the same row are not significantly different at ($p < 0.05$). Values are means of triplicates \pm SD.

Keys: PPN – *Pleurotus pulmonarius* (non-fortified with selenium)

PPS – *Pleurotus pulmonarius* (fortified with selenium)

PON – *Pleurotus ostreatus* (non-fortified with selenium)

POS – *Pleurotus ostreatus* (fortified with selenium)

Table 2 Mineral composition* (mg/100-g) of cultivated *Pleurotus* spp.

Mineral	PPN	PPS	POS	PON
Zinc	0.07 \pm 0.01 ^a	0.25 \pm 0.03 ^b	0.29 \pm 0.00 ^c	0.42 \pm 0.00 ^d
Iron	0.03 \pm 0.00 ^a	1.97 \pm 0.03 ^c	0.73 \pm 0.02 ^b	2.15 \pm 0.04 ^d
Copper	0.09 \pm 0.07 ^a	0.13 \pm 0.00 ^a	0.13 \pm 0.10 ^a	0.21 \pm 0.01 ^a
Calcium	5.66 \pm 0.46 ^b	10.62 \pm 0.56 ^c	20.94 \pm 0.16 ^d	1.78 \pm 0.02 ^a
Magnesium	3.07 \pm 0.05 ^a	4.34 \pm 0.17 ^c	3.84 \pm 0.11 ^b	2.56 \pm 0.89 ^a
Nickel	0.00 \pm 0.00 ^a	0.03 \pm 0.01 ^c	0.01 \pm 0.01 ^b	0.00 \pm 0.00 ^a
Chromium	0.01 \pm 0.01 ^a	0.02 \pm 0.00 ^b	0.05 \pm 0.01 ^c	0.07 \pm 0.00 ^d
Cadmium	0.04 \pm 0.00 ^a	0.01 \pm 0.00 ^a	0.03 \pm 0.02 ^b	0.01 \pm 0.00 ^a
Manganese	0.01 \pm 0.00 ^a	0.17 \pm 0.00 ^b	0.18 \pm 0.00 ^c	0.31 \pm 0.00 ^d
Lead	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a

Values carrying the same alphabets in the same row are not significantly different at ($p < 0.05$). Values are means of triplicates \pm SD.

*selenium concentration of the cultivated *Pleurotus* spp. has been reported in our previous study (Fasoranti et al. 2018)

Keys: PPN – *Pleurotus pulmonarius* (non-fortified with selenium)

PPS – *Pleurotus pulmonarius* (fortified with selenium)

PON – *Pleurotus ostreatus* (non-fortified with selenium)

POS – *Pleurotus ostreatus* (fortified with selenium)

Table 3 Amino acid composition (mg/100g) of cultivated *Pleurotus* spp.

Amino acid	PPS	POS	PON	PPN
Cysteine	0.67±0.13 ^a	1.16±0.13 ^c	1.22±0.12 ^c	0.93±0.12 ^b
Alanine	3.09±0.12 ^a	3.43±0.13 ^b	4.15±0.13 ^c	3.16±0.13 ^a
Glutamic acid	10.61±0.13 ^a	11.79±0.13 ^b	12.27±0.14 ^c	11.18±0.13 ^b
Glycine	3.69±0.13 ^a	4.14±0.13 ^b	4.56±0.13 ^c	3.83±0.12 ^a
Serine	1.67±0.13 ^a	2.67±0.13 ^c	3.33±0.13 ^d	2.34±0.13 ^b
Aspartic acid	4.53±0.13 ^a	5.43±0.13 ^c	7.76±0.13 ^d	5.15±0.13 ^b
Proline	2.36±0.14 ^a	3.38±0.13 ^b	3.77±0.12 ^c	3.17±0.13 ^b
Arginine	4.63±0.13 ^a	5.13±0.13 ^b	5.63±0.13 ^c	4.95±0.14 ^b
Tyrosine	2.54±0.12 ^a	3.22±0.13 ^b	3.57±0.12 ^c	2.73±0.12 ^a
Isoleucine	2.43±0.13 ^a	3.66±0.13 ^c	4.44±0.13 ^d	3.30±0.14 ^b
Histidine	1.28±0.13 ^a	1.63±0.13 ^b	2.17±0.13 ^c	1.34±0.13 ^a
Leucine	3.13±0.13 ^a	3.43±0.13 ^b	5.68±0.08 ^c	3.53±0.13 ^b
Lysine	2.67±0.13 ^a	3.58±0.13 ^c	4.26±0.13 ^d	3.15±0.13 ^b
Tryptophan	1.31±0.13 ^a	1.23±0.13 ^a	1.39±0.13 ^a	1.33±0.13 ^a
Valine	2.91±0.13 ^a	3.64±0.13 ^b	4.13±0.13 ^c	3.34±0.13 ^b
Methione	0.69±0.13 ^a	1.04±0.13 ^b	1.46±0.13 ^c	0.85±0.13 ^a
Phenylalanine	3.31±0.13 ^a	3.49±0.13 ^b	4.13±0.13 ^c	3.68±0.13 ^b
Threonine	2.63±0.12 ^a	2.46±0.16 ^a	3.51±0.12 ^b	2.85±0.13 ^a

Values carrying the same alphabets in the same row are not significantly different at ($p>0.05$).

Values are means of triplicates \pm SD.

Key: PPN – *Pleurotuspulmonarius* (non-fortified with selenium)

PPS – *Pleurotuspulmonarius* (fortified with selenium)

PON – *Pleurotustreatatus* (non-fortified with selenium)

POS – *Pleurotustreatatus* (fortified with selenium)

The minerals found to be present in the cultivated *Pleurotus* spp. include zinc, iron, copper, calcium, magnesium, nickel, chromium, cadmium, and manganese. These minerals are chemical elements that are fundamental for the normal metabolic functioning of the body (Wenk & Bulakh 2004). The minerals play essential structural and metabolic roles. Calcium, magnesium and iron which are nutritionally important were found in reasonable amount, while manganese and copper were in lower amount. The preponderance of calcium in these mushrooms may be due to the absorption and accumulation of this element from their substrate. Minerals generally in the diet are required for metabolic reactions, transmission of nerve impulses, rigid bone formation and regulation of water and salt balance among others (Skinner 2005).

Glutamic acid was the most abundant non-essential amino acid in the cultivated mushrooms. Amino acid profiling revealed that the levels of amino acids in our study were higher as compared to some of the commonly consumed species, *Agaricus bisporus* and *Pleurotus sajor-caju* in the findings of Kayode et al. (2015). A similar kind of increment due to the selenium enrichment has also been reported by Hua et al. (2000). It has been reported that the inorganic selenium compounds get bio-transformed into the seleno-compounds (Vetchinkina et al. 2013). These compounds, in turn, get reduced to hydrogen selenides, which are the key intermediates for facilitating the biosynthesis of selenocysteine and other non-amino acid selenoproteins (Falandysz 2008). This notable difference in the amino acid profile in *Pleurotus* spp. fruitbodies obtained could be due to the activation of biosynthesis of the polysaccharides by the selenium bioaccumulation.

Pleurotus mushrooms are rich in biologically active compounds, which are the important natural antioxidants in biological systems (Chang & Miles 1989). In this study, the total antioxidant content of the ethanol extracts of Se-enriched mushrooms was significantly higher than the non-enriched *Pleurotus* species. Jayakumar et al. (2011) reported that an extract of *P. ostreatatus* exhibit *in vitro* antioxidant activity by virtue of its scavenging hydroxyl and superoxide radicals, inhibiting lipid peroxidation, reducing power on ferric ions, chelating ferrous ions and quenching 2,3-diazabicyclo[2,2,2]oct-2-ene (DBO). Similarly, Zhang et al. (2012) revealed that the fruiting bodies

of *P. ostreatus* exhibited stronger DPPH and superoxide anion radical scavenging activity with increased concentration. Free radical scavenging and Nitric Oxide Synthase (NOS) activation properties of water soluble crude polysaccharide from *P. ostreatus* showed superior antioxidant property, which might be due to presence of carbohydrate component; mostly β -glucan that seems to be responsible for the antioxidant activity (Mitra et al. 2013).

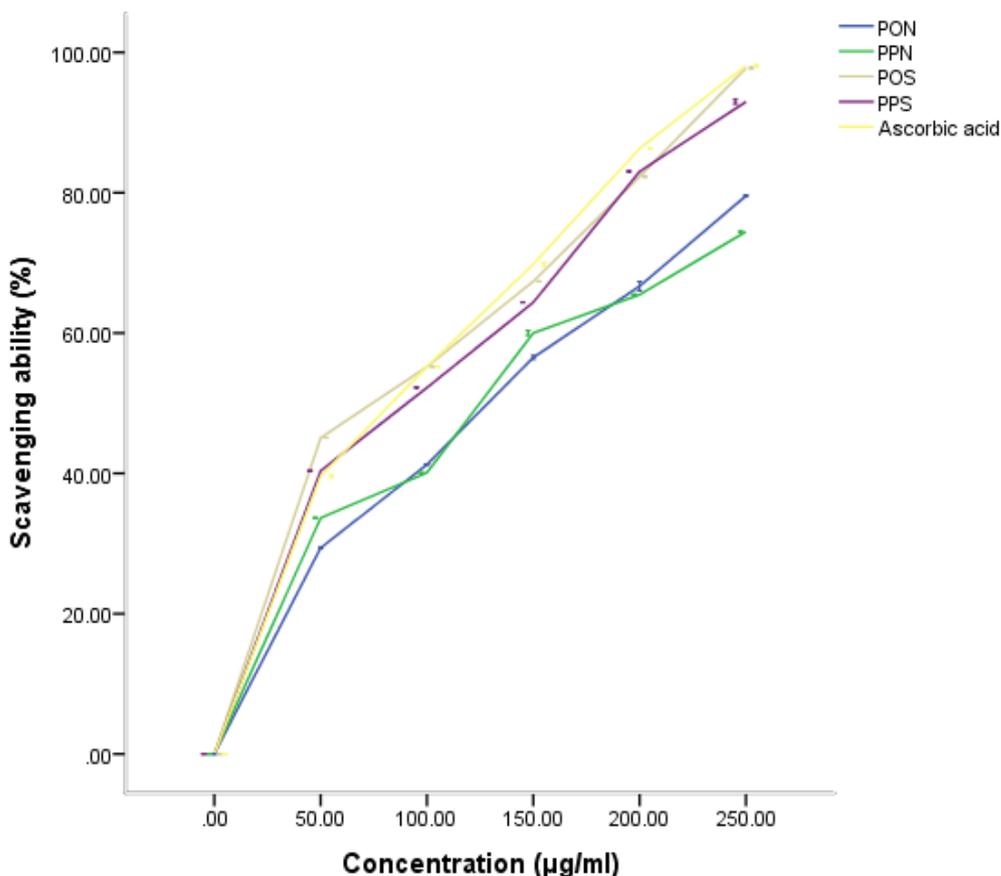


Fig. 1 – DPPH Scavenging ability of cultivated *Pleurotus* spp.

Keys: PPN – *Pleurotus pulmonarius* (non-fortified with selenium)

PPS – *Pleurotus pulmonarius* (fortified with selenium)

PON – *Pleurotus ostreatus* (non-fortified with selenium)

POS – *Pleurotus ostreatus* (fortified with selenium)

The results in the present study revealed that ethanolic extracts of Se-enriched *Pleurotus ostreatus* act as free radical scavengers, indicating the potential use of Se-enrich mushrooms as antioxidants in diet. Selenium enriched *Pleurotus* spp. exhibited moderate and concentration dependent hydroxyl radical scavenging ability than non-enriched *Pleurotus ostreatus*. The inhibitory ability of Se-*Pleurotus* species on hydroxyl radical was directly related to their concentrations. At the concentration of 250 µg/ml, the hydroxyl scavenging activity was 91.58% for POS and 88.48% for PPS respectively. The result of this study is similar to Wei et al. (2015) who reported that mushrooms enriched with selenium displayed stronger scavenging activity of superoxide radical. Similarly, it was reported that Selenium exopolysaccharide from *Lactococcus lactis* and Se-enriched Maitake polysaccharide displayed higher antioxidant activities than the non-selenium fortified polysaccharide (Guo et al. 2013, Mao et al. 2014). The mechanism of scavenging free radicals may be the presence of three-dimensional structure of polysaccharide that has been changed by the selenyl group (SeH) or seleno-acid ester and increasing hydroxyl groups, which affected the hydrogen atom-donating capacity and antioxidant ability (Wang et al. 2016). Otherwise, selenium itself has stronger antioxidant activity.

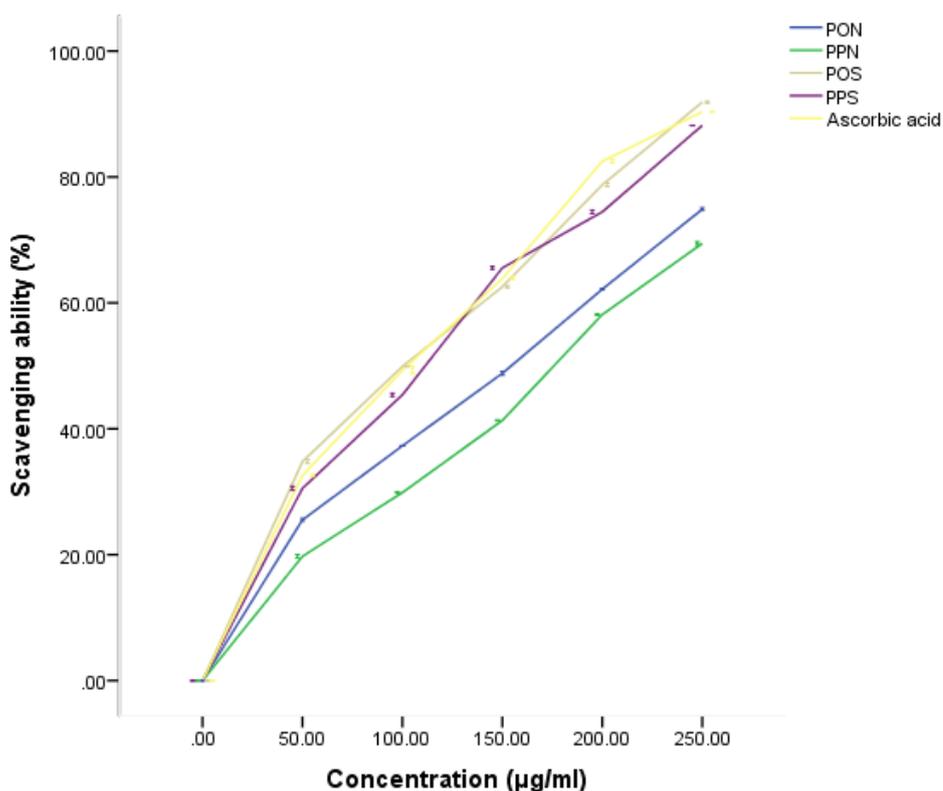


Fig. 2 – Hydroxyl radical scavenging ability of cultivated *Pleurotus* spp.

Keys: PPN – *Pleurotus pulmonarius* (non-fortified with selenium)

PPS – *Pleurotus pulmonarius* (fortified with selenium)

PON – *Pleurotus ostreatus* (non-fortified with selenium)

POS – *Pleurotus ostreatus* (fortified with selenium)

The reductive capacity of the extracts is concentration dependent. Selenium enriched *Pleurotus* spp. exhibited a better and significantly reducing power than non-enriched *Pleurotus* spp. Mushrooms are known to possess antioxidant and reducing ability due to the presence of phenols and terpenoids. A number of studies suggested that the addition of selenium triggers the synthesis of selenoergothioneine by replacing sulphur with the selenium compound from the antioxidative ergothioneine moiety (Beelman & Royse 2006). The current finding is corroborated by the study of Bhatia et al. (2014), where extracts of the Se-enriched *P. sajor-caju* and *Volvariella volacea* mushroom spp. exhibited higher reducing and scavenging activities. A recent study conducted by Poniedzialek et al. (2017) reported that biofortification of *P. ostreatus* and *P. eryngii* with selenium significantly improved their antioxidant and reducing activities, indicating the potential applicability of such bio-fortified ingredients as functional food.

Generally, fruitbodies of *Pleurotus* possessed higher concentration of antioxidants than other commercial mushrooms (Sharareh et al. 2016). This activity was mainly due to presence of polysaccharide pleuran (β -glucan) that has been isolated from *P. ostreatus* showing a positive effect on rat colon with pre-cancerous lesions (Sharareh et al. 2016). Oyster mushrooms are now widely used as ingredients in dietary supplements to maintain health and prevent diseases due to their higher free radical scavenging activities (Yang et al. 2002).

Conclusively, the Selenium-enriched *Pleurotus* spp. showed significant antioxidant property. Therefore, selenium enriched *Pleurotus* spp. could serve as a rich source of natural antioxidant food for the enhancement of the body against oxidative damage. Fortification of *Pleurotus* spp. with essential metals should be encouraged as this can improve its application as health promoting foods.

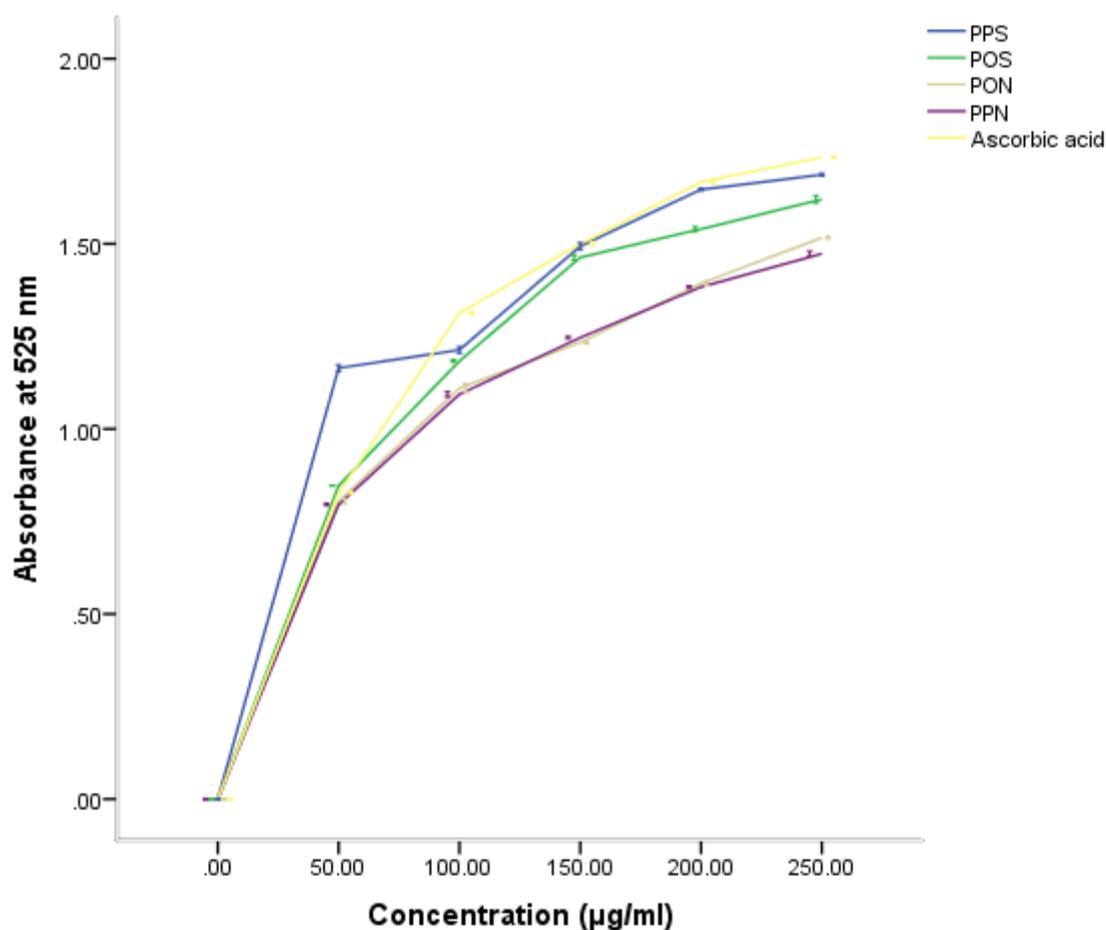


Fig. 3 – Reducing power of cultivated *Pleurotus* spp.

Keys: PPN – *Pleurotus pulmonarius* (non-fortified with selenium)
 PPS – *Pleurotus pulmonarius* (fortified with selenium)
 PON – *Pleurotus ostreatus* (non-fortified with selenium)
 POS – *Pleurotus ostreatus* (fortified with selenium)

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