Microbiological Evaluation of Cultivated Oyster Mushrooms (*Pleurotus ostreatus*) During Storage.

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ABSTRACT

Globally, the cultivation of *Pleurotus ostreatus* has risen tremendously due to their ability to grow on a wide range of cheaply available agricultural wastes. However, they are highly perishable and susceptible to contamination by microorganisms. Contamination of dried food by microorganisms has become a major health concern. The aim of this study was to evaluate the microbiological properties of dried cultivated *Pleurotus ostreatus* during storage. Fresh cultivated *P. ostreatus* were dried in hot air oven at 55°C for 72 hours. A portion was sun dried for five days to constant weight. Samples were taken at day 0, 30, 60, 90, and 120. Moisture content (MC) was determined using standard methods. MC of sun dried varied between 9.00% and 12.20%. MC of oven dried varied from 7.00% and 10.00%. The MC of the sun dried and oven dried samples were within the acceptable limit for dried mushrooms. Isolation, enumeration and identification of microorganisms were done using standard microbiological techniques. Mean total bacterial count (TBC) of sun dried varied from 2.8 x 10^5 CFU/g to 6.5 x 10^5 CFU/g. TBC for oven dried varied from 1.1 x 10^5CFU/g to 4.2 x 10CFU/g. Frequency of occurrence of bacteria: *Escherichia coli* 4(20%), *Staphylococcus aureus* 7(35%), and *Pseudomonas aeruginosa* 9(45%). Mean total fungal count (TFC) for sun dried varied from 2.3 x 10^2CFU/g to 6.2 x 10^2CFU/g. TFC for oven dried was 1.1 x 10^2 CFU/g to 2.0 x 10^2CFU/g. Frequency of occurrence of fungi: *Aspergillus sp* 15(75%), *Fusarium sp* 3(15%), and *Rhizopus sp* 2(10%). Microbial load of sun and oven dried increased with storage time. Post-harvest losses, shortened shelf life and health risk can be ameliorated by evaluating and understanding the microbiological properties of microbial isolates during storage.

Keywords: Pleurotus ostreatus, Microbiological, Cultivated, Fungi, Bacteria

INTRODUCTION

According to Hammond *et al* (2015), the processing and consumption of dried foods and food ingredients have been appreciated in many countries due to convenient storage and transportation in recent times. The process of drying food is a preservation method based on dehydrating water from food, thus suppressing the growth of microorganisms (Deng *et al.,* 2019). The storage temperature, relative humidity, and packaging materials can cause contamination of dried foods (Guo *et al.,* 2023). Dried foods and food ingredients can get contaminated with microorganisms such as *Escherichia coli* (El-Prince *et al.,* 2023), Cronobacter sp (Cechin *et al*., 2022), *Salmonella sp* (Makinde *et al.,* 2020) and *Micrococcus luteus* (Okike *et al*., 2023).

Salman and Mudalal (2022) opined that dried foods can also be contaminated by molds, some of which can produce aflatoxin, ochratoxin, and other mycotoxins. To maintain the quality of dried foods, it is necessary to maintain the recommended storage temperatures and relative humidity (Elik *et al.,* 2019).

Pleurotus ostreatus, belongs to the Kingdom Fungi, Division Basidiomycota, Class Agaricomycetes, Order Agaricales, Family Pleurotaceae and Genus Pleurotus (Deepalakshmi and Mirunalini, 2014).

The nutritional composition of oyster mushroom can be compared to that of eggs, milk and meat and are rich in water (90%), proteins, vitamin (thiamine, riboflavin, folic acid, and niacin) and other minerals (P, Ca, Fe, K, and Na) (Oei, 2023). Mushrooms can provide compounds in sufficient quantities for a human nutritionbalancing diet (Ishara *et al.,* 2018). Oyster mushrooms are well known for their use for food and medicine. They thrive well on substrates made from a wide range of agricultural and industrial wastes, including cotton wastes, sawdust, cereal

stover, corncob, wheat, paddy straw, and sugarcane bagasse (Ragunathan and Swaminathan, 2003; Nnebechukwu *et al.,* 2025). They have a short cultivation cycle when compared to other mushrooms.

Despite all the culinary, nutritional and medicinal properties of *Pleurotus ostreatus*, they have a short shelf life of about 48 to 72 hours at room temperature. According to Jonathan and Osho (2010), mushrooms are very perishable because they are prone to microbial contamination and as a result of this Yoruba people of Nigeria usually collect fresh fruit bodies of mushrooms during the rainy season and preserve them by sun drying and utilize them during dry season when vegetables prices had gone up due to scarcity. Mushrooms cannot be completely sealed in a pack because they will decay resulting to browning, wilting, liquefaction, texture loss, aroma loss, and flavor loss (Kamal *et al*. 2015). Decay in mushrooms could also be attributed to bacterial and fungal contamination of the tissues.

According to Kamal *et al*. (2015), browning in mushrooms might be caused by a synergistic combination of microbial and auto-enzymatic reaction on their tissues. Mushroom cultivation in Nigeria is characterized by low local processing capacity and storage difficulties, due to the unavailability of drying and storage facilities. Mushrooms are highly perishable food sources which results in qualitative and quantitative post-harvest losses. Mushrooms can be dried and converted into value added products that will reduce losses. This includes products such as cookies, biscuits, breakfast cereals and flours. To ensure the safety of stored mushrooms, it is essential to have a clear knowledge of its microbiological and organoleptic peculiarities. Mushrooms can be dried and grounded into powder or left as flakes. They can subsequently be used in making culinary delicacies. During mushroom drying process, unwholesome practices including poor handling, poor hygiene and processing methods can result in microbial contamination. The present investigation therefore seeks to evaluate the microorganisms in different dried *Pleurotus ostreatus* during storage.

MATERIALS AND METHOD

Study location and Duration

This study was carried out in the Mushroom Unit of the Department of Food Science and Industrial Biotechnology, BARC, SHESTCO in Sheda, Kwali Area Council, Federal Capital Territory. Nigeria over a period of one hundred and twenty days.

Processing of mushrooms

Fresh fruit bodies of *Pleurotus ostreatus* cultivated in the Mushroom Unit of BARC, SHESTCO were washed thoroughly to remove sand and other debris. They were sliced into tiny pieces using a clean knife. The sliced mushrooms were divided into two sets. The first set was sundried for five days and the second set was dried in a hot air oven at 55℃ for 72 hours to constant weight. The dried samples were stored in zip-loc bags at an ambient temperature one hundred and twenty days. Samples were collected on day 0, 30, 60, 90, and 120.

Determination of Moisture Content

The moisture content of the dried mushrooms samples was carried out as described by the Standard Methods of the Association of Official Analytical Chemists (AOAC, 2019). Two grams of the sun dried and oven dried samples were weighed into clean dry crucibles and dried at 130 °C for 1 hour. The cooling of the samples was done in a desiccator and weighed using a sensitive analytical weighing balance.

Isolation, Identification and Confirmation of Bacterial Isolates

Serial dilution was carried out as outlined by Cheesbrough (2006). One gram (1g) each of the different dried cultivated *Pleurotus ostreatus* under storage were homogenized in nine (9.0) ml sterile distilled water to form a stock solution. One milliliter (1 ml) of the homogenate was dispensed serially into 9 ml sterile distilled water; the same procedure was repeated serially up to tube 10^{5} dilution factor. Spread plate technique in which 0.1ml aliquots from the 10^{3}, 10^{4} and 10^{5} tubes were inoculated aseptically onto the dried surfaces of sterile plates of Nutrient Agar, Eosin Methylene Blue Agar, MacConkey agar, Mannitol Salt Agar, and Centrimide Agar in duplicates for isolation of bacteria and The plates were incubated at 37°C for 48 hours. (All media were prepared according to manufacturer's instructions).

Mean colony counts were calculated and expressed as colony forming units per gram (CFU/g) of the sample analyzed.

Coliform Forming Unit/Gram was calculated as: Average number of colonies × Total dilution factor divided by volume plated (aliquot). The identity of the bacterial isolates was authenticated by comparing their characteristics with those in Bergey's Manual of Systematic Bacteriology (Prescott *et al.,* 2011).

Purification of Bacterial Isolates

The pure cultures of the bacteria were obtained by repeated streaking aseptically on fresh nutrient agar plates. They were incubated for 24 hours at 37°C after which the isolates were inoculated on nutrient agar slants in bijoux bottles and stored in a laboratory refrigerator at 4°C for future use.

Isolation, Identification and Confirmation of Fungal Isolates

The Total Fungal Count was isolated on Sabouraud Dextrose Agar (SDA) supplemented with

Chloramphenicol. The spread plate technique as described by Prescott *et al*., (2011) was used. A 0.1ml aliquot of 10^2 was inoculated in triplicates on the surface of SDA plates supplemented with Chloramphenicol, and then, spread evenly with a sterile glass spreader. The inoculated plates were incubated at 28 °C for 7 days after which the colonies were counted, and the mean of the count was recorded (Douglas and Robinson, 2019). A sterile inoculating pin was used to pick spores of the fungal isolates. They were then placed on a clean glass slide, and gently spread with inoculating pin and a drop of lactophenol cotton blue. The mixture was covered with a cover slip, and then, observed with a microscope. Microscopic and cultural characteristics were used in the identification of the fungi. Identification was authenticated by comparison with a fungal atlas (Kidd *et al.,* 2016).

RESULT

Table 1: Percentage Moisture Content of Stored Cultivated *Pleurotus ostreatus* (%)

Method of Processing					
	Day 0	Day 30	Day 60	Day 90	Day 120
Sun dried	12.20%	10.12%	10.00%	9.90%	9.00%
Oven dried	10.00%	8.00%	7.70%	7.50%	7.00%

Table 2: Mean Total Bacterial Count in Stored Cultivated Pleurotus ostreatus (CFU/g)

Method of Processing					
	Day 0	Day 30	Day 60	Day 90	Day 120
Sun drying	2.8×10^5	3.5×10^5	4.0×10^5	6.1×10^5	$6{,}5 \times 10^5$
Oven drying	1.1×10^5	2.3×10^5	2.4×10^5	3.0×10^5	4.2×10^5

Table 3: Morphological and Biochemical Characteristics of Bacterial Isolates from Stored Cultivated Pleurotus ostreatus

	Tentative Identity	Tentative Identity	Tentative Identity
	Escherichia coli	Staphylococcus aureus	Pseudomonas aeruginosa
Colony Edge	Irregular	Entire	Entire
Media	Metallic green sheen on Eosin methylene blue agar	Golden yellow on Mannitol salt agar	Bluish green on nutrient agar
Shape	Short red rods	Cocci in clusters	Rods
Grain Stain	-	+	-
Indole	+	-	-
Catalase	+	+	+
Coaglulase	-	+	-
Oxidase	-	-	+

Methyl Red	+	+	-
Voges Proskauer	-	-	+
Citrate	-	-	+
Glucose	+	-	-
Lactose	+	+	-
Fructose	+	+	+
Maltose	+	+	-
Sucrose	+	+	-

Table 4: Frequency of Occurrence of Bacterial Isolates (%)

Bacterial Isolate	**Frequency of Occurrence**	**Percentage %**
Escherichia coli	4	20
Staphylococcus aureus	7	35
Pseudomonas aeruginosa	9	45
	20	100

Table 5: Mean Total Fungal Count in Stored Cultivated *Pleurotus ostreatus* (CFU/g)

Method of Processing	Day 0	Day 30	Day 60	Day 90	Day 120	
Sun drying	2.3×10^2	3.0×10^2	3.3×10^2	3.6×10^2	6.2×10^2	
Oven drying	1.1×10^2	1.5×10^2	1.6×10^2	1.6×10^2	2.0×10^2	

Table 6: Microscopic and Macroscopic Characteristics of Fungi from Stored Cultivated Mushroom

S/N	**Microscopy**	**Macroscopy**	**Probable organism**
1	Having conidial head, septate, smooth conidiophore that ends with swollen vesicles.	White and powdery, rapidly turning black on maturity	*Aspergillus sp*
2	Non septate hyphae, forming stonlons that give rise to rhizoids	Fast growing pink cottony colonies	*Fusarium sp*
3	Branched, septate hyphae forming mycelium and conidia.	Fast growing cottony white turning to yellow with pale reverse.	*Rhizopus sp*

Table 7: Frequency of Occurrence of Fungal Isolates from Stored Cultivated Mushroom

Fungal Isolate	Frequency of Occurrence	Percentage (%)
Aspergillus sp	15	75
Rhizopus sp	2	10
Fusarium sp	3	15
Total	20	100

DISCUSSION

According to Liu *et al.,* (2022), the Shelf-life of food products is determined by the quantity of water in the product. In the present study, the MC of cultivated *Pleurotus ostreatus* for the sun dried samples varied between 9.00% and 12.20%. The MC of the oven-dried sample was 7.00%.to 10.00% as depicted in Table 1. This values fall within the recommended moisture content standard for dried edible fungi if 13% mm as recommended by Codex Alimentarius Commission (1981). In preparing mushrooms for storage, they should be properly dried. The result is in consonance with the result of Ilondu (2017) who observed that sun-dried samples of *C. esculenta* chips retained a high percentage of moisture when compared to the oven-dried ones. The temperature, relative humidity and other factors in an environment can affect the retention of moisture in the food substances. The lower the MC of a food, the longer the shelf life of the food. In the present study, the MC decreased with increasing storage time. Afolabi, (2014) stated that the minimum quantity of moisture at which the product is safe for long time storage is considered a safe moisture level. Zhang *et al.,* (2020) recorded that dried foods tend to absorb water as the relative humidity of the surrounding air is increased.

Table 2 depicts the mean total bacterial count. (TBC) in CFU/g. Microbial pathogens are ubiquitous and abundant in nature; this makes it easier for them to be move for one habitat to the other. There was a gradual increase in the TBC of the samples in each of the drying methods. As shown in Table 2, the TBC sun dried samples varied Between 2.8 x 10^5CFU/g and 6.5 x 10^5CFU/g. The TBC of the oven dried samples ranged from 1.1 x 10^5CFU/g to 4.2 x 10^5CFU/g. The TBC of the sun dried and oven dried samples increased with increase in storage time. Dried mushrooms with low water activity having a total aerobic count (TAMC) of $\leq 10^4$ to 10^5 cfu/g are acceptable (ICMSF, 2011).

As shown Table 3, the morphological and biochemical characteristics of the isolates showed that the isolate phenotypically identified as *Escherichia coli* grew as metallic green sheen on Eosin Methylene Blue Agar. They were gram negative, short red rods. They were indole, catalase, methyl red positive; and coagulase, citrate, oxidase, Voges proscauer negative. They fermented all the sugars tested. The organisms phenotypically identified as *Staphylococcus aureus* presented as golden yellow on Mannitol salt agar. Microscopically they are Gram negative Cocci in clusters. They are indole, Voges proscauer, citrate, oxidase negative. They fermented all the sugars except glucose. Organisms tentatively identified as *Pseudomonas aeruginosa* were bluish green on nutrient agar. They are Gram negative rods. Indole, coagulase, methyl red negative. They are catalase, oxidase, Voges proscauer and citrate positive. They fermented all the tested sugars except fructose.

Table 4 depicts the frequency of occurrence of bacterial isolates from the cultivated mushrooms during storage as *Escherichia coli* 4(20%)*, Staphylococcus aureus* 7(35%), *Pseudomonas aeruginosa* 9(45%). According to Kharel *et al.,* (2016), *Staphylococcus sp, Pseudomonas sp, and Bacillus sp a*re pathogenic. The detection of *E. coli* in the present study indicates faecal contamination. The possible source of contamination is through the raw materials. For example, the rice bran used in this study were, prior to processing were exposed to contamination by grazing animals and faecal droppings from birds. A study presented by Ajis *et al*. (2017), that 15.0% of the tested samples had a load of coliforms that was above 3.0 log cfu/g corroborates with the detection of *E.coli* the presence study. Gavahian *et al.* (2018) stated that processing conditions such as drying and heat treatment might reduce microbial levels, but recontamination could take place during the post processing or storage practices. The presence of these isolates in the samples could also be as a result of the settling of dust and other debris on the surface of the sample during drying especially during sun drying. The presence of these pathogenic organisms in the samples has implications for food safety and quality. Foodborne pathogens can survive for several days in low-water activity foods and in food preparation environments due to inadequate and improper drying as observed in sun-dried samples in this study (Beuchat *et al.,* 2013). The growth conditions for microorganisms depended on specific intrinsic and extrinsic factors such as temperature, water activity, pH, oxidation-reduction potential, microbial interactions, and nutrient content (Guo *et al.,* 2023).

Table 5 shows the mean total fungal count (TFC) from the samples. The TFC of the sun dried samples varied between 2.4 x 10^2 CFU/g and 6.2 x 10^2CFU/g while the oven dried ranged from 1.1 x 10^2CFU/g to 2.2 x 10^2CFU/g. As the storage time increased, the TFC increased in sun and oven dried samples. In a similar work, Jonathan and Osho (2010) reported a total fungal count in the fresh samples of *P. ostreatus* was 3.0×10^1(CFU/g) and increased to 1.35×10^4 (CFU/g) after 105 days of storage and decreased to 1.60×10^3(CFU/g) after 140 days. The acceptable CFU/g for yeasts and molds in dried mushrooms is $\leq 10^2$ - 10^3 Cfu/g. The total fungal count values for the present work fall within the recommended standards.

Table 6 shows the microscopic and macroscopic characteristics of fungal isolates from dried cultivated *Pleurotus ostreatus* under storage. The organisms tentatively identified as *Aspergillus sp* presented macroscopically as rapidly growing colonies rapidly turning black and powdery. *Fusarium sp* appeared as pink velvety colonies. *Rhizopus sp* are fast growing cottony white turning to yellow with pale reverse.

Table 7 depicts the frequency of occurrence of fungal isolates in the sun and oven dried samples during storage as *Aspergillus sp* 15(75%), *Rhizopus sp* 2 (10%), *Fusarium sp* 3(15%).The result of the presence study corroborates with that of Jonathan and Osho (2010) who reported isolating *Aspergillus niger*, *Fusarium oxysporum, A. tamarii, A. flavus, Penicillium chrysogenum, P. oxalicum and F. compaticum* from dried *Pleurotus ostreatus* and *Pleurotus pulmonaris* during storage form 0 to 105 days. The most frequently occurring fungi are *Aspergillus niger* while the least frequently occurring are *Rhizopus sp.*

The detection of these microorganisms in the sun dried and oven dried samples is significant. According to Liu *et al* (2022), bacteria, yeasts, and molds, including foodborne pathogens, are able to survive the drying process and survive for several months or years in low-moisture foods ($a_w < 0.85$).

CONCLUSION

The study revealed the health implications of inadequate and improper processing of cultivated *Pleurotus ostreatus* under storage. The detection of pathogenic microorganisms in the dried mushroom could be a pointer to a lack of adherence to stringent hygienic practices. Future studies should focus on the molecular characterization of these isolates. The susceptibility of the isolates from dried cultivated mushrooms during storage should be subjected to the activity of antibacterial and antifungal agents.

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