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Fortification with Selenium Markedly Affects Biological Efficiency and The Distribution of Essential and Non-Essential Amino Acids in *Pleurotus Ostreatus*

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Abstract

Mineral elements are very important in the metabolic processes of living things; hence their absence often results to deficiency diseases. This study reports the effects of selenium fortification on biological efficiency and the distribution of essential and non-essential amino acids in *P. ostreatus*. Viable spawn of *P. ostreatus* was inoculated into substrate spiced with Se while the control was *P. ostreatus* cultivated on substrate without Se. The biological efficiency was calculated using standard method while the amino acid content of the Se fortified and non fortified *P. ostreatus* was analysed using Applied Biosystems PTH amino acid analyzer. The biological efficiency (31.3%) of Se fortified *P. ostreatus* was lower compared to *P. ostreatus* not fortified with Se (43%). Moreover, there was reduction in essential amino acids in *P. ostreatus* fortified with Se, except Valine (50.0mg/100g) that was higher in Se fortified *P. ostreatus*. Generally, the percentage nitrogen in Se fortified *P. ostreatus* (4.79%) was slightly lower than what was obtained in non-selenium fortified *P. ostreatus* (5.05%). Data gathered from this study revealed that Se fortification markedly affects the biological efficiency, quantities and distribution of essential and non-essential amino acids in *P. ostreatus*.

Keywords: *Pleurotus ostreatus*, Selenium, Fortification, Distribution, Amino acids, Essential, Non-essential.

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INTRODUCTION

Edible mushrooms had been part of human diet from time immemorial. Mushrooms have long been used as a valuable food source and as traditional medicines around the world, especially in Japan and China (Oyetayo, 2011). Generally, edible mushrooms are rich in nutrients such as high quality protein of 10% to 40%, carbohydrate of 3% to 21% and dietary fiber of 3% to 35% on dry weight basis depending on the species (Mallavadhani et al., 2006). Consumption of mushrooms as healthy food is therefore based on their rich proteins, minerals, poor calories and fat (Wang et al., 2018).

Mushrooms also produce secondary metabolites which possess anticancer, antiviral, antibacterial, antifungal and anti-inflammatory properties (Owaid et al., 2015). The presence of mycochemicals such as polysaccharides, proteins, terpenes, phenolic compounds and unsaturated fatty acids, and many other substances of different origin (Barros et al., 2007; Oyetayo et al., 2012; Ogidi et al., 2020) actually confers health promoting properties mentioned above on mushrooms (Oyetayo, 2023). These mycochemicals with pharmacological properties are found in the fruitbodies and the culture filtrates of mushrooms (Oyetayo and Akingbesote, 2022). One unique attribute of mushrooms is their ability to absorb nutrient from substrates on which they are cultivated and bioaccumulate them as functional compounds with nutraceutical, pharmaceutical and cosmeceutical potentials (Ogidi et al., 2020). Mineral elements play very important roles in the metabolic processes of plants and animals. The bio-accumulation of these mineral elements by mushrooms affects the metabolic products generated and their nutraceutical properties (Oyetayo, 2023).

Oyster mushrooms are well known edible mushrooms. *P. ostreatus,* a popular oyster mushroom, is a commonly cultivated edible mushroom (Da Silva *et al*., 2019), and hence, it is of great economic and nutritional importance (Fekry *et al*., 2021). During cultivation, *P. ostreatus* can bio-accumulate mineral elements to form bioactive compounds. Selenium enrichment cultivation is a good strategy for increasing the bioactivity of mushroom since enrichment will enhance the production of Selenium metabolites particularly selenium-polysaccharides, selenium-proteins and seleno-amino acids (Zhu *et al*., 2021). Higher bioactivities in terms of antitumor, antioxidant, antimicrobial, and anti-inflammatory properties of Se enriched mushroom when compared with non-selenium fortified has been attributed primarily to the presence of Se-polysaccharide complexes (Cheng *et al*., 2023). Selenium can be bio-accumulated as selenoprotein which are useful in preventing various types of cancer and diseases like diabetes, age-related immunosuppression and even problems related to fertility (Dwyer *et al*., 2015). Biofortification of *P. ostreatus* and *P. eryngii* with Selenium was observed to significantly improve their antioxidant and reducing activities, indicating the potential applicability of such bio-fortified ingredients as functional food (Poniedzialek *et al*., 2017). Other reports showed that fortifications with mineral elements such as Se, Fe, and Zn positively impact antioxidant and antimicrobial properties of *Pleurotus* species extracts (Fasoranti *et al*., 2018; Fasoranti *et al*., 2019; Oyetayo *et al*., 2021; Oyetayo *et al*., 2024). Moreover, a recent report also revealed that Se and Fe fortification markedly affected the phytochemical and amino acid contents of *P. ostreatus* (Fadugba *et al*., 2024). This study therefore seeks to evaluate the effect of Se fortification on biological efficiency and distribution of essential and non-essential amino acids in *P. ostreatus*.

MATERIALS AND METHODS

Artificial cultivation of Selenium fortified and non-fortified *Pleurotus Ostreatus*

Viable spawn of *Pleurotus ostreatus* purchased obtained from the Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos, Nigeria was artificially cultivated on rice bran and saw dust. The method of Fadugba *et al*. (2024) was adopted for the cultivation of *Pleurotus ostreatus.* Briefly, the substrates (sawdust and rice bran) were mixed together in the ratio 3:1. (60% of saw dust plus 20% rice bran) and moistened with water to prevent dryness.

About 700 g of the substrate was packed into polypropylene bag and sealed with paper with the aid of polyvinyl rings and this was sterilized in an autoclave and allowed to cool to room temperature (26 ± 2^{o}C). Thereafter, 8 ml of Sodium selenite (Na_2SeO_3) at a concentration of 50 mg/kg was injected into the some bag containing for Selenium fortification. A control treatment with no sodium selenite was also prepared. Following this, substrates in separate bags were inoculated with 30 g of spawn. The bags were kept in the dark room with relative humidity of 75% to ramify.

Determination of biological efficiency

The biological efficiency (BE) was calculated using the formular below.
BE = (weight of fresh mushroom/weight of dried substrate) × 100.

Determination of Amino acid content of Selenium fortified and non-fortified *Pleurotus Ostreatus*

The amino acid profile of Selenium fortified and non-fortified *Pleurotus ostreatus* was determined using the method of AOAC (2006). The sample was defatted using chloroform/methanol mixture of ratio 2:1. About 5 g of the sample was put in extraction thimble (or filter paper) and extracted for 15 hours in soxhlet extractor. The sample (200 mg) was weighed and placed in a Kjeldahl flask with 200 mg of different catalysts (Potassium sulphate, Copper sulphate and Selenium powder). Concentrated Sulphuric acid was also added to the content of flask. The mixtures were gently heated for a few seconds until frothing ceased and the heat increased for 1 h 30 min to enhance digestion. It was cooled and distilled water was used to make a known volume (100 cm^3). An aliquot of diluted solution of the digest was piped into distillation chamber of micro Kjeldhal distillation apparatus.

Sodium hydroxide solution (40%) was added and it was steamed into 10.0cm^3 of 4% boric acid containing mixed indicator. It was titrated with standard 0.01N hydrochloric acid. The samples were hydrolyzed by using 7 ml of 6N HCl which was placed in an oven preset at 105 °C ± 5 °C for22 h. The filtrate was evaporated to dryness while the residue was dissolved with 5 ml acetate buffer. 60 µl of each samples were loaded each into the applied biosystems PTH amino acid analyser. The concentrations of the amino acids (in g/100g protein) were calculated from external standards for the different amino acids.

Data Analysis

Data generated were analysed using one-way Analysis of variance (ANOVA), using SPSS 20.0.

RESULTS AND DISCUSSION

Selenium fortification reduced the yield and subsequently the biological efficiency (BE) of *P. ostreatus* fruitbodies (Figure 1). In this study, BE of Se enriched *P. ostreatus* (31.3%) was lower than what was observed in *P. ostreatus* (43%) not enriched with Se. In a previous study, Fekry *et al*. (2021) reported that at 20.0 mg/L Se concentration, *P. ostreatus* biomass reduced from 5.56 g/L to 3.20 g/L, and at concentration of 40.0 and 60.0 mg/L Se concentration, biomass production was significantly suppressed. High Se had been reported to prevent growth and mycelium production in *P. ostreatus* (Da Silva *et al*., 2013). Growth inhibition of another

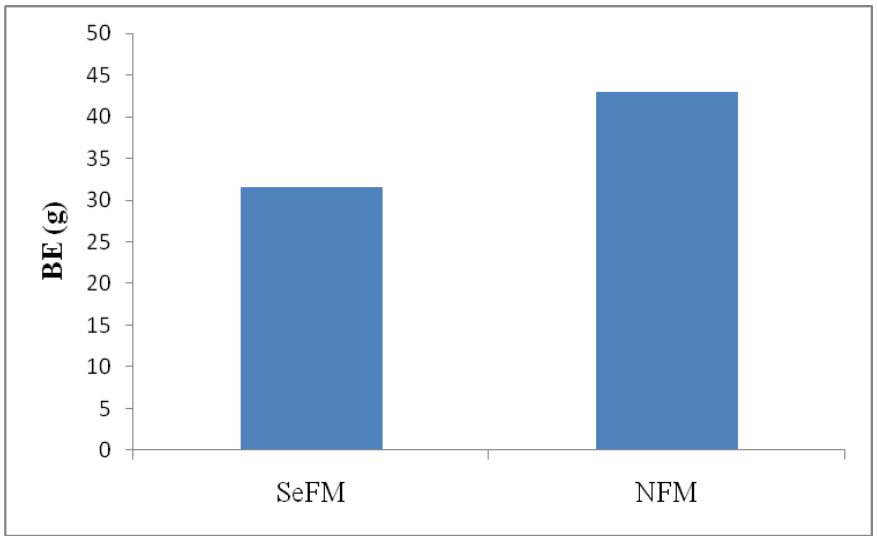

Figure 1. Biological efficiency of Selenium fortified and non-fortified *P. Ostreatus*

SeFM: Selenium fortified mushroom; NFM: Non-selenium fortified mushroom

mushroom, *Inonotus hispidus,* was also observed at higher Se concentration (Song *et al*., 2022). The concentration of 50mg/kg injected into the substrate used in this report was still within the range 40 to 60 mg/l Se concentration that still allowed the growth of the mushroom (Fekry et al., 2021). Tangiadee *et al*. (2023) recently reported that presence of selenium affect oxidative activities in cells and higher concentration of selenium alter biomass production. Generally, the translocation of minerals from substrate affects the productivity, chemical /sensory characteristics and biological efficiency of mushroom (Fekry *et al*., 2021).

Glutamic acid was the most abundant amino acid in Selenium fortified and non fortified *P. ostreatus* with values of 164.3mg/g and 181.1 mg/g respectively. This is in conformity with previous reports that showed glutamic acid is the highest occurring amino acid in *Pleurotus* species (Oyetayo *et al*., 2007; Oyetayo and Ariyo, 2013; Fasoranti *et al*., 2019). However, Se fortification was observed to cause reduction in all the essential amino acids except Valine (Table 1). On the other hand there was increase in the following non essential amino acids, Alanine, Proline, Glycine, Tyrosine, in Se fortified *P. ostreatus* (Table 2).

Table 1. Essential Amino acids (g/100g) in Selenium fortified and Non-fortified *P. ostreatus*

Amino Acid	NFM	SeFM
Valine	45.9 ± 0.01	49.1 ± 0.05*
Leucine	52.9 ± 0.01	47.2 ± 0.06
Lysine	26.2 ± 0.03	22.3 ± 0.03
Isoleucine	34.1 ± 0.01	29.2 ± 0.19
Histidine	13.0 ± 0.02	11.3 ± 0.01
Tryptophan	11.9 ± 0.01	9.1 ± 0.01
Methionine	12.4 ± 0.01	11.4 ± 0.01
Phenylalanine	44.0 ± 0.02	40.7 ± 0.01
Threonine	46.5 ± 0.66	37.1 ± 0.03

*NFM: Non selenium fortified mushroom; SeFM: Selenium fortified mushroom. * Amin acid higher in SeFM*

Table 2. Non-essential Amino acids (mg/g) in Selenium fortified and Non-fortified *P. ostreatus* fruitbodies

Amino Acid	NFM	SeFM
Alanine	41.7± 0.05	44.5 ± 0.03*
Proline	35.2 ± 0.01	37.7± 0.00*
Glycine	33.6 ± 0.02	36.0 ± 0.03*
Tyrosine	29.1 ± 0.01	30.9 ± 0.01*
Glutamic acid	181.1 ± 0.12	164.3 ± 0.13
Aspartic acid	35.5 ± 0.02	36.1 ± 0.10
Arginine	54.6 ± 0.02	49.4± 0.03
Serine	66.4 ± 0.33	52.8± 0.03
Cysteine	13.5 ± 0.03	11.8 ± 0.02

*NFM: Non selenium fortified mushroom; SeFM: Selenium fortified mushroom. * Amino acid higher in SeFM*

Both the total essential and non essential amino acids were lower in Se fortified *P. ostreatus* than Non-selenium fortified *P. ostreatus* (Table 3). This could be as a result of the inhibition of growth of *P. ostreatus* at Se concentration above 20.0 mg/L (Fekry *et al*., 2021). Amino acids which are primary products of metabolism are produced during growth of mushrooms (Yang *et al*., 2020). It was also recently reported by Xiang *et al*. (2023) through transcriptomic profiling that high enrichment for amino acid metabolic pathways in primordia are essential for growth and fruiting body formation in the mushroom, *Hypsizygus marmoreus*. Moreover, It has been reported that metabolism of certain amino acid affects the level of certain amino acids due to the biosynthesis and catabolism of amino acids derived from the same metabolic trunk and /or closely related to other metabolic pathways acting as substrate or intermediate (Song *et al*., 2022). The inhibition / alteration of growth by Se fortification may have affected the quantity and distribution of amino acids produced of *P. ostreatus.*

Table 3. Total essential and non-essential amino acids (mg/g) of Selenium fortified and Non fortified *P. ostreatus* fruitbodies

Amino Acid	NFM	SeFM
Total amino acid	780.6	713.4
Total essential Amino acid	286.9	257.4
Total Non essential Amino acid	490.7	463.5

The quality of dietary proteins can be measured in many ways but it is the ratio of available amino acids in the food compared with the needs as a ratio (Bender, 1992). The quality of proteins in *P. ostreatus* when compared with FAO (2013) standard (Table 4) revealed Phenylalanine/ Tyrosine (1.19 - 1.22); Threonine (0.93 – 1.1) and Valine (0.92 -0.98) were the highest scoring essential amino acid in both Se fortified and non-Se fortified *P. ostreatus* (Table 5). From the data in Table 5, Se fortified and non-Se fortified *P. ostreatus* are good protein sources since they contain essential amino acids in appreciable quantities which are capable of enriching human diets and reducing the incidence of protein-energy malnutrition (Oyetayo *et al*., 2007).

Table 4. Provisional Amino acid scoring pattern

Amino Acid	Suggested level (mg/g protein)	Mg/g N
Isoleucine	250	40
Leucine	440	70
Lysine	340	55
Methionine/ Cysteine	220	35
Phenylalanine/ Tyrosine	380	60
Threonine	250	40
Valine	310	50

Table 5. Calculated amino acid scores of cultivated and wildly obtained *P. ostreatus* fruitbodies.

Amino Acid	NFM	SeFM
Isoleucine	0.85	0.67
Leucine	0.76	0.73
Lysine	0.48	0.48
Methionine/ Cysteine	0.74	0.66
*Phenylalanine/ Tyrosine	1.22	1.19
*Threonine	1.16	0.93
*Valine	0.92	0.98

**Amino acids with the highest score*

In conclusion, Se fortification markedly affected the BE and distribution of essential and non-essential amino acids in *P. ostreatus* though the overall quality of its protein was not too affected when compared with FAO (2013) standard.

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