BACTERIOLOGICAL ASSESSMENT OF COMMERCIALLY RETAILED OYSTER MUSHROOM FOR THE INCIDENCE OF SOME SELECTED PATHOGENS

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BEING A PROJECT WORK SUBMITTED IN THE DEPARTMENT OF BIOLOGICAL SCIENCE LABORATORY TECHNOLOGY, SCHOOL OF APPLIED SCIENCE AND TECHNOLOGY AUCHI POLYTECHNIC, AUCHI, EDO STATE

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF HIGHER NATIONAL DIPLOMA (HND) IN BIOLOGICAL SCIENCE LABORATORY TECHNOLOGY, MICROBIOLOGY OPTION

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CERTIFICATION

I certify that this project work carried out by IGBERASE FAITH OMOYEME (Matriculation Number AST/2382060379) and AIRIHENBUWA SONIA (Matriculation Number AST/2382071050) of the Department of Biological Science Laboratory, Auchi Polytechnic, Auchi. In partial fulfilment of the requirements for the award of Higher National Diploma (HND) in Biology/Microbiology and Microbiology Option.

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DEDICATION

We dedicate this project to the Glory of God Almighty for the knowledge, vision, understanding and his profound mercies towards our lives and the times we spent in Auchi Polytechnic, Auchi.

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Our most sincere gratitude go to God Almighty who has brought us this far in our academic career. We give Him all the thanks for the wisdom and knowledge in putting these scripts together and also for the successful completion of this project work.

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ABSTRACT

This research is aim at investigating the bacteriological assessment of commercially retailed oyster mushroom for the incidence of some selected human pathogens such as *Salmonella* spp, *Staphylococcus aureus* and *Escherichia coli*. The scope of this study covers the bacteriological assessment of commercially oyster mushroom (Pleurotus spp) that influence the microbial growth of oyster mushroom retailed in Benin metropolis. A total of four samples of oyster mushroom were bought from different certified mushroom farms in Ovia North East Local Government Area of Edo State. Two of the samples were dried packaged oyster mushroom while the remaining two were fresh oyster mushroom which were hygienically prepared and labelled A and B. The two dried oyster mushroom were oven dried and labelled C and D. The oyster mushroom samples were analyzed for total viable aerobic plate count and identification of the various contaminating bacteria isolate. The bacteria loads found in the fresh oyster mushroom ranges from 1.6×10^3 and the highest figure being 2.7×10^4 while those of the dry oyster mushroom had the highest amount of microbial contaminants with the least figure being 8.1×10^5 and the highest figure being 8.4×10^6. After the analysis three organisms were isolated *Salmonella* spp, *Staphylococcus aureus* and *Escherichia coli* and only *Staphylococcus aureus* and *Salmonella* spp were present. The bacteria present in oyster mushroom is undesirable and hence may cause several illness to man when consumed. Therefore, oyster mushroom sellers should ensure that when carrying out the oyster mushroom preparation and production should be in a clean and a well sanitized environment.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Oyster mushroom (fungi of genus pleurotus) are the second largest cultivated mushroom species group in the world, constituting approximately 27% of the total global production. The pleurotus species are further divided into subspecies that *includes Pleurotus ostreatus, Pleurotus cornucopiae, Pleurotus cystidiosus, Pleurotus tuber-regium, Pleurotus citrinopileatus* and *Pleurotus flabellatus*. They thrive on most of all hardwoods, wood by-products such as sawdust, paper, pulp sludge, all the cereal straws, corn and corn cobs, coffee residues such as coffee grounds, hulls, stalks and leaves, banana fronds and bottles. The pleurotus species were adapted for cultivation on small and large scale farms as well as in tropical and temperate climates on various lingnocellulosic agro and forest wastes (Myronycheva *et al.*, 2017).

Oyster mushroom *Pleurotus spp* is one of the important edible mushroom on the nutritional, health and environmental levels. On the nutritional level, the fruiting bodies of this mushroom is classified as high-protein materials and high content of most mineral salts and vitamins. On the health level, the fruit bodies are considered a healthy, balanced food described for most diseases of the time. On the environmental level, the cultivation of oyster mushrooms is cultivated on media composed mainly of agricultural residues, such as the residues of most

agricultural crops, and thus contributes to the biological cleaning of these wastes, increasing their economic value, therefore the productivity of this mushroom increased globally to occupy the second place in production and consumption after *Agaricus bisporus* (the white cultivated mushroom) (Abdullah and Maha, 2022).

Oyster mushroom is also used for myco-remediation. Its yield is exposed to many biotic agents like bacterial, fungal, viral and nematodal diseases, causing huge losses that may reach more than 20% in the quantity and quality of mushrooms. The competitions fungi are among the most important of these agents, the growth of competitions fungi such as *Penicillium*, *Aspergillus*, *Fusarium*, and others in mushroom media led to occupy large areas of the medium and deplete nutrients as well as their production of metabolites that inhibit the growth of mushroom, then decreased mycelium growth and consequently a decrease in the yield of mushrooms, while the pathogenic fungi form direct infections on the mycelium and fruit bodies of mushroom, such as green rot disease caused by *Trichoderma* dry bubble disease caused by *Lecanicillium fungicola* and Cobweb disease caused by *Cladobotryum sp* which are the most destructive pathogens of the mushroom industry causes great losses in commercial production of mushrooms (Abdullah and Maha, 2022).

Oyster mushroom is also used in soups and straw. They contain small amounts of arabitol, a sugar alcohol, which may cause gastrointestinal upset in some people. It causes hypersensitivity for individuals in direct contact with its

spores particularly for long time (Ahmed *et al.*, 2019). Therefore, this project work was conducted to evaluate the microbial contents present in oyster mushroom.

1.2 STATEMENT OF PROBLEM

The contamination of oyster mushroom by some selected pathogens such as *Escherichia coli, Salmonella sp* and *Staphylococcus aureus* due to poor personal hygiene of the processor or sellers, poor environmental conditions or from packaging and storage of the oyster mushroom can lead to diseases and infection to humans who consume it.

1.3 AIM OF THE STUDY

The aim of this study was to evaluate the bacteriological assessment of commercially retailed oyster mushroom for the incidence of some selected human pathogens.

1.4 SCOPE OF THE STUDY

This study intends to analyze the bacteriological assessment of commercially retailed oyster mushroom for the incidence of some selected human pathogens such as *Escherichia coli, Salmonella sp* and *Staphylococcus aureus*.

1.6 SIGNIFICANCE OF THE STUDY

The importance of this study is to create awareness and enlighten farmers and consumers on the microbial contaminations of commercially retailed oyster mushroom, the factors that influence the growth of these microorganisms or

pathogens (for the incidence of some selected human pathogens such as *Escherichia coli, Salmonella sp* and *Staphylococcus aureus*), the diseases and infections (caused by these pathogens) consumers and handlers of the oyster mushroom is not hygienically and properly cultivated, harvested, processed and stored.

1.7 LIMITATION OF THE STUDY

Most problems encountered during this research work that limited the efficiency of this research include;

Lack of Finance: Due to increase in prices there was no enough funds to purchase the materials needed to carryout this research work.

Lack of Electricity: Most times there was no power supply during this research work.

Time: The deadline given to carryout this research work was not enough to complete the analysis.

1.8 OPERATIONAL DEFINITION OF TERMS

Microorganism: An organism that is too small to be seen by the aided eye, especially single celled organisms, such as bacterium.

Assessment: It is the action or an instance of making a judgment about something.

Pathogens: They are taxonomically widely diverse and comprise viruses and bacteria as well as unicellular and multicellular eukaryotes.

Oyster Mushroom: It is a common edible mushroom. It is a fruiting body macrofungus. It is retailed to the similarly cultivated king oyster mushroom.

Fruiting Body: It is the spore-producing organ of a fungus, often seen as a toadstool.

Macrofungus (Macrofungi): It refers to all fungi that produce visible fruiting bodies.

Sporophore: It is the spore-bearing structure of a fungus.

Fungus: Any group of spore-producing organisms feeding on an organic matter, including moulds, yeasts, mushrooms and toadstools.

Lamella: A thin layer, membrane or plate of tissue.

Truffles: A strong-smelling underground fungus that resembles an irregular, rough-skinned potato, growing chiefly in broadleaved woodland on calcareous soils.

Toxins: It is a poison of plant or animal origin especially one produced by or derived from microorganisms and acting as an antigen in the body.

Bioconversion: The conversion of organic materials into an energy source by processes involving living organisms.

Organic Substances: Materials that contain carbon bound to other atoms by covalence.

Secondary Metabolites: They are compounds that are not required for the growth or reproduction of an organism but are produced to confer a selective advantage to the organism.

Anti-Inflammatory: Chiefly of a drug used to reduce inflammation.

Anti-Oxidant: It is a substance that inhibits oxidation, especially one used to contract the deterioration of stored food products.

Anti-Cancer: It is a drug or substance used in the treatment of cancer.

Anti-Bacteria: A substance that kills bacteria or stops them for growing and causing diseases.

Immunomodulatory: It is the change in the body's immune system caused by agents that activate or suppress its function.

Glycoprotein: It is any of a class of proteins which have carbohydrate groups attached to the polypeptide chain.

Polysaccharide: It is a carbohydrate (e.g. starch, cellulose or glycogen) whose molecules consist of a member of sugar molecules bounded together.

Food Intoxication: It refers to the consumption of toxic chemicals liberated or produced by bacterial growth in food.

CHAPTER TWO

2.0 LITERATURE REVIEW

Mushrooms are the fruiting bodies of spore bearing fungi, and have been defined as a "Macrofungus with a distinctive fruiting body, which can be either epigeous (grooving above ground) or hypogeous (growing below ground) and large enough to be seen with naked eyes and to be picked by hand. From an economic viewpoint, they are grouped into four categories: (1) edible mushroom (termed as food of the god) - they are fleshy e.g., *Agaricus bisporus*; (2) medicinal mushrooms e.g., *Ganoderma lucidum*; (3) poisonous mushrooms (previously known as 'toadstool') e.g., *Amanita phalloides*; and (4) miscellaneous category - with properties less well defined, and are tentatively grouped together as 'other mushrooms'. They mainly belong to division Basidiomycota and Ascomycota. In India climatic condition favours natural growth of mushrooms, also is very conducive to their cultivation. Some of the well-reported Indian mushrooms are species of - *Astraeus, Auricularia, Tuber morchella, Lycoperdon, Calvatia,* and *Termitomyces*. Mushrooms are commercially grown in both the tropical and sub - tropical regions of the world. Although the term "fruiting" technically applies to flowering plants, it is widely used in mycological literature to describe the formation of fungal sporocarps (Anuradha *et al.,* 2015).

Mushrooms are highly nutritious and contain 20-40% protein on a dry matter basis, which consists of all the essential amino acids required in the human diet. Their taste and delightful aroma make them a delicious and popular food in

restaurants throughout the world. The abundant agricultural waste found in Namibia offers opportunity for mushroom production. Moreover, spent substrate could be used as animal feed after mushroom cultivation and as compost to enrich soil for plant production. Therefore, the cultivation process of oyster mushroom can address the issue of soil waste disposal, economical gain and environmental protection. Cultivation of *Pleurotus* species, commonly known as Oyster mushrooms, is the most popular practice among small-scale farmers; mainly because it can fruit over a wide range of temperatures. The Pleurotus species are regarded as easy to grow and have broad adaptability to the environment in which they grow. This is the reason why their production worldwide has increased rapidly. *Pleurotus sajor caju* and *Pleurotus ostreatus* (oyster mushroom) are two of the choice edible mushrooms which can be cultivated in the tropics. Oyster mushroom production represents an opportunity for farmers interested in an additional enterprise and is a specialty option for farmers without much land (Shivute, 2020).

The term mushroom is generally used to indicate a stem or stalk, a cap (pileus), and the gills (lamellae). The part of the fungus that is usually visible is the sporophore, which is an umbrella-shaped structure that has fertile surfaces on its lamellae. The lamellate fungi are commonly called mushrooms or toadstools, according to whether they are edible or non-edible, respectively. Mushrooms are ancient species and have had different names; for example, Theophrastus used “truffles” for mushroom fruiting bodies (Buba *et al.,* 2018).

Mushroom is a fruiting body of macro fungus that is produced above ground and big enough to be seen with the naked eye and to be plucked by hand. Mushroom are parts of the livelihood of people in different parts of the world. This practice helps rural people reduce vulnerability to poverty and strengthen their livelihoods through a reliable income, the values and roles of mushrooms have been neglected, and all activities related to forest management are focused on maximizing wood products (Tartek *et al.*, 2017).

Figure 1: Diagram of Oyster Mushroom

Source: Hassan *et al.* (2022)

2.1 HISTORY OF EDIBLE MUSHROOM

The word mushroom is derived from the French word for fungi and moulds. However, since then, better and more effective methods have been

developed and there has been a huge increase in mushroom cultivation. In the last 50 years, the Netherlands has grown into the largest mushroom production country within the European Union, with an annual production of 270 million kilograms and more than 10,000 jobs. Next to China and the United States, the Netherlands holds 3rd place in the market. China is the largest producer of mushrooms in the category of top ten nations with a market share of 70 per cent followed by Italy 10.67 per cent, USA 5.29 per cent. Every year, millions of tons of mushrooms are cultivated worldwide. However, Poland was the largest exporter of mushroom in the world. In terms of consumption, Netherland with 11.62 kg per head per annum topped in the list of major mushroom consumers. In China, Japan, Poland and India, the per capita mushroom consumption stood below 1 kg per annum.

Since ancient times, mushrooms have been treated as a special kind of nutritious food. Greeks regarded mushrooms as commodity providing strength for warriors in battle and the Romans regarded mushrooms as the "Food of God". In the earlier times, mushrooms were collected from their natural growing habitats, but with the passage of time, several attempts have been made to domesticate mushrooms under controlled conditions. So far, more than 2,000 edible fungal species are widely accepted for human consumption, but only a few of them are commercially cultivated worldwide and only 5 mushroom species *viz; Agaricus bisporus*, *Pleurotus* spp., *Volvariella volvacea*, *Calocybe indica* and *Lentinula edodes* are popularly cultivated in different parts of India.

Mushrooms have been considered one of the world's greatest natural resources since they have the ability to transform required input into nutritional substance and high protein food. In the event of large increase in population resulting in scarcity of nutritious food, the mushrooms offer a good source of nutrition due to being rich in minerals and vitamins. At present, three mushrooms namely, the button mushroom (*Agaricus bisporus),* paddy straw mushroom (*Volvariella spp.)* and oyster mushrooms (*Pleurotus spp*.) are cultivated in different parts of the world. *Agaricus bisporus* is mostly grown on commercial scale throughout the world. Globally, mushroom is traded mostly in processed form.

2.2 COMMON SPECIES OF EDIBLE MUSHROOMS

Edible mushrooms are the fleshy fruit bodies of many species of macro-fungi that can be used in the human diet. The edibility criteria of mushrooms may depend on the absence of poisonous substances or toxins that are detrimental to human health. Edible mushrooms are mainly consumed for their nutritional value, medicinal features, and sweet taste. Several species of edible mushrooms are found the wild and raised for harvest, but some species are difficult to cultivate. The following are common species of edible mushrooms.

Table 1: Common Species of Edible Mushroom

S/N	Mushroom Species	Common English Name	Name of Family
1.	*Lycoperdon spp*	Puffballs	Agaricaceae
2.	*Coprinus comatus*	Shaggy mane	Agaricaceae
3.	*Pleurotus ostreatus*	Oyster mushroom	Ostreidae or Aviculidae
4.	*Boletaceae*	Boletes	Boletaceae
5.	*Laetiporus sulphureus*	Chicken mushroom	Fomitopsidaceae
6.	*Grifola frondosa*	Hen-of-the woods	Meripilaceae
7.	*Clavariaceae*	Club or antler mushroom	Gomphaceae
8.	*Hericium erinaceus*	Bearded tooth	Hericiaceae
9.	*Volvariella volvacea*	Paddy straw	Pluteaceae
10.	*Morchella*	Morels	Morchellaceae
11.	*Agaricus bisporus*	The white cultivated mushroom	Agaricaceae

Source: (Hassan *et al.*, 2022)

2.3 GENERAL METHOD OF CULTIVATION OF EDIBLE MUSHROOM

Mushroom is commercially cultivated worldwide for its incredible taste and medicinal and nutritional properties. Mushrooms are used as relish in many countries like China, Ghana, and many others. Oyster mushrooms has been reported to be cultivated and grown on wheat straw, grass, wood shavings, sawdust, compost waste and other organic nutrients. *Pleurotus ostreatus*, the pearl oyster mushroom or tree oyster mushroom, was first cultivated in Germany

as a subsistence measure during World War 1, and is now grown commercially around the world for food (Charles *et al.*, 2021).

Mushrooms are seasonal, commercial cultivation is therefore necessary to ensure constant availability. However, large-scale cultivation and processing of mushroom requires a good knowledge of the growth requirements and influence of the substrate. On their growth rate and nutritional composition. It been observed by some researchers that the yield and quality of oyster mushroom depends on the chemical and nutritional content of substrate. The method of cultivating oyster mushroom includes;

1. **Cultivation of Oyster Mushroom:** The pasteurized straw is spawned at 2-3% spawn on wet weight basis. 2-5kg wet substrate can be filled in each bag. Spawn can be mixed thoroughly or put in layers inside the bags. The bags can be kept either inside a room or inside hut and can be kept on ground, in tiers, hung from the roof of rack with the help of nylon rope. Make small perforation in the bags and should be kept at temperature 24 ± 2^0C.It takes two weeks for the whole bags to become white during spawn run. No light or fresh air is required for spawn run, rather keep rooms closed for induction of fruiting, the bags require diffused light and fresh air for 3-4 hours daily for production of normal fruit bodies. Large holes can be made in the bag or the whole polythene can be removed. In 3-4 flushes, one kg of dry straw can produce 0.5 to 1.0kg fresh mushrooms.

The temperature during cropping should be below 20^0C and humidity is maintained above 85%.

2. **Ready to Fruit Bags:** Individuals located in urban areas may not have access or be able to make small quantity of spawn, and also wet and heat straw and fill bags. Hence it is important that such growers are provided ready-to-fruit bags
3. **Harvesting and Post-Harvest Storage:** The mushroom is harvested by twisting and cut any straw of substrate that may be there on the stalk. Do not water the bags before harvesting. (Jannatul *et, al;* 2019).

2.4 HARVESTING AND POST-HARVEST STORAGE OF EDIBLE MUSHROOM

Harvesting mushrooms takes place in "flushes". The first flush is picked in 3 to 5 days and yields 15 to 20 kg/m2. If the mushrooms are mechanically harvest, in the form of once-over harvesting, this yields 22 to 26 kg/m2.

Mushrooms are highly perishable and get spoiled due to browning, wilting, liquefaction, loss of texture, aroma, flavour, etc, making it unsaleable. Most of the mushrooms, being high in moisture and delicate in texture, it cannot be stored for more than 24 hours at the ambient conditions prevailing in the tropics. Therefore, it becomes imperative to adopt scientific post-harvest management practice to extend its shelf life. The secret to mushroom storage is that they stay fresh longer if you take them out of their container. Wrap them in paper towels

placed in open plastic bags (paper bags are even better) and keep them in the fridge.

Fresh mushrooms are delicious, and their umami taste adds a unique richness to almost any dish. But, fresh mushrooms generally don't have a long shelf life. People often leave their fresh mushrooms in the fridge for a few days and then have to throw slimy, spoiled mushrooms into the compost. Fortunately, one can extend mushrooms shelf life by first selecting the freshest mushrooms and then storing them correctly (Jannatul *et al.,* 2019).

2.5 METHOD OF PROCESSING EDIBLE MUSHROOMS

Edible mushrooms can be processed either by:

1. **Cooking:** In order to cook mushrooms they must first be rinsed with water to rid them of dirt and other unwanted matter. Depending on the species of mushroom the feet and skin can either be removed or included. For some species (sticky mushrooms) the cuticle of the pileus (outer skin of the mushroom stipe)is removed or peeled off in order to get rid of the bitter flavor the tissues contains before it is consumed. After proper rinsing the mushroom is then sliced evenly, mix of olive oil and butter is then used. Ensure not to over mix and season with salt and pepper at the end.
2. **Roasting:** In order to roast mushrooms, first rinse the mushrooms properly and cut them into quarters, discard the stems, put the mushroom quarters into a bowl and pour ¼ cup of olive oil, sprinkle salt and freshly grounded

pepper to taste and mix the mushrooms until they are coated with the oil. Spoon the seasoned mushroom into a foil-lined sheet and sprinkle thyme over them and roast the mushroom for 15minutes either using an oven (preheat the oven to 375^0F or 191^0C) or over a hot Comal (a large cast-lorn pan) and drain the liquid from the pan, Roast the mushroom for 30 more minutes and toss the mushrooms with chopped herbs. (Ruan-Soto *et al*., 2017).

2.6 METHOD OF PRESERVING EDIBLE MUSHROOMS

There are several method of preservation of mushrooms and they include:

1. **Drying Method:** Wipe the mushroom clean with a lightly damped towel. Do not wash them under running water. Transfer them to a container that allows air to circulate around them. Do not overcrowd the mushrooms. Place them in a well-ventilated area, preferably in the sun if possible. Allow them to air dry for 7-10 days, until they do not give liquid when squeezed. Drying time vary based on humidity levels and the size of the mushrooms.
2. **Pickling Method:** Clean mushroom thoroughly, bring a large pot of water and vinegar to boil. Add mushrooms and cook for 15minutes. Drain the liquid and set mushrooms aside. Boil the mushroom marinade. Dice up garlic and place in mason jars. Fill up each of the jars with mushrooms and marinade, and cover it. The mushrooms can be stored up to a month.

3. **Freezing Method:** Scrub and brush mushroom to remove dirt, then slice them. Prepare a bowl of iced water and a tray lined with kitchen paper. Once drained and patted dry, lay the mushrooms on a tray and freeze until frozen solid.
4. **Canning Method:** Wash the mushrooms (only select brightly coloured, small to medium size domestic mushroom with short stems, unopened caps, and no discolouration). Cut large mushrooms and leave small mushrooms whole cover with water in a saucepan and boil for 5 minutes. Fill the jars with hot mushrooms, leaving 1-inch headspace. Add salt and Vitamin C (if desired). Top with hot water to 1-inch of headspace. Seal the jars and process in the pressure canner. Can pints for 45 minutes at 10 pounds pressure, when time is up turn off your canner and allows it to return to zero pressure on its own. When ready remove the jars from the canner and place onto a towel for 12 to 24 hour undisturbed. Remove the jar rings and wipe the jars clean. Store your jars in a cool, dry, dark place. Mushrooms are preserved either for consumption or sale. (Ruan-Sole *et al.*, 2017).

2.7 BIOACTIVE, NUTRITIONAL AND ANTI-NUTRITIONAL COMPOSITION OF OYSTER MUSHROOM

2.7.1 Bioactive, Nutritional and Anti-nutritional Composition of Edible Mushroom Bioactive Composition of Oyster Mushroom

The bioactive substance found in mushroom can be divided into:

1. **Secondary Metabolites:** It includes acids, terpennoids, polyphenols, alkanoids, lactones, sterols and vitamins.
2. Glycoproteins and polysaccharides, mainly β-glucose: which are protein complexes from medicinal mushrooms that enhance cell-mediated immune responses and exhibit antitumor activities in humans and animals.
3. New proteins with biological activities which is used for the development of new drugs and biotechnological processes (Valverde *et al*., 2015).

2.7.2 Nutritional Composition of Oyster Mushroom

Oyster mushrooms both add flavour to bland staple foods and are a valuable food in their own right: they are often considered to provide a fair substitute for meat, with at least a comparable nutritional value to many vegetables. The consumption of mushrooms can make a valuable addition to the often unbalanced diets of people in developing countries. Fresh mushrooms have a high-water content, around 90 percent, so drying them is an effective way to both prolonged their shelf life and preserve their flavour and nutrients. Mushrooms are a good source of vitamin B, C and D, including niacin, riboflavin, thiamine, and folate, and various minerals including potassium, phosphorus, calcium, magnesium, iron and copper. They provide carbohydrates, but are low in fat and fibre, and contain no starch. Furthermore, edible mushrooms are an excellent source of high-quality protein (reportedly between 19 percent and 35 percent), and white button mushrooms contain more protein than kidney beans.

In addition to all the essential amino acids, some mushrooms have medicinal benefits of certain polysaccharides, which are known to boost the immune system (Atul *et al.,* 2019).

Mushrooms are highly nutritious, contrary to popular belief. Often grouped with vegetables, mushrooms provide many of the attributes of produce as well as attribute more commonly found in meat, beans and grains. It was further stressed that the nutrients present in mushroom are considered to be typical of meat. The food value of mushroom is considered to lie between meat and vegetables. It was further emphasized that, mushrooms have been proved by experiments to be well suited to supplement diets which lack protein and in this sense; they have rightly been called “vegetable meat”.

Mushrooms supply proteins, amino acids, B group vitamins, vitamin C, K, mineral elements such as copper, magnesium, phosphorus, potassium, selenium and iron. It was further reported that mushrooms are among the few food sources rich in the trace element germanium, which is thought to promote efficient use of oxygen in the body and protect against damage from free radicals. Some species of mushrooms even provide carotene, a powerful antioxidant. Among the vitamins reportedly found in mushrooms are riboflavin, thiamin, niacin, biotin, cobalamin, pantothenic acid, vitamin C and K. Although, they are not significant sources of vitamin D, some mushrooms can become significant sources after exposure to ultra violet light. This also darkens their skin.

Mushrooms are low in calories. In view of their low calories, they are considered as the number one diet to be recommended to weight watchers and heart patients as the ideal food to lose weight and maintain a healthy heart. mushrooms, as compared with fruits and vegetables, are a better source of proteins, containing lysine, arginine, histidine, and threonine in high concentrations. All the essential amino acids required by an adult are present in mushrooms. The high concentration as compared with cystein and methionine. Many novel sources of food, particularly of protein, mushrooms apart from being famous for their appetizing flavour offer themselves as potential protein source to bridge the protein gap.

The small amount of fat in mushroom consists mainly of unsaturated fatty acids. In the study of some wild species of mushrooms. Unsaturated fatty acids, particularly oleic and linoleic acids, predominate in the total free fatty acids. Mushrooms are very low in sodium and are cholesterol free. The digestibility of mushrooms protein is as high as 72-83%. Mushrooms must be cooked to take advantage of their nutritional values.

2.7.3 Anti-Nutritional Composition of Oyster Mushroom

Anti-nutritional are defined as natural or synthetic compounds which can interfere with the absorption of nutrients by humans apart from the many advantages resulting from the consumption of mushrooms, some reports discussed how mushroom products can be hard to digest due to their content of anti-nutrients such as:

1. **Chitin:** Edible mushrooms in general contains about 1.87-7.25% chitin and many studies involve chitin in mushrooms from different point of view such as the production of chitinase, wound treatment, production of chitin and isolation of chitin-glucan complex.
2. The consumption of edible mushrooms may be associated with lower risks of mortality.
3. Mushrooms also contain other antinutrients like glucosinolates, lectins, tannins, which plants need in order to protect themselves from the surrounding stressful conditions (Hassan *et al.*, 2022).

2.8 ECONOMIC VALUE OF OYSTER MUSHROOM

Mushrooms are an indispensable way of income for numerous poor societies and of worth to competitive foragers, especially in Nigeria, most of the development has been consolidated on mushrooms due to their nutritious qualities and medicinal significance. The production of mushrooms creates a large number of direct and indirect employment opportunities in cultivation as well as in marketing activities as a labor-intensive management and offering opportunities for processing enterprises. Mushroom farming needs low capital, low technical knowledge and even in an indoor setting it is possible to cultivate mushrooms in small scale and one can easily get high return with low investment capital. The global market of mushroom industry in 2005 was valued at over $45 billion, in 2013 at $63 billion, the world market for edible mushrooms continues

to rise from USD 34.1 billion, in 2015 to US$69.3 billion by the end of 2024 due to the nutritional, culinary and health benefits of mushrooms. Therefore, mushroom cultivation not only empowers rural inhabitants (mostly women) but also alleviates poverty from the grass root level (Abdul and Aneequa, 2021).

2.9 MEDICINAL IMPORTANCE OF THE OYSTER MUSHROOMS

This genus includes more than 40 species, commonly referred to as the "Oyster mushroom", including *P. ostreatus* and *P. eryngii,* which has attracted special attention because of its high nutritional values and medicinal attributes. It is well known for its anti-oxidative, anti-carcinogenic, anti-inflammatory, anti-viral, anti-hypercholesteremic and immune-stimulating properties, as well as its ability to regulate glucose levels and blood lipids (Hassan *et al.,* 2022).

Mushrooms have a wide array of medicinally important compounds, these medicinal properties include:

1. **ANTI-INFLAMMATORY:** Chaga mushrooms have been found to help fight inflammation. It decreases inflammation in our gut, which can cause problems like irritable bowel syndrome.
2. **ANTIOXIDANT:** Enoki and oyster mushrooms are great source of antioxidants. These compound can help neutralize harmful free radicals to protect the cells from damage and oxidative stress. It is mostly used in south India.

3. **ANTIBACTERIAL:** In New Zealand native brown oyster mushroom have been proved to have the ability to inhibit the growth of five common bacterial strains.
4. **ANTIDIABETIC:** Ganoderma lucidum extract (Reishi mushroom) exhibited the best dose dependent inhibitory activity against diabetics in Asia.
5. **IMMUNOMODULATORY:** Chestnut mushroom, golden oyster mushroom and Pleasants back mushroom, etc. contain immune regulatory compounds they are used for immune modulating.
6. **ANTICANCER:** In Asia reishi mushrooms turkey tail mushroom, shiitake and maitake mushrooms are used to treat cancer. Mushrooms after great hope for production of new drugs for ailments like HIV/AIDS, Avian influenza and the many cancers that afflict humanity today (Odediran *et al.,* 2020).

2.10 ENVIRONMENTAL AND ECOLOGICAL IMPORTANCE OF OYSTER MUSHROOM

Mushroom play an important ecological role in the management of ecosystems (environments). Indirectly mushroom farming is a bioconversion process of organic substances which provides opportunities for the recycling of organic matter thus reduces pollution. Substances used in edible mushroom farming are applied as organic manures to the land after harvesting of

mushrooms. Mushroom cultivation is an appropriate technology for management of agricultural and agro-industrial residues (Jannatul *et al.*, 2022).

2.11 MICROBIAL CONTAMINATION OF OYSTER MUSHROOM

The microbiological or microbial contamination of mushroom is determined by the isolation of *Escherichia coli*, *Salmonella sp* and *Staphylococcus aureus*. The presence of these bacteria in mushroom (*Astacus leptodactylus*) confirms microbial contamination from mushroom, poor personal hygiene of the processor or sellers, poor environmental conditions, or from packaging and storage of the mushroom (*Astacus leptodactylus*).

Escherichia coli, also known as E. coli, is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms.

Salmonella sp, is a genus of rod-shaped Gram-negative bacteria of the family Enterobacteriaceae. The two species of Salmonella are *Salmonella enterica* and *Salmonella bongori*. *S. enterica* is the type species and is further divided into six subspecies that include over 2,600 serotypes.

Staphylococcus aureus, is a Gram-positive spherically shaped bacterium, a member of the Bacillota, and is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin.

CHAPTER THREE

3.0 MATHERIALS AND METHODS

3.1 MATERIAL USED

The materials that were used for this project practical include the following; Beaker, conical flasks, pipettes, petri- dishes, nutrient agar, mannitol salt agar, eosin methylene blue agar, salmonella-shigella agar, blender, weighing scale, colony counter, cotton wool, incubator, autoclave, detergent, Dettol, inoculating wire loop, lactophenol cotton blue, wooden clip (slide holder), masking tape, crystal violet stain, oil immersion, gloves, safranin stain, lugol's iodine, acetone, wash bottle, droppers, work bench, water, spatula, gloves autoclave, bunsen burner, aluminum foil paper, distilled water, test tubes, test racks, cover-slips and sterile bottle water.

3.2 METHODS

3.2.1 Source and Collection of Samples

Four (4) samples of oyster mushroom were purchased from certified mushroom farms in Benin, Ovia North East Local Government Area, Edo State, South-South of Nigeria. In a polythene bag and later taken to the school laboratory for immediate analysis.

3.2.2 Processing of Samples

10g of each of the oyster mushroom samples were weighed and hornogenized in 90ml of sterile distilled water with the aid of a blender. The

homogernate of each of the samples were aseptically collected with the aid of sterile pipette and kept for use as inoculum.

3.3 MICROBIOLOGICAL ANALYSIS

3.3.1 Sterilization of Apparatus

All glassware, apparatus, and laboratory work bench/table were sterilized before use. While the glassware and apparatus were sterilized with the aid of the autoclave operated at 120^0C for 15 minutes, the laboratory work bench/table was sterilized with the aid of cotton wool soaked with methylated spirit.

3.3.2 Media Preparation

The media used for the analysis were; Nutrient Agar (NA), Manitol Salt Agar (MSA), Salmonella Shigella Agar (SSA) and Eosin Methylene Blue Agar (EMBA). All the media were prepared according to the manufacturer's instructions and sterilized with the aid of an autoclave operation at 120^0C for 15 minutes before use.

3.3.3 Serial Dilution of Samples

1ml aliquot of each of the dried and fresh oyster mushroom inoculation (obtained for 3.3.2 above) were used for serial dilution of each of the samples. 9ml of sterile distilled water were aseptically dispensed into six sterile test tubes. Each of the 1ml aliquot of inoculum were separately transferred and diluted in the test tubes with the aid of sterile pipettes. The six fold serial dilution were used, where 9mls of sterile distilled water were aseptically dispensed into 6 sterile test tubes labelled 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}.

3.3.4 Inoculation of Media

The method used for media inoculation was the pour plating technique 1ml of aliquot of the serially diluted samples of dried and fresh oyster mushroom which were transferred into sterile petri dishes labelled accordingly and for about 10-15ml cooled molten agar medium was aseptically dispensed into the petri dishes containing the inoculum, the petri-dish was then swirled to ensure even distribution and mixture of the inoculum and medium. The medium was allowed to harden before the plates were incubated.

3.3.5 Incubation of Culture Plates

All the inoculated culture plates were transferred to the incubator for incubation at 37^0C for 24 hours before enumeration and identification of isolates in order to get the total viable count.

3.3.6 Enumeration of Colonies

Colonies that were developed after 24 hours of incubation were enumerated as colony-forming unit for all the samples of dried and fresh oyster mushroom. Colonies were enumerated with the aid of digital colony counter. Only culture with colonies of between 30-300 were used for enumeration.

3.3.7 Identification of Microorganisms

The bacterial isolates that was obtained after enumeration was thereafter identified based on their cultural, morphological and biochemical characteristics. The bacterial species were separately identified in the cultural characterization,

the shape, color, size, elevation and margin of individual colonies noted on their specific agar.

GRAM STAINING

A thin smear of the bacteria was made on a clean grease free microscopic slide, air-dried and heat fixed by passing it through the Bunsen flame. The slide was placed on a staining rack and flooded with crystal violet for one minute and was rinsed with sterile distilled water, the slide was then flooded with lugols iodine solution for one minute and was rinsed with sterile distilled water, the slide was decolorized with ethanol for thirty seconds (60 secs) and rinsed with clean sterile distilled water. Grams iodine was added (mordant) for 60 seconds. The smear was greatly rinsed with tap water. Alcohol (70% ethanol) was applied to decolorize it for 30 seconds. Lastly, the slide was filled with safranin for the next 1 minute and rinsed with sterile distilled water. The slide was drained and allowed to air dry, before viewing under the lens microscope using immersion oil, objective lens (x100). The gram-positive bacteria appeared purple while gram-negative bacteria colony appears red or pink.

3.3.8 Biochemical Test (Bacteria)

INDOLE TEST

To detect if bacteria possess enzymes trytophanase which degrades amino acid tryptophan to indole, pyruvic acid and ammonia.

Procedure: The test organism was inoculated into peptone water both in test tube and incubated at 37^0C for 48-96 hours. 0.5ml of Kovac's reagent is then

added and shaken gently. A pink red colour in the alcohol layer indicates a positive reactive.

METHYL RED TEST

To determine the ability of bacteria to produce and maintain stable acid end products from glucose fermentation. The test detects the production of acid

Procedure: The test organism was inoculated in glucose phosphate broth in a test tube and incubated at 37^0C for 2 to 5 days about 5 drops of 0.4% solution of alcoholic methyl red solution was added and mixed thoroughly and the result was read immediately. A positive test gives bright red colour and yellow colour indicating negative test.

UREASE TEST

The test detects the ability of organisms to produce urease enzyme.

Procedure: The test organism is inoculated on the agar and incubated at 37^0C, observation was after 4 hours and overnight incubation, development of purple coloration indicates the production of urease i.e. positive test. *Staphylococcus aureus* appeared positive in urease test while *Salmonella* spp came out negative.

MOTILITY TEST

It is aimed at identifying motile bacteria, motility can sometimes be referred to as the way an organism grow on solid media and it is determined by the presences or absence of flagella. The test is performed to distinguish the motile organism from the non-motile one.

Procedure: The semi-solid agar was dispensed into test tube and autoclave. The test organism was isolated (stab inoculated) in the medium. The nominated medium was inoculated at 37^0C for 24 to 48hours. A diffused and rapid growth that spread through the medium is an indication of motility.

CATALASE

The test was used to determine the ability of the organism to produce enzyme catalase that breaks down hydrogen peroxide to water and oxygen.

Procedure: A small bacteria colony was taken from sample by using a sterile wire loop and a drop of catalase reagent (hydrogen perioxide) was added to the slide using a sterile pipette. The presence of catalases observed by bubbling indicated a positive result which gave oxygen production within 10 seconds while negative result test result of bacteria.

COAGULASE

It is an enzyme-like protein and cause plasma to dot by converting fibrinogen to fibrin. The test is performed on gram positive, catalase positive species to identify the coagulase positive *Staphylococcus aureus*. Coagulase is a virulence factor for *Staphylococcus aureus*.

Procedure: A drop of physiological saline was placed on a sterile test tube using a sterile pipette. With the sterile urine loop, a portion of the isolated colony is placed on each test-tube human plasma is added to the suspension and mix gently looking clumpy of the organism within 10 seconds. The presence of

particles indicates positive result while the absence of particles indicate negative result

OXIDASE TEST

Test was carried out to identify bacteria species. This test is used to determined if an organism possess the cytochrome oxidase enzyme. The test is use as an aid for the differentiation of bacteria species.

Procedure: A piece of filter paper was placed in a clean sterile petri dish and 2 to 3 drops of fresh or nascent oxidase reagent was added. A colony of test organism was collected using a sterile wire loop and smeared on the filter paper and observation of blue purple colour within few seconds shows positive test and absence of color gives negative results.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

The results obtained for the Total Variable aerobic plate count in colony forming unit per 1ml of each of the Oyster mushroom (*Pleurotus* spp). The samples are presented in Table 4.1 below.

Table 4.1: Total Viable aerobic plate count (cfu/ml) of samples of Oyster mushroom (*Pleurotus* spp)

Samples	Total Viable aerobic counts (cfu/ml)
A	2.7×10^4
B	1.6×10^3
C	8.1×10^5
D	8.4×10^6

Key: A, B = Fresh Oyster Samples

C, D = Dried Packaged Oyster Samples

The results obtained for the Total Viable Aerobic Plate Count in colony forming unit per ml of each of the oyster mushroom samples is as presented in two table above. From the results shown in the table above fresh oyster mushroom samples had the lowest amounts of microbial contaminants with the least figures being 1.6×10^3 and the highest figure being 2.7×10^4 while those of the dry oyster mushroom had the highest amount of microbial contaminants with the least figure

being 8.1 x 10^5 and the highest figure being 8.4 x 10^6. It therefore implies that the dried oyster mushroom samples contained more contaminants compared with those of the fresh oyster mushroom samples. The occurrence of these microorganisms in the handling of the oyster mushroom from cultivation down to the area of processing. Unhygienic process in the processing of the dried and fresh oyster mushroom such as talking, sneezing, using the hands to hold the oyster mushroom without proper washing could be a source of contamination, since the human skin harbor lots of microorganisms such as *Staphylococcus aureus* which is a normal microbiota of the skin. It was reported that food can be infected with microorganisms as a result of coughing, careless sneezing among harvesters and processors.

Table 4.2: Incidence of *Escherichia coli, Staphylococcus aureus* and *Salmonella* spp in Dried and Fresh Oyster Mushroom (*Pleurotus* spp)

Samples	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella* spp
A	-	+	+
B	-	+	+
C	-	+	+
D	-	+	+

Key: A, B = Fresh Oyster Samples

C, D = Dried Packaged Oyster Samples

Key: + = Positive, - = Negative

Table 4.3: The Cultural, Morphological and Biochemical Evaluation of Dried and Fresh Oyster Mushroom

Cultural characteristics	Yellowish pin, head shaped, round colonies on mannitol salt agar	Thin transparent pale colonies on Salmonella-shigella agar
Morphological characteristics	Cocci arrange graphite clusters	Short rods occurring singly and in pairs
Gram reaction	+	-
Catalase	+	+
Citrates	+	-
Methyl red	+	+
Voges pastuer	-	-
Oxidase	-	-
Sugar utilization	+Glucose +Lactose +Manitol +Sucrose +Fructose	 + - + -
Identity of isolates	*Staphylococcus aureus*	*Salmonella* spp

4.2 DISCUSSION

From the incidence of the selected microorganisms in fresh and dried oyster mushroom samples. Table 4.2 shows that while *Staphylococcus aureus* was found in all of the sample both fresh and dried oyster mushroom as well as

Salmonella spp, *Escherichia coli* was not found in any of the samples. It therefore, shows that the sample were contaminated by *Staphylococcus aureus* and *Salmonella* spp. *Staphylococcus aureus* must have gotten in contact with the oyster mushroom and proliferated because of poor hygiene and indiscriminate handling of the oyster mushroom after preparation, *Staphylococcus aureus* is a normal flora of human being so the oyster mushroom handlers may not have sanitized their hands properly and where the oyster mushroom were kept must have harbored a lot of contaminants which may have led to the reason why *Staphylococcus aureus* was found in all of the samples of both the fresh and dried oyster mushroom.

Escherichia coli is a faecal contaminant, its presence in samples shows that faeces got in touch with the samples, for it not to be present shows that the oyster mushroom samples does not have any faecal contamination.

Salmonella spp is a causative agent of typhoid fever and food borne disease or illness, its presence in food is not desirable. *Salmonella* spp may have come in contact with the fresh and dried oyster mushroom probably due to exposure of the samples to the atmosphere.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

From the outcome of the study carried out on the samples of commercially retailed oyster mushroom (fresh and dried packaged) collected from Benin City and labelled sample A, B, C and D, a variety of microorganisms were detected. The bacteria present in the oyster mushroom include *Salmonella* spp and *Staphylococcus aureus*. Therefore, the detection of pathogenic bacteria in particular *Salmonella* spp, *Staphylococcus aureus* and *Escherichia coli*, their viable densities and the presence of their virulence factors is necessary before oysters enters the consumer's market.

5.2 RECOMMENDATION

1. It is recommended that oyster mushroom farmers and seller should ensure that when carrying out their mushroom production and preparation, it should be in a clean and sanitized environment.
2. All equipment's and utensils used in the preparation of oyster mushrooms should be properly washed and sanitized before and after use.
3. Oyster mushroom consumers should ensure that the mushroom is properly washed and well cooked before intake.
4. Government should establish a certification program in oyster farming areas that classifies oysters based on microbial testing results and should

also enforce strict compliance to enable oyster farmers and sellers to sell good oysters to the general public as these measures can help to ensure food security when consuming raw oyster mushrooms.

5. Finally, most bacteria cannot survive at high temperature including *Salmonella* spp, *Staphylococcus aureus* and *Escherichia coli*. Hence, oyster consumers are advised to avoid eating raw oysters to reduce the risk of infection and oyster processors should avoid wound contact and faecal contact with these mushrooms.

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