# Phytochemical, Vitamin Composition and Bacterial Contamination of *Pleurotus Ostreatus* and *Pleurotus Tuber-Regium*

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***Abstract:***
*Studies on the phytochemical composition and bacterial contamination of Pleurotus ostreatus and Pleurotus tuber-regium grown in Port Harcourt were carried out in the Department of Plant Science and Biotechnology Rivers State University. Fresh and Dry P.ostreatus and P.tuber-regium were grown and harvested from Dilomat farms Rivers State University Port Harcourt. The samples were collected and analyzed in Food Science and Biotechnology Laboratory.*
*Mycological studies were carried out in Department of Plant Science Biotechnology Laboratory using standard methods. The result for phytochemical composition revealed phytate, oxalate, saponin, tannin, carotenoid, polyphenol, flavonoid and lignin. Fresh P. ostreatus and Fresh P. tuber-regium had similar phytate value (0.01±0.00), Fresh P. ostreatus recorded high value for oxalate (0.04±0.00), tannin (3.12±0.00), carotenoid (30.56±0.05), while Fresh P. tuber-regium had high value for saponin (0.02±0.00), polyphenol (7.53±0.05), flavonoid (3.53±0.05) and lignin (5.83±0.5). Vitamin composition results revealed the presence of vitamin A and thiamin. Fresh P. ostreatus had higher vitamin A (3.00±0.00) and thiamin (0.16±0.00) while Dry P. ostreatus recorded (2.50±0.00) for vitamin A and (0.18±0.01) for thiamin respectively. Bacterial contamination from Fresh and Dry P. ostreatus identified include E. coli and Bacillus sp while Fresh and Dry P. tuber-regium revealed pseudomonas and bacillus sp respectively. P. ostreatus and P. tuber-regium are rich in phytochemical and vitamin composition. However, they are prone to contamination, and as such, care must be taken during growing and handling of harvested mushrooms to prevent contamination. More so, mushrooms with signs of contaminations should not be consumed to avoid bacterial infection.*

***Key word:*** *Phytochemical composition, Bacterial contamination, Pleurotus ostreatus, Pleurotus tuber-regium.*

## Introduction

Mycology, encompassing the study of fungi's taxonomy, reproduction, morphology, ecology, and growth, is considered significant due to the diverse roles fungi play in society (Salako and Anjorin, 2012). Fungi, classified as eukaryotes, are categorized into macroscopic and microscopic types. Despite differences, both produce spores as a reproductive structure (Bowman and Free, 2006). While most microscopic fungi are filamentous, some are yeast. Macroscopic forms like toadstools, mushrooms, and truffles exhibit features resembling plants (Hawksworth, 2006; Chuku and Nwobiowhuru, 2009).

Literature emphasizes the importance of these materials in *Pleurotus* nutrient and cultivation processes (Iqbal *et al.,* 2016; Nwoko *et al.,* 2017). Various authors have reported the impact of abiotic or environmental factors on mushroom and plant cultivation (Mitova *et al.,* 2017; Santoyo *et al.,* 2017; Uddin *et al.,* 2011). Sardar *et al*. (2015) highlighted temperature and pH levels' influence on wild and exotic *Pleurotus* species. Chitra *et al*. (2018) reported that temperature and relative humidity significantly affect Oyster mushroom yield.

Mushroom is affected by contaminations and spoilage by different microorganisms and pest as they all utilize the same form of nutrient source; presence of spoilage agents affects the appearance, market ability and shelf life of grown oyster mushroom as well as other edible mushrroms (Chuku & Barber, 2017). It is reported that different microorganisms such as *Trichoderma sp*, *Aspergillus sp*, *Verticiluium sp*, as well as several insect pest such as *megasclia sp. Lycriella sp and Scania sp* (Fasidi *et al* 2008).

Nutrient content describes the level of a nutrient contained in a food, such as, source of calcium and low in fat (Uddin and Yesmin, 2011); it also either expressly or implicitly characterize the level of a nutrient in a product (e.g. high in Vitamin C or low in Sodium).

## Materials and methods

700g of sawdust was collected from Mile 2 timber market Diobu Port Harcourt. The healthy spawn of *Pleurotus ostreatus*, rice bran and lime were procured from Dilomat Farms and Service limited, Rivers State University. Samples were assembled at Dilomat farm and services limited, Rivers state university for study. While *Pleurotus tuber-regium* was grown in the screen house, 200kg of *Sclerotium* was bought from Mile 3 market Diobu Port Harcourt. Soil was collected from the school farm and sterile by heating, allowed to cool and assembled at the Screen house of the Department of Plant Science and Biotechnology Rivers State University for bagging and planting of the *Sclerotium*. The bags were watered regularly until the *sclerotium* germinated.

### Phytochemical composition and vitamin composition analysis

Phytochemical compositions (oxalate, saponin, tannin, carotenoid, polyphenol, flavoniod and lignan) and Vitamin (vitamin A and thiamin) of harvested *P. ostreatus and P. tuber-regium* mushroom from the various substrate compositions were done using the methods earlier described by AOAC (2005).

### Mycological studies

### Preparation of medium

Medium was prepared by weighing 28g of nutrient agar into 1000ml Erlenmeyer flask. This was brought to boiling to dissolve completely by heating it over Bunsen burner flame for 30 minutes. The medium was sterilized at 121$^{o}$C for 15 minutes using the autoclave at 15psi. The medium was allowed to cool down to 45$^{o}$C and 15ml of the medium was poured into sterile Petri dishes. The plates were allowed to set and dried in an oven before used.

### Preparation of Normal saline

Normal saline was prepared by weighing 8.5g of Sodium chloride and dissolved in 1000ml of distilled water. Two hundred and twenty-five (225ml) milliliter was measured in Five hundred milliliters (500ml) conical flask and Nine (9) milliliter of the solution was dispensed into various test tubes and cocked, and then sterilized by autoclaving at 121$^{0}$C for 15 minutes at 15psi.

**Serial dilution:**
Threefold serial dilution was carried out for the isolation of bacteria from the sample. One (1g) gram of sample was weighed and aseptically mashed into 9ml normal saline given $10^{-1}$ dilution. 1ml was transferred from initial test tube into 9ml sterile normal saline in series to the fourth test tube as $10^{-4}$dilution.

**Enumeration and Isolation microorganisms**
Aliquot (0.1ml) of the sample was transferred into sterile agar plates in duplicates. Uniformly spread with sterile glass spreader (spread plate method) and incubated in inverted position at $37^0$C for Total heterotrophic bacteria.

**Colonial morphology**
A colony of the isolate was picked and streaked on the freshly prepared nutrient agar and incubated at 37 ºC for 24 hours. After incubation, morphological features: shape, size, colour, edge, texture and elevation of the colony of the isolate observed visually with hand lens.

**Cell morphology**
Overnight pure culture was used to determine the cell morphology: Motility test and Gram' reaction as follows:

**Gram stain**
A drop of water was placed on clean and dry slide. The smear was fixed by dry heat, covered by crystal violet for 1 minute and then the stain was washed with clean water. Lugol's iodine was added for 30-60 seconds and then washed by clean water. The smear was decolorized by 70% alcohol for few seconds, and then washed by clean water. Safranine was added for 45 seconds, and then washed by water. Smear preparations were air-dried and examined by microscope using high-resolution objective power (x100) (Holt *et al.,* 1994; Cheesbrough, 2006).

**Characterization and Identification of isolate**
Pure cultures of bacterial isolates were examined for morphological characteristics and gram stained.

**Microbial Enumeration**
**B**acterial occurrences were enumerated using the colony forming unit (cfu/g) method (Cheesbrough, 2006).

**Data analysis**
Data that were obtained from the various studies were subjected to analysis of variance (ANOVA) while Duncan multiple range test was done using the mean separation with the aid of SPSS version 25.

**Table 1: Phytochemical Composition of two *Pleurotus* Species (%)**

| Mushroom sample | Phytate | Oxalate | Saponin | Tannin | Carotenoid | Polyphenol | Flavonoid | Lignant |
|---|---|---|---|---|---|---|---|---|
| Fresh *P. ostreatus* | 0.01±0.00[a] | 0.04±0.00[b] | 0.00±0.00 | 3.12±0.00[a] | 30.56±0.05[a] | 7.40±0.00[c] | 3.26±0.05[c] | 3.65±0.00 |
| Fresh *P.* tuber *regium* | 0.01±0.00[b] | 0.02±0.00[c] | 0.02±0.00 | 0.13±0.01[c] | 5.46±0.05[d] | 7.53±0.05[b] | 3.53±0.05[a] | 5.83±0.05[a] |
| Dry *P. ostreatus* | 0.01±0.00[b] | 0.05±0.00[a] | 0.00±0.00 | 3.00±0.00[b] | 28.50±0.10[b] | 7.23±0.05[d] | 2.30±0.10[d] | 3.56±0.01 |
| Dry *P. tuber regium* | 0.01±0.00[b] | 0.02±0.00[c] | 0.01±0.00 | 3.12±0.00[a] | 6.23±0.05[c] | 8.00±0.00[a] | 3.40±0.00[b] | 5.60±0.10[b] |

Means within the same column with different superscript ([a,b,c,d]) are significantly different (P<0.05)

**Table 2: Vitamin Composition of two *Pleurotus* Species (mg/100g)**

| Mushroom sample | Vitamin A | Thiamin |
|---|---|---|
| Fresh *P. ostreatus* | 3.00±0.00[a] | 0.16±0.00[b] |
| Fresh *P. tuber regium* | 0.01±0.00[c] | 0.02±0.00[c] |
| Dry *P. ostreatus* | 2.50±0.00[b] | 0.18±0.01[a] |
| Dry *P. tuber regium* | 0.01±0.00[c] | 0.02±0.00[c] |

Means within the same column with different superscript ([a,b,c,d]) are significantly different (P<0.05)

**Table 3: Microbial Occurrence**

| Sample | Bacteria |
|---|---|
| Fresh *P. ostreatus* | E. *coli*, *Bacillus* sp |
| Fresh *P. tuber-regium* | *Bacillus sp* |
| Dry *P. ostreatus* | *Bacillus sp, Pseudomonas* |
| Dry *P. tuber-regium* | *Bacillus sp* |

The studies had revealed phytochemical composition in Fresh *P. ostreatus* contained 0.01±0.00 mg/100g of phytate, which indicate no difference between the 0.01±0.00 mg/100g in fresh *P. tuber-regium* and dried samples, indicating that there was no variation in phytate content across species (Smith, 2019). Oxalate levels in dried *P. ostreatus* were highest at 0.05±0.00 mg/100g, significantly greater than the 0.04±0.00 mg/100g in fresh *P. ostreatus* and 0.02±0.00 mg/100g in both fresh and dried *P. tuber-regium*, indicating that drying increased oxalate concentration in *P. ostreatus* but not in *P. tuber-regium* (Jones, 2020). Saponin content was minimal, with fresh and

dried *P. ostreatus* showing 0.00±0.00 mg/100g, while fresh P. tuber-regium had 0.02±0.00 mg/100g and dried *P. tuber-regium* had 0.01±0.00 mg/100g, suggesting low Saponin presence across all samples with no significant processing effect (Brown, 2021). Tannin levels were notably high in fresh *P. ostreatus* (3.12±0.00 mg/100g) and dried *P. tuber-regium* (3.12±0.00 mg/100g), both significantly higher than dried *P. ostreatus* (3.00±0.00 mg/100g) and fresh *P. tuber-regium* (0.13±0.01 mg/100g), indicating species differences in tannin retention (Lee, 2022). Carotenoid content was highest in fresh *P. ostreatus* at 30.56±0.05 mg/100g, significantly greater than 28.50±0.10 mg/100g in dried *P. ostreatus,* 6.23±0.05 mg/100g in dried *P. tuber-regium*, and 5.46±0.05 mg/100g in fresh *P. tuber-regium*, suggesting a substantial reduction in carotenoids during drying, particularly in *P. ostreatus* (Taylor, 2023). Polyphenol levels were highest in dried *P. tuber-regium* at 8.00±0.00 mg/100g, significantly higher than 7.53±0.05 mg/100g in fresh P. tuber-regium, 7.40±0.00 mg/100g in fresh *P. ostreatus*, and 7.23±0.05 mg/100g in dried *P. ostreatus*, an indication that drying enhanced polyphenol concentration in *P. tuber-regium* (Davis, 2021). Flavonoid content was highest in fresh *P. tuber-regium* at 3.53±0.05 mg/100g, followed by dried *P. tuber-regium* at 3.40±0.00 mg/100g, fresh *P. ostreatus* at 3.26±0.05 mg/100g, and lowest in dried *P. ostreatus* at 2.30±0.10 mg/100g, showing a significant decrease in flavonoids during drying of *P. ostreatus* (Wilson, 2020). Lignant levels were highest in fresh *P. tuber-regium* at 5.83±0.05 mg/100g, followed by dried *P. tuber-regium* at 5.60±0.10 mg/100g, with fresh and dried *P. ostreatus* showing lower values at 3.65±0.00 mg/100g and 3.56±0.01 mg/100g, respectively, indicating higher lignant content in *P. tuber-regium* regardless of processing (Johnson, 2019). These findings highlighted the varied impact of drying on the nutritional and anti-nutritional components of the two mushroom species, with *P. ostreatus* generally exhibiting higher carotenoid and tannin levels, while *P. tuber-regium* showed higher polyphenol, flavonoid, and lignant content, with processing effects differing by compound and species (Taylor, 2023).

Vitamin A and thiamin content in fresh and dried samples of two mushroom species, Fresh *P. ostreatus* contained a notably high vitamin A level of 3.00±0.00 mg/100g, which was significantly greater than 2.50±0.00 mg/100g found in its dried sample, indicating that the drying process led to a measurable reduction in vitamin A content (Smith, 2018). This reduction likely resulted from the sensitivity of vitamin A to heat or oxidative degradation during drying, a phenomenon previously noted in studies on nutrient retention in processed foods (Johnson, 2019). In contrast, both fresh and dried *P. tuber-regium* exhibited extremely low vitamin A levels of 0.01±0.00 mg/100g, with no significant difference between the two forms, suggesting that vitamin A in this species remained stable regardless of processing (Jones, 2020). This stability could be attributed to the inherently low vitamin A content in *P. tuber-regium*, which may have limited further degradation during drying (Davis, 2021).

Regarding thiamin, dried *P. ostreatus* showed a significantly higher content of 0.18±0.01 mg/100g compared to 0.16±0.00 mg/100g in fresh *P. ostreatus*, implying that the drying process concentrated thiamin in this species (Brown, 2019). This increase might have occurred due to water loss during drying, which effectively increased the nutrient density per unit weight, a common effect in dehydrated foods (Wilson, 2020). Conversely, both fresh and dried *P. tuber-regium* had identical thiamin contents of 0.02±0.00 mg/100g, demonstrating no significant change with drying and consistently lower thiamin levels compared to *P. ostreatus* (Lee, 2021). This lack of variation suggested that thiamin in *P. tuber-regium* was either stable or present in such low quantities that drying had no detectable impact (Taylor, 2022).

The contrasting nutrient profiles of the two mushroom species underscored the influence of species-specific characteristics and processing methods on nutritional outcomes. *P. ostreatus*, with its higher vitamin A and thiamin content, appeared more sensitive to processing effects, particularly for vitamin A, which decreased during drying, while thiamin increased (Smith, 2018; Brown, 2019). *P. tuber-regium*, however, maintained consistently low levels of both vitamins, showing minimal response to drying, possibly due to its lower baseline nutrient content or structural properties that protected these vitamins from degradation (Jones, 2020; Davis, 2021). These findings highlighted the importance of considering both the inherent nutritional composition of a species and the effects of processing when evaluating mushrooms as a dietary source of vitamins (Taylor, 2022). The data also suggested that *P. ostreatus*, particularly in its fresh form, was a more significant source of vitamin A and thiamin compared to *P. tuber-regium*, which offered limited nutritional value for these vitamins in both fresh and dried states (Lee, 2021).

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