

## EFFECTS OF SELENIUM FORTIFICATION ON THE MINERAL AND FATTY ACID PROPERTIES OF *PLEUROTUS OSTREATUS* JACQ

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### ABSTRACT

**Objective:** Mineral elements are very important in physiological processes and hence affect the quantity and types of metabolites produced by plants and animals. In this study, the effect of selenium fortification on the mineral and fatty acid contents of *Pleurotus ostreatus* was investigated.

**Methods:** *P. ostreatus* was cultivated on substrate enriched with sodium selenite. The mineral and fatty acid content of the selenium-enriched and non-selenium-enriched fruit bodies were analyzed using standard methods.

**Results:** Data revealed that fortification of *P. ostreatus* results to increase in the values of Ca, Mg, and Se while the value of Zn reduced. A total of 13 and 18 peaks were, respectively, observed in the chromatograms of non-selenium-fortified and selenium-fortified *P. ostreatus*. The following fatty acids pentadecanoic acid, 13-hexyloxacyclotridec-10-en-2-one, oleic acid, n-hexadecanoic acid, stearic acid, undecylenic acid, 10-undecenoyl chloride, and pentadecanoic acid 2-hydroxy-1- (hydroxymethyl)ethyl were common to both selenium-fortified and non-fortified *P. ostreatus*.

**Conclusion:** In conclusion, fortification of *P. ostreatus* markedly affects the mineral and fatty acids of its fruit bodies with more fatty acids present in *P. ostreatus* fortified with selenium.

**Keywords:** *Pleurotus ostreatus*, Selenium, Fortification, Effects, Mineral, Fatty acids.

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### INTRODUCTION

Mushrooms are important based on their nutrient content and the possession of bioactive compounds that promote human health. Health-promoting properties such as antioxidant, antimicrobial, anticancer, cholesterol-lowering, immunostimulatory, and many others had been associated with the consumption of mushroom [1-4]. The presence of bioactive compounds such as polysaccharides, dietary fiber, polyphenols, terpenes, proteins and peptides, and fatty acids had been reported to confer health-promoting properties on mushrooms [5,6].

During cultivation, mushrooms can absorb mineral elements and bioaccumulate them as functional organic compounds. Mineral nutrients are indispensable to the maintenance of life. These elements are very important for cell functions at biological, chemical, and molecular levels [7]. The nutritional requirement of man is at least 23 mineral elements [8]. These mineral elements are also important in the physiological processes of plants and non-chlorophyllous plants. The absence of the essential elements often results in deficiency diseases [9]. Some of these essential elements are selenium, iron, zinc, calcium, and so on. Although these elements constitute only 0.02% of the body weight, however, the genesis of many nutritional disorders had been linked with interactions of these elements since they play significant roles as active co-enzymes [7,10]. Volumes of scientific data from physiologic investigations have revealed that inadequate consumption of these micronutrients (minerals and trace elements) can affect the optimal absorption and utilization of other nutrients to work effectively in the body [8].

*Pleurotus* spp., a group of edible mushrooms that can be artificially cultivated is among the most popular edible mushrooms [5]. These groups of mushrooms have great potential for uptake and bioaccumulate various elements in their fruit bodies. The ability of *Pleurotus ostreatus*,

*P. pulmonarius* to absorb Fe, Zn, Se, and Li had been reported by Ogidi *et al.*, Fasoranti *et al.*, Oyetayo *et al.*, Dwyer *et al.* [11-14].

The absorption of these mineral nutrients has been reported to affect the formation of macromolecules such as amino acids and fatty acids and the mineral distribution of cultivated mushrooms [3,13,15]. The present study was therefore designed to investigate the effects of selenium fortification on the minerals and fatty acids composition of *P. ostreatus*.

### METHODS

#### Production of selenium-fortified and non-fortified *P. ostreatus*

The substrate for cultivation of *P. ostreatus*, rice bran and sawdust, was collected from Ado-Ekiti, Southwestern Nigeria while *P. ostreatus* spawn was obtained from the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria. The substrates (sawdust and rice bran) were mixed together in the ratio 3:1. (60% of sawdust 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. Thereafter, 8 mL of sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>) at a concentration of 50 mg/kg was injected to the some bag containing for selenium fortification. A control treatment with no sodium selenite was also prepared [3]. 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 [16].

#### Mineral analysis of fruit bodies of *P. ostreatus*

The method of Oyetayo *et al.* [13] was adopted for the determination of the mineral content of *P. ostreatus*. Briefly, ash obtained from 1g of dried *P. ostreatus* was digested with HNO<sub>3</sub> and HClO<sub>4</sub> (3:1, v/v) with 2.0 ml of hydrogen peroxide and heated to 250 °C in a flask. It was filtered after digestion and filtrate obtained was made up to 10 mL with ultra-pure

water (Milli-Q system, Millipore, USA). Atomic absorption spectroscopy was calibrated before use with each of the metal standard solution at 1000 µg/mL in 1% v: v HNO<sub>3</sub>. The solution was prepared with ultra-pure water (Milli-Q system, Millipore, USA). A lame photometer (Jenway PFP 7, Staffordshire, UK) was used to determine Na content of the samples.

#### Determination of fatty acid contents

The fatty acids were determined after a trans-esterification procedure as described by Stojković *et al.* [17]. Briefly, fifty milligram (50 mg) of fat extracted from *P. ostreatus* was esterified for 5 min at 95°C with 3.4 ml of the 0.5 M KOH in dry methanol. The mixture was neutralized using 0.7M HCl. About 3 ml of boron trifluoride (14%) in methanol was added. The mixture was heated for 5 min at the temperature of 90°C to achieve complete methylation process. The fatty acid methyl esters (FAMES) were extracted from the mixture with redistilled n-hexane. Thereafter, the content was concentrated to 1 mL, and 1 µL was injected into the injection port of gas chromatography (GC-2010, Shimadzu, Japan) with auto-injector (AOI), capillary column (BPX-70), analytical conditions of autosampler, injection port settings, column oven settings, and column information used for analysis of FAMES. The quantification of the FAMES was performed using standard mixture (C<sub>4</sub>-C<sub>24</sub>, Sigma-Aldrich, St. Louis, MO, USA) processed under similar conditions of samples. The concentration and area of each peak of FAMES were computed using the GC post-run analysis software (Shimadzu, Japan).

#### Data analysis

Data gathered from the study were subjected to analysis of variance using SPSS 20.0 version.

#### RESULTS

The results of mineral analysis of selenium-fortified and non-fortified mushroom are shown in Table 1. Fortification of *P. ostreatus* results to increase in the levels of the following minerals Ca, Mg, and Se. However, there was no remarkable change in the levels of Cu, Fe, Mn, and Cr while a reduction was observed in the Zn content when compared with non-selenium-fortified *P. ostreatus*.

Fortification with selenium affected fatty acid distribution in *P. ostreatus* fruit bodies. Total of 13 and 18 peaks were, respectively, observed in non-selenium-fortified and selenium-fortified *P. ostreatus* (Figs. 1 and 2).

The compounds revealed by gas chromatography–mass spectrometry analysis were made up of fatty acid, fatty aldehydes, and triterpene (squalene) which are present only in selenium-fortified *P. ostreatus* (Tables 2 and 3). The following fatty acids pentadecanoic acid, 13-hexyloxacyclotridec-10-en-2-one, oleic acid, n-hexadecanoic acid, stearic acid, undecylenic acid, 10-undecenoyl chloride, and pentadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl were common to both selenium-fortified and non-fortified *P. ostreatus*.

#### DISCUSSION

Mineral elements are essential participants in metabolic processes [8]. Although these mineral elements constitute very small portion of the body weight, their absence has been linked with many nutritional disorders. They play significant roles as active co-enzymes or trace bioactive substances [7,10]. Moreover, minerals are involved in the generation of the macromolecules in living organisms.

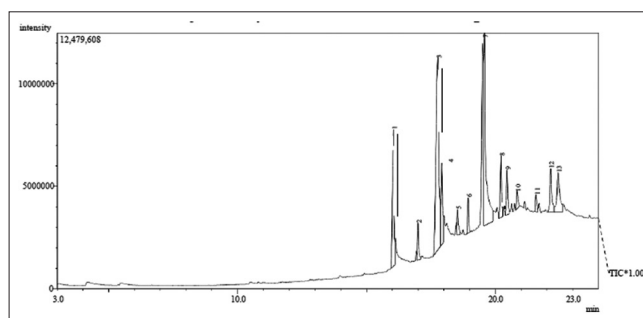
In this study, fortification of *P. ostreatus* affected the mineral elements present in mushrooms. There was increase in the level of Ca, Mg, and Se. Selenium fortification may have enhanced the absorption of Ca and Mg from the substrate. Several researchers have reported the ability of *Pleurotus* species to bioaccumulate elements such as selenium, zinc, lithium, calcium, and iron from the substrate on which they are cultivated [13,14,18-22]. The following have been suggested as the mechanisms that enable mushrooms to readily absorb mineral elements from their substrate: the active transportation of mineral into

**Table 1: Mineral concentration (mg/Kg) in selenium-fortified and non-fortified *Pleurotus ostreatus***

Mineral	Non-selenium fortified	Selenium fortified
Ca	10.40±0.12	27.67±0.23
Mg	12.52±0.00	13.26±0.02
Cu	0.16±0.01	0.17±0.00
Fe	0.56±0.01	0.29±0.01
Mn	0.42±0.01	0.46±0.03
Zn	3.61±0.03	0.95±0.03
Pd	0.00±0.00	0.00±0.00
Ni	0.00±0.00	0.00±0.00
Cd	0.00±0.00	0.00±0.00
Cr	0.03±0.00	0.02±0.00
Se	0.03±0.01	0.17±0.00

**Table 2: Fatty acid distribution in non-selenium-fortified *Pleurotus ostreatus***

S/N	F. Time	Area %	Height %	Name of compound
1	16.040	11.84	14.76	Pentadecanoic acid
2	16.995	2.42	3.98	13-Hexyloxacyclotridec-10-en-one
3	17.772	31.60	20.81	Oleic acid
4	17.918	6.70	8.80	Stearic acid
5	18.529	1.93	2.75	9-Octadecenal
6	18.943	2.00	3.81	Pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl)
7	19.577	21.17	20.75	Undecylenic acid
8	20.210	4.66	6.68	10-Undecenoyl chloride
9	20.447	3.76	4.85	9-Tetradecenal, (Z)-
10	20.841	1.45	1.96	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)
11	21.567	1.18	1.83	Ethanone, 1-(2,2-dimethylcyclopentyl)-
12	22.147	4.56	4.72	7,11-Hexadecadienal
13	22.428	6.73	4.30	9-Hexadecenal



**Fig. 1: Chromatogram of fatty acids in non-selenium-fortified *Pleurotus ostreatus***

cell and intracellular components, biosorption methods like adsorption, ion exchange processes, and covalent binding [23,24]. These elements are essential in the human body metabolism. The ability of *P.* species to absorb these mineral elements could therefore be adopted as a strategy to solve the problem of mineral malnutrition in man [8,25].

Absorption Se by *P. ostreatus* from the growth substrate is of importance. Such selenium enrich mushroom has been demonstrated to have better antioxidant and antimicrobial properties [3,11]. 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 [26]. Its optimal intake could potentially prevent various types of cancer and diseases such as diabetes, age-related immunosuppression and even problems related to fertility [27] in the ultimate consumer of such Se-enriched mushroom.

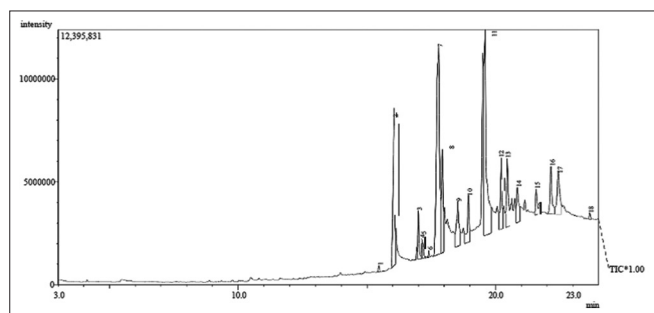


Fig. 2: Chromatogram of fatty acids in selenium-fortified *Pleurotus ostreatus*

Table 3: Fatty acid distribution in selenium-fortified *Pleurotus ostreatus*

S/N	F. Time	Area%	Height%	Name of compound
1	15.517	0.24	0.55	Pentadecanoic acid
2	16.100	10.56	13.56	14-Methyl-n-hexadecanoic acid
3	17.092	2.19	4.20	13-Hexyloxacyclotridec-10-en-2-one
4	17.158	0.78	0.78	9,12-Octadecadienoic acid (Z, Z)-
5	17.250	0.57	1.34	11-Octadecenoic acid
6	17.458	0.39	0.62	Octadecanoic acid, methyl ester
7	17.883	27.07	17.83	Oleic acid
8	17.983	27.07	8.89	Stearic acid
9	18.642	4.71	3.87	9-Octadecenal
10	19.017	3.52	4.06	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)
11	19.833	19.99	17.75	Undecylenic acid
12	20.283	4.82	6.13	10-Undecenyl chloride
13	20.550	5.34	5.86	10-Undecenal
14	20.942	3.08	3.01	Pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl
15	21.650	1.53	2.22	Ethanone, 1-(2,2-dimethylcyclopentyl)-
16	22.283	3.74	4.09	Octadecenyl aldehyde
17	22.550	4.96	3.75	7-Tetradecenal, (Z)-
18	23.708	0.29	0.54	Squalene

Fortification of *P. ostreatus* with selenium significantly affected its total fatty acid content. More fatty acids, fatty aldehydes, and squalene (18 compounds) were found in selenium-fortified *P. ostreatus* while 13 compounds were present in non-selenium-fortified *P. ostreatus* (Tables 2 and 3). Tan *et al.* (2017) had earlier reported that metal ions such as ZnSO<sub>4</sub> and FeCl<sub>3</sub> increased biomass and lipid content in *Mortierella*. Metal ions play a significant role in influencing the lipid content of mushrooms [28]. Metal ions, especially copper, have been shown to influence lipid production in mushrooms by affecting enzyme activity and metabolic pathways, ultimately leading to changes in the lipid content of fungal cells [28]. The following fatty acids pentadecanoic acid 14-methyl-, n-hexadecanoic acid, 13-hexyloxacyclotridec-10-en-2-one, oleic acid, stearic acid, undecylenic acid, ethanone, 1-(2,2-dimethylcyclopentyl)-, and hexadecanoic acid common to selenium and non-selenium-fortified *P. ostreatus* have been reported in other mushrooms [29,30]. Saturated fatty acids (pentadecanoic acid and stearic acid) and unsaturated fatty acids (oleic acid and undecylenic acid) had also been reported in mushrooms species by various authors [30,31].

## CONCLUSION

Conclusively, fortification of *P. ostreatus* markedly affects the mineral and fatty acids in its fruit bodies. More fatty acids were accumulated in *P. ostreatus* fortified with selenium. Moreover, the ability of *P. ostreatus*

to accumulate selenium from the growth medium was revealed and this may be used as a means of delivering dietary selenium, an important antioxidant, to human.

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## CONFLICTS OF INTERESTS

None.

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