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Comparative Analysis of Vitamin, Amino Acid, and Fatty Acid Profiles in *Pleurotus ostreatus* Cultivated on Iron-Fortified Wood Substrates

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ABSTRACT

Background and Objective: Iron deficiency and protein-energy malnutrition remain widespread in developing regions, necessitating affordable dietary interventions. *Pleurotus ostreatus* (oyster mushroom) offers potential as a functional food due to its rich nutrient composition. This study investigated how iron fortification of different tropical wood substrates influences the vitamin, amino acid, and fatty acid profiles of *P. ostreatus*. **Materials and Methods:** The experiment involved six treatment groups using three tropical wood substrates (*Canarium* sp., *Ceiba pentandra*, and *Pycnanthus angolensis*), each with iron-fortified and non-fortified variants. Standard analytical methods were used to determine vitamin, amino acid, and fatty acid contents. The data were analyzed using ANOVA, and treatment means were compared with Duncan's Multiple Range Test, with the t-value evaluated at a 95% confidence interval. **Results:** Vitamin content varied significantly across treatments. Mushrooms cultivated on non-fortified *Pycnanthus angolensis* recorded the highest vitamin A level (8.08 mg/100 g), while iron-fortified samples on the same substrate exhibited the highest vitamin C content (204.91 mg/100 g). Glutamic acid was the most abundant amino acid, reaching 10.70/100 g in fortified Pycnanthus samples. Essential amino acids such as leucine (2.37/100 g), arginine (6.32/100 g), and alanine (3.77/100 g) were also elevated with fortification. Fatty acid analysis showed oleic (33.95%) and palmitic acids (29.30%) as the most prevalent, with higher concentrations in fortified mushrooms. **Conclusion:** Iron fortification significantly enhanced the vitamin, amino acid, and fatty acid composition of *P. ostreatus*, particularly when cultivated on *Pycnanthus angolensis*. The fortified mushrooms demonstrate strong potential for improving iron and protein intake in resource-limited populations.

KEYWORDS

Zinc, fortification, *Pleurotus ostreatus*, fatty acids, amino acids

INTRODUCTION

Mushrooms have long been recognized as nutritious, functional foods with significant potential for addressing malnutrition, especially in resource-limited settings. Among cultivated species, *Pleurotus ostreatus* (the oyster mushroom) stands out for its efficient growth on agricultural and forestry residues, its rich profile of vitamins, minerals, amino acids, and fatty acids, and its capacity to bioaccumulate micronutrients when cultivated on supplemented substrates[1,2].

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The global burden of malnutrition, particularly micronutrient deficiencies such as iron deficiency anemia, continues to threaten public health, especially in developing regions[3]. Iron is an essential mineral critical for oxygen transport, immune function, and cellular metabolism, yet it remains one of the most common nutrient deficiencies worldwide[4]. In response to this challenge, researchers have been exploring sustainable and food-based approaches to enhance micronutrient intake among vulnerable populations. Among the promising strategies is the mineral element enrichment of edible fungi like *Pleurotus ostreatus*, commonly known as the oyster mushroom[5,6].

Pleurotus ostreatus is valued not only for its culinary appeal and ecological sustainability but also for its rich nutritional profile, including essential vitamins, amino acids, and unsaturated fatty acids[7,8]. *Pleurotus* mushrooms are widely consumed due to their attractive taste, aroma, with nutritional and medicinal values[9]. It is a fleshy fungus with spore spore-bearing fruiting body belonging to the family Pleurotacaea, and the second largest cultivated mushroom. Its ability to grow on lignocellulosic agricultural and forestry wastes makes it an economically viable crop, especially in low-resource settings. Nutritional composition of *Pleurotus ostreatus* can vary depending on environmental factors such as temperature and substrate type[10]. This fungus can absorb mineral elements and bioaccumulate them as functional organic[11]. More importantly, its mycelial network has demonstrated the capacity to absorb and accumulate minerals from the growth substrate[12], making it a suitable candidate for nutrient fortification.

However, there is still scanty information on the effect of substrate modification with mineral elements such as iron, zinc and selenium on the fatty acid and other contents of *Pleurotus ostreatus* is limited[13]. Previous studies reported the influence of mineral enrichment on the nutritional profile of mushrooms, but there remains limited literature on how iron fortification specifically affects the vitamin, amino acid, and fatty acid content of *P. ostreatus* cultivated on various tropical wood substrates. Understanding these interactions is crucial for optimizing mushroom-based interventions against malnutrition. This study, therefore, investigates the influence of iron fortification on the vitamins, amino acids, and fatty acids composition of *P. ostreatus* cultivated on wood substrates fortified with iron.

MATERIALS AND METHODS

Study area: This study was carried out in the Department of Microbiology, Federal University of Technology, Akure, Nigeria. *Pleurotus ostreatus* was cultivated on different wood substrates between the months of June and August, 2024.

Substrate preparation and cultivation of *Pleurotus ostreatus*: *Pleurotus ostreatus* was cultivated for several weeks on the sawdust obtained from *Canarium* sp., *Ceiba pentandra,* and *Pycnanthus angolensis*. The dry substrates were checked for consistency and were mixed thoroughly with water. The substrates were moistened with water to prevent dryness. About 800 g of medium was filled into polypropylene bags and sealed using paper and polyvinyl rings. The substrates in bags were sterilized in the autoclave and were left to cool down to ambient room temperature.

Fortification of *Pleurotus ostreatus* with zinc chloride: Eight milliliters of Ferrous sulphate ($FeSO_4$) at a concentration of 50 mg/kg was injected to the bags containing substrates for iron fortification. A control treatment with no ferrous sulphate was also prepared[14]. Following this, substrates in separate bags were inoculated with 30 g of spawn. The bags were kept in the dark room with a relative humidity of 75% to maintain.

The growth of mycelium in each bag was observed. Once the mycelium fully covered the Substrate, the bags were left open in the growing house to allow fruit body formation. The mushroom fruit bodies were harvested, air-dried, and then ground into powder using a grinding machine (Lexmark Mixer Grinder KP- 4055).

Determination of the vitamins A and C content of cultivated *Pleurotus ostreatus*: Vitamin A and C content was determined according to the method of Achikanu *et al*.[15]. The mushroom samples were collected and thoroughly cleaned to remove soil and debris. They were freeze-dried to preserve vitamin content. The freeze-dried mushrooms were ground into a fine powder and stored in airtight containers at -20°C until analysis.

Vitamin A extraction: One gram of the powdered sample was weighed and added to 25 mL of ethanol, and homogenized for 2 min. A 25 mL of hexane was added and shaked for 5 min. The upper hexane layer was collected in separate phases, and the hexane was evaporated under reduced pressure, and the residue was reconstituted in methanol[16].

Vitamin C extraction: One gram of the powdered sample was weighed and added to 50 mL of 0.1 M HCl, and homogenized for 2 min. A 25 mL of hexane was added and shaken for 5 min. It was heated in a water bath at 95°C for 30 min and centrifuged at 5000 rpm for 10 min, and the supernatant was collected[17].

Determination of the vitamins A and C content of cultivated *Pleurotus ostreatus*: Standard solutions for vitamins A and C were prepared. The extracts were injected into a High-Performance Liquid Chromatography (HPLC) system with UV/Vis detector at 265 nm for vitamin A and 254 nm for vitamin C. Retention times and peak areas were compared with standards to determine concentrations. The LC-MS was used for precise quantification and confirmation. Mass Spectrometry (MS) parameters were optimized for each vitamin[18]. Quality control was performed with standard solutions and blanks. Methods for linearity, accuracy, precision, and sensitivity was validated[19]. Triplicate analyses for reproducibility were conducted. Vitamin content was calculated using calibration curves from standards and the results expressed as mg or µg per 100 g of dry mushroom weight.

Fatty acid analysis of cultivated *P. ostreatus*: Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GLC-FID)/capillary column based on the ISO 5509 (2000) trans-esterification method. The analysis was conducted using a split-splitless injector, a FID, and a Chrompack CP-9050 autosampler. The injector and detector temperatures were both set to 250°C. A 50 m×0.25 mm i.d. fused silica capillary column coated with a 0.19 µm film of CP-Sil 88 (Chrompack) was used for separation. Helium was employed as the carrier gas at an internal pressure of 120 kPa. The column temperature was initially set at 140°C for 5 min and then programmed to increase to 220°C at a rate of 4°C/min, where it was held for 10 min. The split ratio was 1:50, and the injected volume was 1.2 µL. The results are expressed as the relative percentage of each fatty acid, calculated by internally normalizing the chromatographic peak area. Fatty acid identification was performed by comparing the relative retention times of FAME peaks from samples with standards. A Supelco (Bellefonte, PA) mixture of 37 FAMEs (standard 47885-U) was utilized, and some fatty acid isomers were identified using individual standards also obtained from Supelco following the procedure of Barros *et al*.[16].

Total amino acid analysis of cultivated mushrooms: The mushroom sample (1-3 g) was hydrolyzed under nitrogen gas with 10 mL of 4 N NaOH and 200 µg of ascorbic acid as an antioxidant in an autoclave at 110°C for 16 hrs and adjusted to pH 9.00. The hydrolysate was then filtered through a 0.45 µm cellulose acetate membrane filter before being injected into the HPLC for analysis. The amino acid composition was determined using reversed-phase HPLC and gradient elution[20]. The analysis was performed using the Agilent 1100 HPLC system (Agilent Technologies) with an autosampler, a Zorbax-Eclipse XDB-C18 column (4.6×150 mm, 5 µm) with a Zorbax Eclipse -AAA guard column (4.6×15.5 mm, µm) and a fluorescence detector. Chemstation Rev.A.09.03 (1417) (Agilent Technologies 1990-2002) was used for data acquisition and analysis. The sample was subjected to automatic pre-column derivatization with a combination of

OPA-3MPA for primary amino acids and FMOC for secondary amino acids. Mobile phase A contains 40 mmoL/L Na_2HPO_4 at pH 7.8 and B contains 45% acetonitrile, 45% methanol, and 10% deionized water. The temperature of the chromatographic column was set at 40°C with a flow rate of 2 mL/min. The detector was set to 340/450 (Ex/Em) at 0 min and 266/305 (Ex/Em) at 15 min.

Calculation of amino acid values from the chromagram peaks: The net height of each peak produced by the TSM chart recorder (each representing one amino acid) was measured following Horwitz and Latimer[21] procedure. The half-height of the peak on the chart was found, and the width of the peak at half height was accurately measured and recorded. The approximate area of each peak was calculated by multiplying the height by the width at half height. The nor leucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$NE = \frac{\text{Area of norcleucin}}{\text{Area of each amino acid}}$$

A constant (S) was calculated for each amino acid in the standard mixture:

$$S_{std} = NE_{std} \times \text{moL. Weight} \times \mu MAA_{std}$$

$$\text{Amino acid (g/100 g protein)} = NH \times W \text{ @ } NH/2 \times S_{std} \times C$$

$$C = \frac{\text{Dilution} * 16}{\text{Sample wt (g)} \times N\,\% * \text{Vol. loaded}} \div NH \times W \text{ (Nleu)}$$

where:

NH = Net height
W = Width at half height
Nleu = Norcleucine
MAA = Molar absorption coefficient of the amino acid standard

Statistical analysis of data: The data obtained were subjected to statistical Analysis of Variance (ANOVA) and Duncan's Multiple Range Test was used to compare means. The 't' value was tested at 95% confidence interval.

RESULTS

Non-iron fortified *P. ostreatus* cultivated on *Pycnanthus angolensis* has the highest vitamin A (8.08 mg/100 g) and vitamin C (204.91 mg/100 g) contents, as shown in Fig. 1. There are notable variations in the levels of both vitamins across the different samples.

Table 1 shows the amino acid content of the cultivated *Pleurotus ostreatus*. Glutamic acid was the highest amino acid recorded and the values range from 9.07 to 10.70/100 g, with iron fortified *P. ostreatus* cultivated on the wood substrate *Pycnanthus angolensis* recording the highest value of 10.70 g/100 g.

Fatty acid content (%) of cultivated mineral fortified and non-mineral fortified *P. ostreatus*: Table 2 shows the variations in the fatty acid content in all the samples. Palmitic acid content was significantly high in fortified *P. ostreatus* (B, D and F), but the highest value was recorded in *P. ostreatus* cultivated on non-fortified wood substrate (*Pycnanthus angolensis*). Oleic have the highest value of 33.33% in *P. ostreatus* cultivated on wood substrate (*Canarium* sp.) fortified with iron. This suggests that both substrate type and micronutrient fortification influence the accumulation of specific fatty acids.

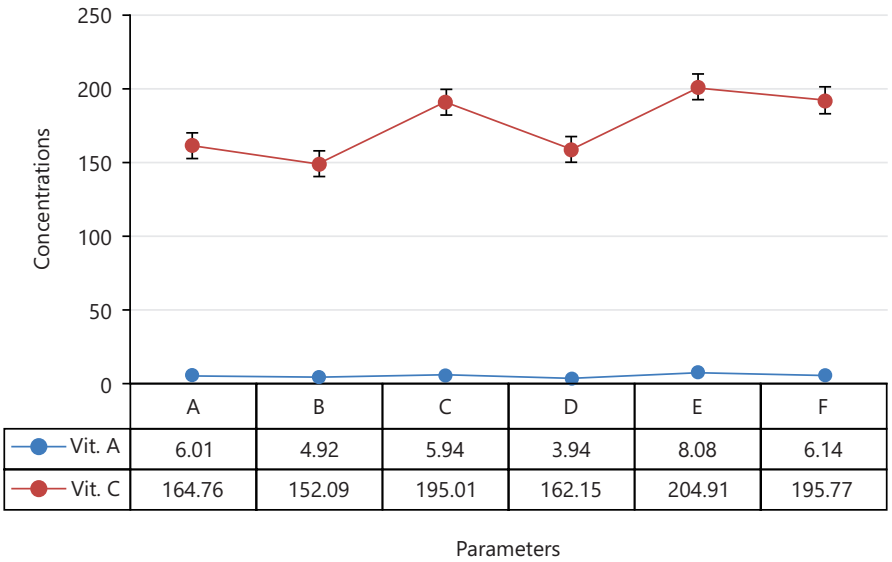

Fig. 1: Vitamin A and C content (mg/100 g) of cultivated iron fortified and non-iron fortified *P. ostreatus*
(a) *Pleurotus ostreatus* cultivated on wood substrate (*Canarium* sp.) without fortification, (b) *Pleurotus ostreatus* cultivated on wood substrate (*Canarium* sp.) fortified with iron, (c) *Pleurotus ostreatus* cultivated on wood substrate (*Ceiba pentandra*) without fortification, (d) *Pleurotus ostreatus* cultivated on wood substrate (*Ceiba pentandra*) fortified with iron, (e) *Pleurotus ostreatus* cultivated on wood substrate (*Pycnanthus angolensis*) without fortification and (f) *Pleurotus ostreatus* cultivated on wood substrate (*Pycnanthus angolensis*) fortified with iron

Table 1: Amino acid composition (g/100 g) of cultivated mineral fortified and non-mineral fortified *P. ostreatus*

Amino acid	A	B	C	D	E	F
Alanine	3.56±0.09^{a}	3.53±0.02^{a}	3.56±0.03^{a}	3.58±0.02^{a}	3.76±0.03^{b}	3.77±0.01^{b}
Arginine	5.04±0.02^{a}	5.03±0.02^{a}	5.75±0.02^{b}	5.76±0.02^{b}	6.30±0.02^{c}	6.32±0.03^{c}
Aspartic acid	3.82±0.02^{a}	3.83±0.03^{a}	4.10±0.02^{b}	4.10±0.01^{b}	4.32±0.03^{b}	4.39±0.01^{b}
Cystine	0.47±0.02^{a}	0.48±0.01^{a}	0.54±0.02^{b}	0.53±0.03^{b}	0.54±0.01^{b}	0.55±0.02^{b}
Glutamic acid	9.09±0.02^{a}	9.07±0.03^{a}	9.94±0.01^{b}	9.93±0.02^{b}	10.40±0.20^{c}	10.70±0.20^{c}
Glycine	0.44±0.02^{a}	0.45±0.02^{a}	1.54±0.03^{b}	1.53±0.01^{b}	1.64±0.02^{c}	1.67±0.02^{c}
Histidine	1.05±0.02^{a}	1.04±0.02^{a}	1.13±0.03^{b}	1.11±0.01^{b}	1.12±0.03^{b}	1.12±0.03^{b}
Isoleucine	1.12±0.03^{a}	1.12±0.02^{a}	1.21±0.02^{a}	1.22±0.02^{a}	1.24±0.02^{a}	1.29±0.03^{a}
Leucine	1.77±0.02^{a}	1.76±0.01^{a}	2.19±0.02^{b}	2.17±0.02^{b}	2.33±0.02^{b}	2.37±0.02^{b}
Lysine	1.42±0.02^{a}	1.41±0.03^{a}	1.44±0.02^{a}	1.44±0.03^{a}	1.48±0.02^{a}	1.52±0.03^{b}
Methionine	0.48±0.01^{a}	0.43±0.03^{a}	0.44±0.02^{a}	0.45±0.02^{a}	0.55±0.02^{b}	0.54±0.03^{b}
Phenylalanine	1.28±0.02^{a}	1.31±0.02^{b}	1.45±0.01^{c}	1.45±0.02^{c}	1.28±0.02^{a}	1.30±0.02^{b}
Proline	0.44±0.02^{a}	0.47±0.01^{a}	0.45±0.03^{a}	0.46±0.02^{a}	0.44±0.01^{a}	0.44±0.02^{a}
Serine	1.96±0.02^{a}	1.96±0.02^{a}	2.06±0.02^{b}	2.07±0.01^{b}	2.12±0.02^{b}	2.15±0.02^{b}
Threonine	2.10±0.02^{a}	2.09±0.02^{a}	2.27±0.03^{b}	2.28±0.03^{b}	2.30±0.03^{b}	2.30±0.02^{b}
Tyrosine	0.84±0.03^{a}	0.84±0.02^{a}	0.99±0.02^{ab}	0.99±0.01^{ab}	1.15±0.02^{c}	1.15±0.02^{c}
Valine	1.44±0.02^{b}	1.44±0.01^{a}	1.62±0.02^{b}	1.65±0.01^{b}	1.62±0.02^{b}	1.63±0.01^{b}

Values are means of triplicate±SD, Samples carrying the same superscripts in the same row are not significantly different at ($p>0.05$), (a) *Pycnanthus ostreatus* cultivated on wood substrate (*Canarium* sp.) without fortification, (b) *Pycnanthus ostreatus* cultivated on wood substrate (*Canarium* sp.) fortified with iron, (c) *Pycnanthus ostreatus* cultivated on wood substrate (*Ceiba pentandra*) without fortification, (d) *Pycnanthus ostreatus* cultivated on wood substrate (*Ceiba pentandra*) fortified with iron, (e) *P. ostreatus* cultivated on wood substrate (*Pycnanthus angolensis*) without fortification and (f) *Pycnanthus ostreatus* cultivated on wood substrate (*Pycnanthus angolensis*) fortified with iron

DISCUSSION

The substrate and most especially the nutrient therein used in cultivating *Pleurotus ostreatus* significantly affect its nutrient composition. Mushrooms are known to possess the unique attribute of absorbing nutrient from the substrates on which they are cultivated and bioaccumulate them as functional compounds with nutraceutical, pharmaceutical and cosmeceutical potentials[22]. In this study, the vitamins C and A levels of *P. ostreatus* varies across the different substrates used in its cultivation. Vitamin C content was far higher than vitamin A in all the *P. ostreatus*. In a previous report, a comprehensive analysis on the nutritional composition of two *Pleurotus* spp., revealed that vitamin C was the most abundant[23]. Vitamins are essential for human health, and their levels can be influenced by substrate type and mineral supplementation[24].

Table 2: Fatty acid content (%) of cultivated *P. ostreatus*

Fatty acids	A	B	C	D	E	F
Myristic	0.62±0.03^{b}	0.67±0.03^{b}	0.52±0.01^{a}	0.56±0.03^{a}	0.60±0.03^{ab}	0.65±0.04^{b}
Pentadecanoic	0.25±0.0^{a}	0.29±0.01^{b}	0.24±0.02^{a}	0.30±0.02^{b}	0.25±0.01^{a}	0.31±0.02^{b}
Palmitic	26.86±0.30^{b}	28.21±0.23^{c}	21.83±0.49^{a}	25.29±0.38^{b}	29.30±0.37^{c}	29.29±0.39^{c}
Palmitoleic	0.45±0.04^{b}	0.64±0.09^{c}	0.37±0.05^{a}	0.55±0.03^{bc}	0.43±0.05^{a}	0.57±0.04^{bc}
Stearic	26.15±0.89^{b}	29.41±0.89^{c}	23.92±0.16^{a}	26.44±0.62^{b}	27.37±0.20^{cd}	28.31±0.35^{c}
Oleic	30.88±0.54^{d}	33.33±0.75^{a}	30.24±0.39^{d}	33.95±0.18^{a}	29.94±0.17^{b}	32.33±0.40^{c}
Linoleic acid	0.24±0.02^{a}	0.31±0.02^{c}	0.27±0.02^{b}	0.28±0.04^{b}	0.27±0.01^{b}	0.31±0.02^{c}

Values are means of triplicate±SD, Samples carrying the same superscripts in the same row are not significantly different at (p>0.05), (a) *Pycnanthus ostreatus* cultivated on wood substrate (*Canarium* sp.) without fortification, (b) *Pycnanthus ostreatus* cultivated on wood substrate (*Canarium* sp.) fortified with iron, (c) Pycnanthus ostreatus cultivated on wood substrate (Ceiba pentandra) without fortification, (d) *Pycnanthus ostreatus* cultivated on wood substrate (Ceiba pentandra) fortified with iron, (e) *Pycnanthus ostreatus* cultivated on wood substrate (*Pycnanthus angolensis*) without fortification and (f) *Pycnanthus ostreatus* cultivated on wood substrate (*Pycnanthus angolensis*) fortified with iron

Moreover, studies have demonstrated that iron fortification of substrates can meaningfully alter the nutritional composition of *P. ostreatus*, enriching its mineral and phytochemical makeup, and enhancing amino acid profiles[13,14,25]. Amino acid composition is a key nutritional indicator of edible mushrooms such as *Pleurotus ostreatus*. Past studies revealed that glutamic acid typically dominates the profile, contributing to both nutritional value and umami flavour[26,27]. However, the implications of mineral fortification, particularly with iron on amino acid composition remain largely unexplored.

In this present study, iron fortification generally increased the level of most amino acids across substrates. Glutamic acid was the most abundant amino acid in all *P. ostreatus* samples. This is in conformity with previous studies that revealed glutamic acid as the most abundant amino acid in *Pleurotus* spp.[28-30].

Amino acids, which are primary products of metabolism, are produced during the growth of mushrooms[31]. The value of glutamic acid content of iron-fortified *P. ostreatus* cultivated on *P. ongoleubis* was the highest and it ranges from 9.07 to 10.70/100 g. This suggest that iron supplementation during the cultivation of mushrooms enhances amino acid accumulation in synergy with certain substrates like *Pycnanthus angolensis*. This finding correlates with previous studies, which reported glutamic acid as the most abundant amino acid in *P. ostreatus*, with levels frequently ranging from 7-9/100 g in fruiting bodies cultivated on sawdust, straw, or agricultural wastes[26,27]. The observed range of 9.07 to 10.70/100 g for glutamic acid is significantly higher, especially in the iron-fortified samples. This shows the potential synergistic effects of the substrate used and the iron fortification during mushroom cultivation. The interaction between these factors appears to enhance the amino acid profile, contributing to improved nutritional quality.

The fatty acid profile of iron and non-iron fortified *P. ostreatus* revealed notable differences across different substrates and fortification conditions. Essential fatty acids present in *P. ostreatus* include myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, pentadecanoic acid, and linoleic acid. *Pleurotus ostreatus* fortified with iron exhibited higher concentrations of certain fatty acids compared to non-fortified *P. ostreatus*. The observed variations correlate with previous researches on the influence of substrate and mineral fortification on fungal lipid metabolism[32]. Studies have highlighted that substrate composition affects fungal lipid biosynthesis due to variations in nutrient availability and metabolic pathways[32]. Additionally, mineral fortification has been shown to enhance fungal growth and lipid accumulation[33]. In a previous study, it was observed that more fatty acids, fatty aldehydes, and squalene (18 compounds) were found in selenium fortified *Pleurotus ostreatus* while 13 compounds were present in non-selenium fortified *Pleurotus ostreatus*[29]. Generally metal ions are known to influence lipid production in mushrooms by affecting enzyme activity and metabolic pathways, ultimately leading to changes in the lipid content of fungal[34].

Previous researches consistently report palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids as dominant components of *P. ostreatus* lipids[35]. Oleic and linoleic acids often define the unsaturated fraction, with oleic typically ranging between 25-35% and palmitic around 20-30%. This study correlates with the past studies with iron- fortified *P. ostreatus* cultivated on *Canarium* sp and *Ceiba pentandra* show oleic levels within expected upper limits (≈ 33-34%), and palmitic values (~29%).

CONCLUSION

Conclusively, this study shows that iron fortification of wood substrates used for the cultivation of *P. ostreatus* significantly affected its vitamins, fatty, and amino acid content. The effect of iron fortification on the vitamins, fatty, and amino acid content of *P. ostreatus* was more pronounced in *Pycnanthus angolensis* wood. The iron-enriched *P. ostreatus* could be used to combat iron deficiency and macronutrient deficiency in human, especially in areas reliant on cereal grains and starchy foods as their main staple food.

SIGNIFICANCE STATEMENT

This study discovered the nutrient-enhancing effect of iron fortification on *Pleurotus ostreatus*, which can be beneficial for improving dietary iron, vitamins, amino acids, and fatty acids in nutritionally vulnerable populations. By demonstrating that iron-enriched tropical wood substrates, particularly *Pycnanthus angolensis*, significantly elevate essential nutrients such as vitamins A and C, glutamic acid, leucine, and key fatty acids, the study highlights the potential of fortified mushrooms as an affordable functional food. These findings also provide new insight into using substrate modification to biofortify edible fungi for public health nutrition. Moreover, this study will help researchers uncover the critical areas of mushroom-based biofortification that many researchers were not able to explore. Thus, a new theory on nutrient-optimized fungal cultivation may be arrived at.

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