

**EVALUATING THE IMPACT OF CHEMICAL PRESERVATIVES ON
THE SHELF LIFE OF EDIBLE MUSHROOM SAMPLES**

BY

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LSC2009756

DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

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**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF
MICROBIOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL
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HONS) DEGREE**

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CERTIFICATION

This is to certify that this project was carried out by **Emmanuella Oluoma IGWE (Miss)** with the Matriculation number **LSC2009756** of the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, under adequate supervision, in partial fulfillment for the award of a Bachelor of Science degree in Microbiology.

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Date

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(Head of Department)

Date

DEDICATION

This research work is dedicated to Almighty God who made it possible for me to reach this height in life. May His name be praised forever.

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ABSTRACT

Mushrooms serve as the fruiting bodies of fungi. Unlike higher plants, they lack chlorophyll, the green pigment found in leaves that enables plants to utilize water, carbon dioxide, and solar energy for photosynthesis. Consequently, mushrooms are unable to synthesize their own food and rely on higher plants for sustenance. They possess high nutritional value, characterized by elevated levels of protein, vitamins, minerals, fiber, and trace elements, alongside low or negligible calorie and cholesterol content. Due to these factors, mushrooms are particularly prone to spoilage. The objective of this study was to investigate the impact of chemical preservatives on the shelf life, sensory evaluation, bacteriological quality, and nutritional composition of mushrooms, with the aim of assessing their suitability for extended storage. Freshly harvested oyster mushrooms (*Agaricus* spp.) were sourced from the African Centre for Mushroom Research and Technology Innovations (ACMRTI) at the University of Benin, Benin City. The preservatives utilized included hydrogen peroxide and citric acid, administered at concentrations of 0.5%, 1.5%, 2.0%, and 2.5%, with water serving as the control. Samples were stored in a refrigerator for a duration of six days and analyzed at two-day intervals. The analyses conducted encompassed sensory evaluation, bacterial count, biochemical tests, antibiotic susceptibility tests, and virulence factor assessment. The results indicated that a concentration of 2.5% hydrogen peroxide yielded the lowest bacterial count, recorded at $1.3 \pm 8.5 \times 10^2$ cfu/g, and exhibited the highest overall appearance score of 3.9 ± 1.7 . Identified bacterial isolates included *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Pantoea agglomerans*, *Bacillus subtilis*, *Cronobacter sakazakii*, *Bacillus cereus*, *Escherichia coli*, *Corynebacterium jeikeium*, *Serratia marcescens*, *Aeromonas hydrophila*, and *Enterococcus faecalis*. The isolates that occurred most frequently (11 instances) were *Bacillus subtilis*, *Escherichia coli*, and *Enterococcus faecalis*. The Multiple Antibiotic Resistance Index (MARI) revealed that *Escherichia coli* had the highest value of 0.9, while *Corynebacterium jeikeium* had a MARI of 0.4. The study illustrates that chemical preservatives effectively reduce microbial load, significantly slowing microbial growth, mitigating spoilage, and consequently extending the storage period of mushrooms.

CHAPTER ONE

INTRODUCTION

Mushroom is the fleshy, spore-bearing fruiting body of fungus, typically produced above ground, on soil, or on its food source. They belong to the fungi, *Ascomycota* and *Basidiomycota*. Edible mushrooms play an essential role in both nature and the human economy. They have been used medicinal materials for thousands of years, which is believed to have health benefits (Tbee *et al.*, 2021). The health effects and mechanisms of edible mushrooms in maintaining consumer health have been gradually seen in recent decades (Vamanu *et al.*, 2021). Chemical food spoilage by enzymatic and nonenzymatic mechanisms may be controlled by some additives like antioxidants and anti-browning agents, which are described in the relevant articles (Grondi *et al.*, 2021). Mushrooms are packed with large numbers of bioactive ingredients, such as polysaccharides, proteins, and phenols. Due on the presence of these active ingredients, more recently, mushrooms are used as probiotics and food supplements to improve consumer health. Over 700 edible mushrooms have been found to have therapeutic properties and could be a good source of bioactive compounds for the pharmaceutical industry. Mushrooms have been considered as a valuable food source due to its nutritional and medicinal characteristics. They contain various bioactive components such as proteins, polyunsaturated fatty acids, polysaccharides, dietary fibers, amino acids, vitamins, and minerals. They have essential health effects, such as antioxidant, antimicrobial, immune-stimulatory, and anticancer, cholesterol-lowering properties (Nowak *et al.*, 2022).

The fleshy, umbrella-shaped, fruiting body (spore-bearing) of a fungus that is normally developed above the soil surface or on self-food supply is known as a mushroom or toadstool. It belongs to the phylum Basidiomycota and the kingdom Fungi. The term "mushroom" is most

customarily used for fungus (Basidiomycota, Agaricomycetes) that have gills (lamella) on the underside of the cap, a stem (stipe), and a cap (pileus). In other words, the term "mushroom" describes the fleshy fruiting bodies, with or without stems, of a variety of Ascomycota and other gilled fungi (Onuoha *et al.*, 2019). Small spores produced by these gills help the fungus spread across the surface of the earth or its host. Bolete, puffball, stinkhorn, and other names for forms that vary from conventional morphology are a few examples. Gilled mushrooms are frequently called "*Agaricus*" itself. In contrast, the term "mushroom" is applied to describe the complete fungus as it is grown, the species itself, the thallus called (a mycelium), or the fruiting bodies known as mushrooms (Patra *et al.*, 2015). Mushroom mycelia may live for hundreds of years or die in a matter of months, depending on the accessibility of food. A mycelium will produce a fresh crop of sporophores each year during its fruiting season if food is available and temperature and moisture are favorable.

Lectins are carbohydrate-binding proteins that can be found in many types of edible mushrooms such as *Agaricus campestris*, *Agaricus bisporus*, *Grifola frondosam*, *Boletus satanus*, *F. velutipes*, *Tricholoma mongolicum*, *Ganoderma lucidum*, and *Volvariella volvacea*. Lectins have been shown to increase insulin secretion, activate the immune system, and have anticancer effects. Lectins can also play essential roles in physiological processes such as dormancy, growth, morphogenesis, morphological changes and molecular recognition in the early stages of mycorrhization (Maradny *et al.*, 2021). Edible mushrooms are considered an important source of food for humans for thousands of years. The medicinal properties provided by the chemical composition and nutritional value of edible mushrooms have been investigated by several researchers. *Agaricus bisporus*, *lentins edodes*, and *Pleurotus* spp. are presently the most common cultivated edible mushrooms, with China as the largest producer of these mushrooms in

the world (Jacinto-Azevedo *et al*, 2021). Oyster mushroom (*Pleurotus* species) which belongs to Class Basidiomycetes and Family *Agaricaceae* is prominently known and grows naturally in the temperate and tropical forests on dead and rotting wooden logs or sometimes on dying trunks of deciduous or coniferous wood. They also grow on decaying organic substance. The fruiting bodies of this mushroom are distinctly shell or spatula shaped with different shades of white, cream, grey, yellow, pink or light brown depending upon the species. *Pleurotus florida*, a species of Oyster mushrooms that grows easily in warmer condition serves as the best substitute for the all year supply of mushrooms in summer seasons. Oyster mushrooms are the second most widely grown mushrooms in the world (Sánchez *et al*, 2019), valued for nutritional, medicinal, and income contribution. They are also valued for their antioxidant properties. They contain phenolic compounds, flavonoids, reduced glutathione, ergothioneine, and low amounts of vitamin antioxidants (Selvi *et al.*, 2013). Oyster mushroom extract has been shown to possess antioxidant activity in vitro (Barros, *et al.*, 2013) and in vivo (Jayakumar *et al.*, 2018).

Mushroom cultivation can improve livelihoods in poor communities by providing nutritious food and possibly generating income (Nair *et al*, 2019). Oyster mushrooms are especially suited for this role as their production does not require high expertise, or high capital investment. One major challenge is that oyster mushrooms have the shortest shelf life among cultivated mushrooms due to its water content, delicate nature and proneness to bacterial and fungal contamination (Marshall *et al*, 2019). Mushroom are exceptionally perishable produce with post-harvest life running from 24-48 hrs. because of browning, textural changes, weight reduction and microbial rot (Fernandes *et al.*, 2012). Oyster mushroom has very short time span of usability and maintenance at the dimension of producer, while merchant, retailer and customer for one season or the other may result in the deterioration in the quality of produce leading to almost 100

percent monetary loss. A few investigations have been led to expand the shelf life of oyster mushroom by chemical preservation. In any case, more efforts are required toward this path. Additionally, efforts are expected to upgrade the shelf life by subjugating the fruit bodies to low temperature and packaging materials of various densities. The processing of mushroom is necessary to develop suitable post-harvest techniques for its prolonged preservation and usage. Many times, grower faces problem of over saturation of market and distress at highly non-remunerative prices. The retention of fresh mushroom at various level such as grower, whole seller, retailers and consumers further results in deterioration in mushroom helps to extend shelf-life as well as to retain whiteness. Mushrooms are macro-fungi with visible structures that produce spores otherwise known as fruiting bodies. They offer tremendous applications as they can be used as food and medicines besides their key ecological roles. Numerous species are consumed throughout the world as a delicacy particularly for their specific aroma, texture, and taste (Wani *et al.*, 2019).

Mushrooms are low in fat, high in complex carbohydrates and protein (Bano *et al.*, 2013) and they also lack cholesterol and are good sources of vitamins and minerals (Matilla *et al.*, 2021). Mushrooms have received critical scientific and socio-economic attention in the last few decades. Nowadays, it is increasingly being utilized as an important food product for their crucial role in human health, nutrition and disease control. Currently, common mushroom genera (*Agaricus spp*, *Lentinus spp*, *Auricula spp*, *Flammulina spp*, *Volveriella spp*, *Grifola spp*, *Pholiota spp* and *Pleurotus spp*) are cultivated, utilized, processed, identified, characterized, and preserved in different parts of the world (Zhang *et al.*, 2014). Mushrooms are highly perishable food items that tend to lose their unique organoleptic properties immediately after harvesting. Their short shelf-life is mainly explained by the high losses of water vapour that allows dehydration, high

respiration and microbial colonization by bacteria or fungi. Furthermore, enzymatic activity and biochemical alteration lead to mushrooms quality losses (Simon *et al.*, 2015). The microorganisms most often associated with mushroom spoilage and colonization are Gram-negative bacteria especially those belonging to Pseudomonad family such as *Pseudomonas fluorescens* (Masson *et al.*, 2012).

1.2 AIMS AND OBJECTIVES

The aim of this study was to study the impact of chemical preservatives on the shelf life, quality, and nutritional composition of mushrooms to evaluate their suitability for extended storage period.

OBJECTIVES

The main objectives were to;

- assess the effect of chemical preservatives on the shelf life of mushrooms under different storage conditions.
- evaluate the changes in physical attributes, such as texture, color, and appearance, caused by the preservatives.
- conduct sensory evaluations, assessing taste, aroma, texture, and visual appearance of mushrooms after preservation.
- measure microbial growth (e.g., bacteria and fungi) on preserved mushrooms over a period of time.

CHAPTER TWO

LITERATURE REVIEW

2.1 Mushroom

Mushrooms is known as a cuisine ingredient across the globe due for their unique flavor and have been valued by humans. It is said that more than 2,000 species of mushrooms are in existence, but around 25 are widely accepted as food and few are commercially cultivated (Valverde *et al.*, 2015). Mushrooms are considered as a delicacy with high nutritional value, and they are also known as nutraceutical foods. They are of good interest because of their organoleptic merit, medicinal properties and economic significance (Ergonul *et al.*, 2013). However, there is not an easy distinction between edible and medical mushrooms because many of the common edible species have therapeutic properties and several used for medical purposes are also edible (Guillamon *et al.*, 2010).

Mushrooms are healthy and is a sumptuous food by a large population residing throughout the globe. Since ancient times mushrooms have been an important part of the human diet. It was even considered as “Food of the God” by the Romans. Mushrooms were believed to provide strength during the battle by the Greek and according to Chinese, it was considered as a health food or “Elixir of Life” (Valverde *et al.*, 2015). By definition, a nutraceutical is a part of a food substance or whole food having medicinal benefits used for treatment or prevention of diseases. Mushrooms, due to its diverse chemical complexity act as an reservoir for drug formulation and as a nutraceutical source (Fasido *et al.*, 2013).

Mushrooms are globally appreciated for their nutritional value and medicinal properties. Their cultivation is an effective bioconversion technology of transforming wastes and woods into potentially valuable resources and could also be an important part of sustainable agriculture and forestry. The per capita consumption of mushrooms in India is also very meager and is even less than 100 g per year. They have been part of the human culture for thousands of years and have considerable interest in the most important civilizations in history because of their sensory characteristics; they have been recognized for their attractive culinary attributes. Mushroom is a macro fungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand. It is perhaps the most well-known and documented edible forest product (Kumari *et al.*, 2014). Mushrooms are the common components in folk medicine, especially in Africa, the Middle East, China and Japan since ages. Earlier, edible mushrooms were only harvested wild and were difficult to domesticate and cultivate. Collection from wild woodlands is still important in the world and particularly in southern Asia and other developing countries. Mushrooms belong to Basidiomycetes and Ascomycetes with a cell cycle including the formation of sexual spores and have two growth phases, i.e, the vegetative phase (mycelia) and the reproductive phase (fruit bodies) (Valverde *et al.*, 2015).

Mushroom develops from a nodule or pinhead, less than two millimeters in diameter, called a primordium, which is typically found on or near the surface of the substrate. It is formed within the mycelium, the mass of threadlike hyphae that make up the fungus. The primordium enlarges into a roundish structure of interwoven hyphae roughly resembling an egg, called a “button”. The button has a cottony roll of mycelium, the universal veil that surrounds the developing fruit body. As the egg expands, the universal veil ruptures and may remain as a cup

or volva, at the base of the stalk or as warts or volval patches on the cap. Many mushrooms lack a universal veil therefore they do not have either a volva or volval patches. Often, a second layer of tissue, the partial veil, covers the bladelike gills that bear spores. As the cap expands, the veil breaks and remnants of the partial veil may remain as a ring or annulus, around the middle of the stalk or as fragments hanging from the margin of the cap (Gupta *et al.*, 2018). The fungal spores are located in a special structure called the basidium (for Basidiomycetes) or the ascus (for Ascomycetes). The mushroom continues its life cycle in three key stages viz., vegetative growth, reproductive growth, and spore production by fruiting bodies of the mushrooms (Kumar *et al.*, 2020).

The most cultivated mushroom worldwide is *Agaricus bisporus*, followed by *Lentinus edodes*, *Pleurotus spp.* and *Flammulina velutipes*. Mushrooms production continuously increases, China being the biggest producer around the world. However, wild mushrooms are becoming more important for their nutritional, sensory and especially pharmacological characteristics (Aida *et al.*, 2019). Mushrooms could be an alternative source of new antimicrobial compounds, mainly secondary metabolites, such as terpenes, steroids, anthraquinones, benzoic acid derivatives and quinolones, but also of some primary metabolites like oxalic acid, peptides and proteins. *Lentinus edodes* is the most studied species and seems to have an antimicrobial action against both Gram-positive and Gram-negative bacteria (Alves *et al.*, 2012). Mushrooms have a long history of use in the oriental medicine to prevent and fight numerous diseases (Carneiro *et al.*, 2013). Nowadays, mushroom extracts are commercialized as dietary supplements for their properties, mainly for the enhancement of immune function and antitumor activity (Guillamon *et al.*, 2015). In this work, we aimed to review the nutritional value as well as the chemical and

nutraceutical composition and commercial potentialities of the most cultivated edible mushrooms worldwide.

2.2 HISTORY

The consumption of mushrooms occurred during prehistory, in the hunting and gathering period. Unlike plants, mushrooms could not be cultivated at first and were collected for a long period of time. Even today, relatively few species of mushrooms can be cultivated compared to the number of edible species (Tundey *et al.*, 2020). Mushrooms were thought to be special and supernatural in origin – 4600 years ago, the Egyptians believed mushrooms to be plants of immortality; the Pharaohs decreed that only they could eat mushrooms (Abdel *et al.*, 2016). Many people collect mushrooms for the purpose of consumption, but lots of myths and false concepts still survive today (Valverde *et al.*, 2015). Mushrooms have been consumed since earliest history; Ancient Greeks believed that mushrooms provided strength for warriors in battle and the Romans perceived them as the “Food of the Gods.” Although time to time, small efforts and simple research were conducted to introduce mushroom cultivation in India, scientific and systematic research only started in 1961, when the Indian Council of Agricultural Research, New Delhi, first cultivated *Agaricus bisporus* at Solan in Himachal Pradesh, a hilly state of North India. India, is rich in terms of agro-wastes that are not properly utilized by the nation’s farmers (Gupta *et al.*, 2018). India produces nearly 700 million 543 tons of agricultural residues which can profitably be utilized for mushroom cultivation. Even if India uses 2% of its total agro-residues for mushroom production, the production would be 7.0 million tons of fresh mushrooms, which will be equal to current global button mushroom production (Gupta *et al.*, 2016).

Currently, India is using only 0.03% of these residues to produce about 0.13 million tons of mushrooms and contributes of the oyster and paddy straw mushrooms in India. The country's production in 2010 was 1.00 lakh metric tons, of which button mushroom accounted for 89% of the total production, followed by oyster (6%), milky (1%) and others (4%). Punjab, Uttarakhand, Haryana, Uttar Pradesh and Tamil Nadu were the leading producers of the mushroom in the country at the time of 2010. The present production status revealed that Punjab, Haryana and Odisha are emerging as the leading states in mushroom production (Thakur *et al.*, 2021).

Mushrooms have been present on earth from a long time and are an important, indispensable part of global cuisine. Mushrooms are exploited for their beneficial health properties. There are about 2000 mushroom species worldwide, but only few of them are edible and nutraceutical. A species of button mushroom *Agaricus bisporus* is the most widely grown mushroom, followed by *Lentinus edodes* and *Flammulina velutipes*. Mushrooms contain various metabolites, such as terpenes, steroids, anthraquinone, phenolic acid, and benzoic acid, while primary metabolites contain proteins, oxalic acid, and peptides. Mushrooms have been reported to have an action against both Gram-positive and Gram-negative bacteria (Alves *et al.*, 2012). Nutritionally, they are rich in protein and amino acids (Reis *et al.*, 2012). However, they contain a significant amount of vitamins such as B1, B2, B12, C, D, and E. Structurally, mushrooms comprise the pileus, lamella, stipe, mycelium, and roots. The roots are mainly responsible for absorbing and gathering nutrients (Jiang *et al.*, 2020). Earlier, there was a misconception regarding the classification of mushrooms as plants. Later, with advancement in science, they were added under the independent kingdom known as *mycota*, mainly characterized by chitin inside the cell walls (Fischer *et al.*, 2016).

2.3 HEALTH BENEFITS OF MUSHROOMS

Mushrooms are globally known for their great taste and amazing health benefits. They are packed with a ton of essential vitamins and minerals and they make for an excellent addition to your diet, adding flavor to many different recipes. Mushrooms are a low-calorie food that packs a nutritional punch loaded with many health-boosting vitamins, minerals, and antioxidants. They have long been recognized as an important part of any diet. For instance, mushrooms grown in exposure to ultraviolet light are a good source of Vitamin D, an important component in bone and immune health. Mushrooms are a particularly excellent source of zinc, an essential trace element. Zinc is a vital nutrient for the immune system and is also needed for ensuring optimal growth in infants and children (Elliot *et al.*, 2021). Mushrooms contain protein, vitamins, minerals and antioxidants. For example, antioxidants are chemicals that help the body eliminate free radicals. Free radicals are toxic byproducts of metabolism and other bodily processes. They can accumulate in the body and if too many collect, oxidative stress can result. This can harm the body's cells and may lead to various health conditions (Kumari *et al.*, 2016). Moreover, researchers have found a number of other excellent reasons for incorporating mushrooms into your diet, such as:

Lower blood pressure: Mushrooms are good sources of potassium. Potassium lessens the tension in blood vessels, potentially helping to lower blood pressure (Thakur *et al.*, 2021).

Boost immune system: The anti-inflammatory effect of mushrooms has been shown to greatly improve the efficiency of the immune system. Mushrooms help stimulate microphages in the immune system, enhancing its ability to defeat foreign bodies and making you less susceptible to serious illnesses (Nahata *et al.*, 2017).

Weight loss: Long research have shown that frequent intake of mushrooms, in combination with exercise and other lifestyle changes, can have an important impact on weight loss. For example, after being asked to substitute 20% of their beef consumption with mushrooms, research participants showed improvements in their BMI and belly circumference. The antioxidants in mushrooms are also thought to reduce the risk of hypertension and other metabolic disorders (Kumar *et al.*, 2018).

Cancer: Mushrooms serves as an antioxidant that helps to neutralize free radicals, thus preventing cell damage and reducing the risk of cancer and other diseases. Mushrooms contain more selenium than any other form of agricultural produce (Thakur *et al.*, 2021).

Diabetes: Dietary fiber may help manage a number of health conditions, including type 2 diabetes (Dilani *et al.*, 2012). The Dietary Guidelines for Americans recommend that adults consume 22.4–33.6 g of dietary fiber each day, depending on sex and age. Mushrooms, beans, some vegetables, brown rice and whole-grain foods can all contribute to a person’s daily requirement of fiber.

Heart health: The fiber, potassium and vitamin C in mushrooms contributes to cardiovascular health. Potassium can help regulate blood pressure and this may decrease the risk of hypertension and cardiovascular disease (Salido *et al.*, 2016). The American Heart Association (AHA) recommends reducing the intake of added salt in the diet and eating more foods that contain potassium. According to current guidelines of National Institutes of health, people should consume around 4,700 milligrams (mg) of potassium each day. Mushrooms appear on the AHA’s list of foods that provide potassium. A 2016 study of National Institutes of health

concluded that people with a vitamin C deficiency were more likely to experience cardiovascular disease and suggested that consuming vitamin C may help prevent this illness.

Nutritional value: The nutritional value of edible mushrooms is due to their high protein, fiber, vitamin and mineral contents, and low-fat levels (Barros *et al.*, 2018). They are very useful for vegetarian diets because they provide all the essential amino acids for adult requirements; also, mushrooms have higher protein content than most vegetables. Besides, edible mushrooms contain many different bioactive compounds with various human health benefits (Valverde *et al.*, 2015).

It is paramount to know that the growth characteristics, stage and postharvest condition may influence the chemical composition and the nutritional value of edible mushrooms. Also, great variations occur both among and within species (Kalac *et al.*, 2013). Mushrooms contain a high moisture percentage that ranges between 80 and 95 g/100 g, approximately. They are a good source of protein, 200–250 g/kg of dry matter, leucine, valine, glutamine, glutamic and aspartic acids. Mushrooms are low-calorie foods since they provide low amounts of fat, 20–30 g/kg of dry matter, being linoleic (C18:2), oleic (C18:1) and palmitic (C16:0) the main fatty acids. Edible mushrooms contain high amounts of ash, 80–120 g/kg of dry matter (mainly potassium, phosphorus, magnesium, calcium, copper, iron and zinc). Carbohydrates are found in high proportions in edible mushrooms, including chitin, glycogen, trehalose and mannitol; besides, they contain fiber, β -glucans, hemicelluloses and pectic substances. Additionally, glucose, mannitol and trehalose are abundant sugars in cultivated edible mushrooms, but fructose and sucrose are found in low amounts.

Mushrooms are also a good source of vitamins with high levels of riboflavin (vitamin B2), niacin, foliates and traces of vitamin C, B1, B12, D and E. Mushrooms are the only nonanimal food source that contains vitamin D and hence they are the only natural vitamin D ingredients for vegetarians. Wild mushrooms are generally excellent sources of vitamin D2 unlike cultivated ones; usually cultivated mushrooms are grown in darkness and UV-B light is needed to produce vitamin D2 (Reis *et al.*, 2012). The amount of vitamin D mushrooms contain varies widely and depends on how long the mushrooms are exposed to UV light. Estimates show that fresh wild mushrooms like chanterelles and morels can contain up to 1200 IU of vitamin D per 3.5 ounce serving, whereas mushrooms grown in darkened conditions like white button, shiitake and oyster contain less than 40 IU (Cardwell *et al.*, 2018).

Edible mushrooms are important sources of food, they form very nourishing meals especially for invalids, for they are easily digestible. They are consumed not only for their innate flavor and taste, but also for their important nutritional value. On fresh weight basis mushrooms are superior in protein content (Aremu *et al.*, 2019) to all vegetables and fruits, but are inferior to meat and dairy products, which are the conventional protein sources. On dry-weight basis, however, mushrooms are similar with respect to dried-yeast and superior to dried peas and beans. The nutrient content varies from species and depends on their growth requirement. Mushrooms have a high percentage of water 93-95% as compared to lean beef (70%) and fresh vegetables (92%). They also contain valuable minerals such as iron, potassium, phosphorus, calcium and copper, 56% carbohydrate, 30% protein, 2% fat and also 10% ash on dry weight basis. They are also rich in vitamin B and vitamin D. Mushrooms provide a high protein and low caloric diet and can thus be recommended to heart patients. They also contain all the essential amino-acid required by an adult (Koyyalamudi *et al.*, 2009). Tryptophan and lysine are present in high

concentrations as compared to cystine and methionine. Mushrooms is reported to be an excellent source of riboflavin and nicotinic acid; a good source of pantothenic acid and ascorbic acid (Ukpebor *et al.*, 2017). The carbohydrate and fat contents of edible mushrooms are quite low. The absence of starch in mushrooms makes it an ideal food for diabetic patients and for persons who wants to shed excess fat. Edible mushrooms known as the meat of the vegetable world (James *et al.*,2019) can be prepared into a variety of delicious dishes and as flavors for other dishes. Among the Nigerian mushroom dishes are common such as mushroom with vegetable, in melon soup, in okro soup and mushroom in stew. These soups are used to eat a variety of foods. Some people use mushrooms as a substitute for meat in their stews (Abulude *et al*, 2015).

2.4 MEDICINAL VALUE OF EDIBLE MUSHROOMS

Mushrooms have been present on earth from a long time and are an important, indispensable part of global cuisine. Mushrooms are exploited for their beneficial health properties. There are about 2000 mushroom species worldwide, but only few of them are edible and nutraceutical. A species of button mushroom *Agaricus bisporus* is the most widely grown mushroom, followed by *Lentinus edodes* and *Flammulina velutipes*. Mushrooms contain various metabolites, such as terpenes, steroids, anthraquinone, phenolic acid, and benzoic acid, while primary metabolites contain proteins, oxalic acid, and peptides. Mushrooms have been reported to have an action against both Gram-positive and Gram-negative bacteria (Alves *et al.*, ss2012). Nutritionally, they are rich in protein and amino acids (Reis *et al.*, 2012). However, they contain a significant amount of vitamins such as B1, B2, B12, C, D, and E. Structurally, mushrooms comprise the pileus, lamella, stipe, mycelium, and roots. The roots are mainly responsible for absorbing and gathering nutrients (Jiang *et al.*, 2020). Earlier, there was a misconception regarding the classification of mushrooms as plants. Later, with advancement in science, they were added

under the independent kingdom known as *mycota*, mainly characterized by chitin inside the cell walls (Fischer *et al.*, 2015). Since thousands of years, edible fungi have been revered for their immense health benefits and extensively used in folk medicine. Specific biochemical compounds in mushrooms are responsible for improving human health in many ways. These bioactive compounds include polysaccharides, triterpenoids, low molecular weight proteins, glycoproteins and immunomodulating compounds. Hence mushrooms have been shown to promote immune function; boost health; lower the risk of cancer; inhibit tumor growth; help balancing blood sugar; ward off viruses, bacteria and fungi; reduce inflammation and support the body's detoxification mechanisms. Increasing recognition of mushrooms in complementing conventional medicines is also well known for fighting many diseases (Manikandan *et al.*, 2017)

Good for heart: The edible mushrooms have little fat with higher proportion of unsaturated fatty acids and absence of cholesterol and consequently it is the relevant choice for heart patients and treating cardiovascular diseases. Minimal sodium with rich potassium in mushroom enhances salt balance and maintaining blood circulation in human. Hence, mushrooms are suitable for people suffering from high blood pressure. Regular consumption of mushrooms like *Lentinula*, *Pleurotus spp.* were shown to decrease cholesterol levels (Salvido *et al.*, 2016).

Low caloric food: The diabetic patients choose mushroom as an ideal food due to its low calorific value, no starch and little fat and sugars. The lean proteins present in mushrooms help to burn cholesterol in the body. Thus it is most preferable food for people striving to shed their extra weight (Manikandan *et al.*, 2015).

Bioactive materials of solvent extracts of some mushrooms has been discussed below;

Hericium erinaceus

Erinacines (A-I) is very important and well-studied bioactive metabolite. It belongs to a group of cyathin diterpenoids. It was extracted from the mycelium and fruiting body of *Hericium erinaceus* and demonstrated for its neurotropic and neuroprotective effects. It can induce nerve growth factor (NGF) synthesis, both in vitro and in vivo. However, this medicinal mushroom also has antioxidative, anti-inflammatory, anticancer, immunostimulant, antidiabetic, antimicrobial, hypolipidemic, and antihyperglycemic properties; although it's most frequent use is for the treatment of neurodegenerative diseases and cognitive impairment (Venturella *et al.*, 2021). *Erinacin A*, from the erinacine group, has been proven to have an effective protective activity against Parkinson's disease. This metabolite was also found to be effective against ischemic stroke, as reported in a study on rats (Lian *et al.*, 2018).

Agaricus bisporus

Agaricus bisporus contains beta-glucans, ergosterol, ergothioneine, vitamin D, and flavonoids, with varying concentrations (Blumfield *et al.*, 2020). Besides, essential amino acids, peptides, glycoproteins, nucleosides, triterpenoids, lectins, fatty acids, and their derivatives make this mushroom of considerable importance for its potential application as an antimicrobial, anticancer, antidiabetic, anti-hypercholesterolemic, antihypertensive, hepatoprotective, and antioxidant agent (Atila *et al.*, 2021). The consumption of *Agaricus. bisporus* in the diet is recommended to prevent prostate cancer due to the action of conjugated linoleic acid (CLA) since it inhibits proliferation in prostate cancer cell lines in vivo (Adams *et al.*, 2016). The nephroprotective effects of *Pleurotus ostreatus* and *Agaricus. bisporus* aqueous extracts on hyperoxaluria-induced urolithiasis induced in Wistar rats have been investigated. The mushroom

extracts inhibited the progression of nephrolithiasis and showed nephroprotective effects against ethylene glycol-induced kidney dysfunction (Ahmed *et al.*, 2020).

Pleurotus spp.

Several studies have been carried out to assess the biological activities of *Pleurotus* species. *Pleurotus florida* and some other related species were found to have antioxidative, antimicrobial, antidiabetic, anticancer, anti-inflammatory, immunomodulatory, anti-hypercholesterolemic, antihypertensive, antimicrobial, hepatoprotective, and antiaging properties; although very few mechanisms and specific metabolites responsible for these activities have been elucidated, identified and characterized. The effect of water-soluble proteoglycan fractions extracted from *P. ostreatus* on a sarcoma-180-bearing mouse model had been evaluated. Treatment with this extract, it resulted in a quantitative reduction of tumor cells and their arrest in the pre-G0/G1 phase of the cell cycle. Moreover, increased cytotoxicity of NK cells was also recorded. In a study by Jedinak and Sliva comparing the impact of different medicinal mushrooms on the growth of breast and colon cancer cells, *P. ostreatus* proved to be the most effective, suppressing cell proliferation via the p53- dependent and p53-independent pathways. More specifically, the methanolic extract of the mushroom induced the suppression of the proliferation of the human breast cancer cell lines MDA-MB-231 and MCF-7 and colon cancer cell lines HCT-116 and HT-29, and caused cell cycle arrest in the G0/G1 phase in MCF-7 and HT-29 cells (Jedinak *et al.*, 2013).

Grifola frondosa

Grifola frondosa or maitake mushroom is a medical mushroom with numerous medicinal properties. Metabolites from *Grifola frondosa* were extracted and its main bioactive metabolite

was named D-fraction or GFP which is a β -glucan proteoglycan compound. Several studies have demonstrated its antitumor effect (Alonso E.N *et al.*, 2013). Two polysaccharide fractions obtained from GFPs and named as F2 and F3 showed promising hypoglycemic effects in vitro (Xiao *et al.*, 2015). Similarly many other biologically active compounds have, however, been extracted from maitake mushroom and investigated for their effects (Cui *et al.*, 2016).

Ganoderma lucidum

Several researchers reported the protective action of this mushrooms on experimentally induced liver injuries. Morel mushrooms have been reported to have beneficial action against the CCl₄ and ethanol-induced hepatotoxicity (Nitha *et al.*, 2017). The oyster mushroom, also known as *dhingri* in India, is a species of *Pleurotus* that belongs to the Basidiomycetes class and the *Agaricaceae* family. It is primarily found in temperate and tropical forests, especially on decaying organic materials. Major components of oyster mushrooms are described in the literature as having a spatula-shaped top (pileus), a central stalk (stipe; short or long), and lengthy ridges along with furrows beneath the pileus (i.e., gills or lamellae), but this species' mycelium is completely white in color. The button mushroom, on the other hand, is a well-known species of mushroom that has already been reported in practically every country in the world (*Agaricus* species; family *Agaricaceae*). In India, production of this species earlier was limited up to the winter season, but with advancements and development in technology, these are produced and reported throughout the year (Liang *et al.*, 2016).

All mushrooms can be grouped into a select few major categories. These categories overlap as well because some mushrooms frequently fall under more than one heading. These categories overlap as well because some mushrooms frequently fall under more than one heading.

The following are classifications apply to types of mushrooms;

- Cultivated mushrooms- Commercially grown mushrooms are referred to as "cultivated mushrooms." Mushroom producers use a wide range of diverse techniques and settings to take care of their crops as they cultivate mushrooms in large quantities for consumers. Any variety of mushrooms that customers can purchase at the market is considered to be cultivated. These include shiitake, oyster mushrooms, enoki, portabello, etc.
- Wild mushrooms- Those mushrooms are obtained by mushroom *shikarionce* from their natural growing spot in undomesticated areas. Some mushroom varieties, like those that enlarge on the living root structures of trees, only originate in nature. While some mushrooms are grown commercially, others are foraged by foragers from the wild. Many times, the qualities of wild mushrooms that can only be found in nature make it difficult or practically impossible to cultivate them on a large-scale agricultural scale. It is difficult to recognize the species of mushroom before harvesting. You must be mushroom hunting alongside an expert who can confirm your findings and make sure your finds are safe to eat if you don't have experience recognizing mushrooms. Most of the wild mushrooms are poisonous but exactly look like edible mushrooms.
- Medicinal mushrooms- In literature, varieties of mushrooms were reported and showed several health advantages. Shiitake, lion's mane, and porcini are a few well-known edible mushrooms with health advantages. Other medicinal mushrooms cannot be consumed because they are either too bitter or too woody. To benefit from the health advantages of these kinds, are prepared into teas and used as supplements or capsules. *Reishi*, turkey tail mushrooms are examples of beneficial fungi. Scientific studies have demonstrated the

numerous advantages and applications of medicinal mushrooms, from the treatment of cancer to the boosting of the immune system.

- **Poisonous mushrooms-** Several wild varieties of mushrooms are identified as poisonous species; it is essential to make sure those foraged specimens are positively recognized without a doubt before ingesting them. Poisonous mushrooms can cause a person to become extremely unwell or even permanently harm their organ systems. Some poisonous mushrooms have the potential to be fatal if eaten.
- **Useful mushrooms-** While some types of mushrooms are not consumed by people, they nonetheless serve a vital purpose, such as breaking down oil and other environmental pollutants or being added to compost. Every year, new ideas utilizing mushrooms are made and launched by scientists, including the use of mushrooms in biofuels, packaging, cleaning goods, and other applications.
- **Psychoactive mushrooms-** Mushrooms that can induce hallucinations and have a psychotropic impact are known as psychoactive mushrooms. The hallucinogenic component psilocybin is present in most of them. Most nations forbid the cultivation of certain kinds of mushrooms, and doing so can be harmful to people's health.

2.5 Conditions Required for Mushroom Growth

The general requirements for growing mushrooms are stated below, however, bear in mind that each variety has specific needs: (Iqbal *et al.*, 2015).

- **Light:** Although mushrooms don't need darkness to thrive, mushrooms don't need light either. However, darkness promotes the moist conditions that mushrooms require, individuals frequently grow mushrooms in low light or complete darkness.

- **Moisture:** The important condition for healthy mushroom growth is moisture. Mushrooms should be kept out of direct sunlight to promote wetness. A wet growing medium, such as manure or compost, is beneficial for button mushrooms. On logs, shiitake mushrooms thrive best with dry bark and moist internals. Every time the logs start to dry out, soak them for 48 hours to bring the moisture level back to 35 to 45 percent.
- **Nutrients:** Mushrooms obtain their sustenance through the breakdown of organic matter, as opposed to plants, which obtain it through photosynthesis. In addition to lipids, lignin, nitrogen, protein, starch, and sugar are required for mushroom growth.
- **Temperature:** The improper temperature can assassinate growing mushrooms, therefore it's crucial to keep an eye on this situation, adjusting the temperature as necessary with heaters or fans, and protecting mushrooms from draughts and direct heat. The temperature range that button mushrooms require to survive is between 55 and 60 degrees Fahrenheit, where most species thrive. Shiitakes thrive in temperatures between 72 and 78, but can survive between 40 and 90 degrees. The ideal air temperature for enoki mushrooms is 45 degrees.

2.6 TYPES OF MUSHROOMS

Mushrooms are easily cultivable in hilly regions due to abundant moisture but can also be grown in artificial environment with proper temperature and humidity control. Varieties must be identified thoroughly as some of them might cause food poisoning or allergy upon consumption. They are among the most fascinating plant species, which are commercially grown variations, wild varieties, or even both. Antioxidant-rich edible mushrooms add an earthy flavor to practically any prepared food. However, many mushrooms are either dangerous or tasteless.

Given that there are 10,000 different kinds of mushrooms known to exist, a complete list of all of them would be impossible to maintain. Some of the major varieties consumed in India are as follows:

2.6.1 Button Mushroom

Button mushroom (*Agaricus bisporus*) belongs to Class Basidiomycetes and Family *Agaricaceae* and is native to Europe and North America. It is of two types white and brown, out of which white button mushroom is commonly grown in India (Wikipedia, 2018). According to ICAR - Directorate of Mushroom Research, this variety contributes more than 85% to mushroom production. It is the most relished variety used in eateries and households (ICAR, 2008). Button mushroom (*Agaricus bisporus*)- known as white mushrooms are the most frequently convenient mushrooms in the food mart. However, they appear poisonous in the wild.

2.3.2 Shiitake Mushroom

Shiitake Mushrooms (*Lentinula edodes*) is an Asian mushroom that is well renowned for its culinary versatility as well as its therapeutic properties. They are native to East Asia and are highly consumed in Asian countries. Shiitake Mushrooms readily grow on wood of deciduous and hard wood trees such as Oak, Chestnut, and Maple etc. and require moist and warm climate. In rare cases they may cause allergic reaction like itching but can be eliminated by thorough cooking. These are used in Asian cuisines and traditional medicines. Patients with modern stomach cancer who received chemo coupled with shiitake-derived chemicals lived longer than those who received chemotherapy alone. The maitake mushroom, commonly known as hen of the woods, has a long history of being beneficial in the treatment of cancer patients. Researchers found that maitake extract reduced malignant tumors by 75% when paired with low doses of interferons. Maitake and shiitake mushrooms, which are also rich sources of minerals including

B vitamins, have also been linked to the immune system. Additionally, rich in fiber and low in fat, mushrooms also have vitamin D in them. They also include a tonne of healthy chemicals and antioxidants, as well as the nutrients copper, niacin, phosphorus, potassium, and selenium. It is understandable why mushrooms have been used in traditional medicine for so long.

2.3.3 Oyster Mushroom

Oyster Mushrooms (*Pleurotus ostreatus*) belongs to *Pleurotus* species. It is known as “*dhingri*” in India and has fan or oyster-shaped cap . They grow easily on decaying wood or straw (Wikipedia, 2018). The oyster mushroom can be found in a diversity of habitats, although other closely related species, like the branching oyster mushroom, can only be found on trees. In the UK, they can be spotted all year long. Although dead hardwood trees have been seen to have this fungus growing on them, it does so saprophytically rather than parasitically. As the tree dies for other reasons, *P. ostreatus* grows rapidly on the pile of dead and dying wood. By rotting the dead wood, they replenish the ecosystem with essential nutrients and minerals that may be used by other plants and animals. As a delicacy, the oyster mushroom is extensively utilized in cuisine. It is typically consumed alone, in soups, stuffed, or soy sauce-based stir-fry dishes. In some cases, oyster mushrooms are turned into an oyster sauce-like sauce that is utilized in Asian cuisine. The flavor of the mushroom has been characterized as moderate, with a faint anise-like aroma. The oyster mushroom is finest when collected when it is still young because as it ages, the meat gets tough and loses flavor (Patra *et al.*, 2015).In Kerala, India, pearl mushrooms are extensively cultivated and utilized in a wide range of recipes. The majority of oyster mushrooms are raised in large, clear polyethylene bags that have been divided into layers with hay and spawn. Oyster mushrooms contain very small amounts of arabitol, a sugar alcohol that some individuals may

find upsetting. Scientists are now learning what herbalists have known for centuries. In addition to being a vital source of nutrients, mushrooms can also boost the immune system, aid in the treatment of cancer, lower blood sugar and excessive cholesterol in diabetics, and even fight the HIV virus. In many temperate and subtropical woods around the world, oyster mushrooms are common in the Pacific Northwest of North America, *Pleurotus pulmonarius* and *Pleurotus populinus* have taken their place.

It is a saprophyte that mostly decomposes wood, notably beech trees and other deciduous trees. This fungus causes white-rot wood degradation. The oyster mushroom is one of the few known predatory mushrooms. It is believed that the mushroom obtains its nitrogen through the ability of its mycelia to kill and digest nematodes (Obodai *et al.*, 2012). Oyster mushrooms have an intricate look and a flavorful, mild flavor. They are readily available in stores and simple to cultivate at home. Despite not being the most popular oyster mushroom in the world, *Pleurotus populinus* and *Pleurotus pulmonarias* are both nonetheless regarded as oyster mushrooms. One of the more popular wild mushrooms is the oyster mushroom, though it can be grown on straw and other materials. It has an odor like benzaldehyde, which is bittersweet (which is also characteristic of bitter almonds). Greek term "*pleurotus*" relates to the stem's lateral extension concerning the cap, whilst the Latin term "*ostreatus*" (and the English common name, "oyster") alludes to the shape of the cap that resembles the bivalve of the same name. Many people also say that the name is appropriate because the food has an oyster-like flavor (Islam *et al.*, 2019). This mushroom is usually found in fields and grassy areas around the world after rain, particularly when combined with manure, from late spring to October. It is frequently harvested and consumed throughout most of the world, despite similarities to dangerous or lethal look alike. Amanita species can be confused with ordinary mushrooms in their juvenile forms, but you can

distinguish them by their volva or cup at the base of the mushroom and their pure white gills (as opposed to the pinkish or brown of *Agaricus bisporus*). As a result, it is crucial to always clean away debris, look at the base of identical mushrooms, and sever the gills of immature specimens to inspect them. In addition to flourishing in mossy forests, the destructive angel gets along well with spruce. *Agaricus xanthodermus*, an inedible mushroom that can be found in grassy areas all over the world, is a more frequent and less hazardous mistake than *Agaricus bisporus*. *Agaricus xanthodermus* has a phenol-like odour, and when damaged, its flesh turns yellow. Some persons who have this fungus experience nausea and vomiting (Guillamon *et al.*, 2015).

Other varieties are as follows;

- *Portabello*-This big, muscular mushroom may spread out to a maximum of 6 inches. They are frequently used as a meat replacement and taste great when grilled.
- Morel (*Morchella*)-Early to late April is when this delectable, edible mushroom blooms in forest regions. It appears to be a sponge. Though toxic, false morels look like morels.
- Chanterelle (*Cantharellus*)-is called a first class mushroom. It has a powerful flavor, is brilliant orange or yellow, and has a soft texture. Chanterelle is gathered in the fall from the ground beneath hardwood trees like oaks, identical are toxic.
- Enokitake- These tiny edible mushrooms may be purchased in most stores and are easily recognised by their thin, pin-like form and white color. Enoki mushrooms are used in a variety of ways, such as pickled or quickly fried, to give any dish a texture.
- Cordyceps (*Cordyceps militaris*)-is a type of edible fungus that is mostly renowned for its therapeutic uses. It has been researched for its potential anti-inflammatory and anti-cancer properties.

- *Laetiporus sulphureus* -Unsurprisingly, this mushroom also known as the chicken discovered in big, fan-shaped clusters that are occasionally linked to some kind of tree, either alive or dead. Mushrooms are frequently vividly colored and appear in orange and yellow tones mushroom, tastes like chicken. Depending on the species, these mushrooms are typically.
- Green-spored *Lepiota* (*Chlorophllum*)-This white, upright mushroom, which can be found in grassy regions, can get up to 8 inches across. Grey-green spores are what it leaves behind. Avoid interacting with this dangerous species.
- Destroying Angel (*Amanita* sp.)- From summer to fall, this extremely deadly mushroom thrives in woodlands. It resembles edible button mushrooms that you can get at the supermarket almost exactly. It is deadly, as the name suggests.
- Matsutake mushroom- In contrast to its relative obscurity in the west, the matsutake, or pine, mushroom is highly used in the east. Since it exclusively grows under specific trees and is sometimes hidden by leaves and other vegetation on the forest floor, the matsutake is an uncommon find in the wild. Since rabbits, deer, squirrels, and other animals frequently consume pine mushrooms before they can be harvested, humans are not the only species to desire them. The matsutake cultivar has a strong spicy flavor and aroma.
- Truffles- Despite being difficult to come by, the truffle mushroom is called one of the best tastings in the world. These fungi lie underground and used by specially trained canines with exceptionally refined senses of smell. As the underground fruiting body of a fungus, truffles are difficult to see with the human eye because they lack an external stem or cap that would protrude from the ground. Truffles are fairly unsightly and knobby, yet foodies all over the world adore their rich, nutty, earthy flavor.

- Shimeji- Identifiable characteristics of these little edible mushrooms include their tall stems and concave, tight caps. Mushrooms should never be eaten raw since they can be hard to digest until they have been roasted for a while. tree oyster mushroom or pearl oyster mushroom. Today, it is produced commercially for food all around the world. They respond well to practically all cooking methods, whether they are used at high, low, quick, or slow temperatures. So go ahead and fried or braises them and enjoy the delicate earthy taste of the shimeji
- *Porcinis mushrooms*: The porcinis mushrooms are known as a good source of antioxidants that prevent aging and may even lower the risk of neurological disorders like Parkinson's and Alzheimer's. Recent studies have found a link between the most often consumed mushroom, the white button mushroom, and has the potential to cure breast cancer and prostate cancer. One of the few foods that naturally contain vitamin D, button mushrooms are also high in antioxidants.

2.7 CULTIVATION

The basic requirements for mushroom cultivation are manure/compost, spawns, right temperature and humidity. Favorable growing conditions involve 80%- 90% of relative humidity, ample ventilation, a temperature range of 20-280 C during spawn run and 12-180 C for reproductive growth. Initially for a week temperature must be maintained at 23 ± 20 C and then it can be reduced to 16 ± 20 C for subsequent weeks. The CO₂ concentration should be 0.08-0.15 % (NHB). If the above stated conditions are maintained appropriately the pin heads start to appear within few days and progressively mature into button stage. Apart from these insecticides, nutritional supplements like nitrogen, vermiculite, water are also required for a healthy harvest. The following steps are to be followed for mushroom cultivation:

2.4.1 Compost Preparation

The compost (synthetic or natural) used for mushroom growth usually comprises of wheat straws, horse manure, poultry manure, rice bran, gypsum etc. (Datha *et al.*, 2019). Utmost care is taken to protect the raw compost against rain or external moisture, as it might introduce undesirable microbes. The chopped wheat straws or rice bran are mixed with horse dung, sprinkled with water and are heaped in a pile to allow fermentation. The fermentation process along with heat development breaks down the chemical compounds in small components. Frequent turnings and watering is done at a specific interval so as to avoid the drying up of compost. Gypsum is sometimes added to the compost to reduce greasiness and allow more aeration (Pennstate Extension, 2016). Within 15 to 20 days the compost gets all set to be used as bed, it is then spread onto wooden trays and sowed with spawns (TIMEIS, 2010).

2.4.2 Spawning

Spawns refers to the mycelium carefully propagated on agars or grains. Spawning is a process of sowing or mixing spawns in compost. Although mushroom produces spores which acts as a seed for further propagation but are not used generally due to uncertain germination and growth (RR Mushroom, 2013). The spawns are thoroughly mixed with the compost, are covered with newspaper and is watered sufficiently to maintain the moisture. Throughout the cultivation period humidity is kept high to avoid loss of moisture. Gradually they grow into white cottony mycelium growth (RR, 2013).

2.4.3 Casing

Casing is a kind of sterilized soil or dressing containing cow manure which is spread onto the spawn mixed compost. It is applied when the mycelium growth commences on the compost

surface. After 15 to 20 days of its application mushroom head or pins start becoming visible on the surface. They are allowed to mature for a specific time period and are harvested before opening of the cap. Mushrooms with opened cap (looks like an umbrella after opening of cap) are undesirable and are considered of menial quality (RR 2013).

2.8 Mushroom Cultivation and Production

The first mushrooms were produced in India in the 1970s, but as environmental control technology and a better understanding of cropping systems have evolved, mushroom output has increased significantly (Kakraliya *et al.*, 2020). Following oyster (6%), milky (1%), and other (4%), button mushrooms made up 89% of all the mushrooms produced in India in 2010. 5 total of 94,676 metric tons of white button mushrooms are now produced in India from both seasonal and high-tech cultivation units, and they account for roughly 73% of the country's total mushroom production (Kakraliya, *et al.*, 2020). The mushroom industry in the United States (U.S) generated \$1.22 billion in revenue in 2017. Since 2007, this indicates an 8% gain in value. The crop's total weight was 929 million pounds, a 2 percent decrease from the year before but a 12 percent (12%) gain over the preceding ten years. Around 60 % of all mushroom sales are produced in Pennsylvania, a percentage that has not changed over the past ten years. The industry is consolidating at the same time that production value is rising. The industry is consolidating at the same time that production value is rising. Between 2007 and 2017, the number of *Agaricus* and specialist mushroom growers fell by 32%. Growers have increased production through acquiring already-existing operations, extending their own operations, and both constructing new production facilities and improving their already-existing operations (AMI, 2018; Ali, 2003). Although commercial production of mushrooms began in Europe at the turn of the century, Bangladesh has only recently begun to produce them. The oyster (*Pleurotus spp.*)

and white button mushroom (*Agaricus bisporus*) are the two species that are most suitable for cultivation (Banglapedia, *et al.*,2019). Due to its good environment, cheap production costs, accessibility to growth substrates, and high market value, Bangladesh is one of the most suited countries in the world for mushroom farming (Banglapedia, 2019; Bangladesh, 2019; Ultimate Mushroom, 2021).

Although there is a great potential for mushroom production in Bangladesh, there are several issues with cultivation and marketing that must be resolved before actions can be taken to increase production (Rahman *et al.*, 2018). Due to people's unfavorable attitudes and their misconception that mushrooms are Halal, they have not received much attention as a dietary item in Bangladesh (Easin, *et al.*, 2019). Although mushroom cultivation technique is a relatively new development, incorporating this unconventional crop into the current agricultural system can help to improve the social and economic status of rural farmers and suburban residents. In 2014, the government of Bangladesh built the Mushroom Development Institute (MDI), formerly known as the National Mushroom Development and Extension Centre (NAMDEC), in Saver, Dhaka, to provide training and promote mushroom farming. The Bangladeshi population is becoming more and more interested in producing and eating mushrooms. Its production in Bangladesh is rising daily, as seen by the nearly four times bigger number of mushrooms produced in 2018–19 compared to 2009–10. Over the past ten years, a nascent trend in mushroom production has been seen. Through the use of low-cost, farmer-friendly equipment and MDI-developed technology, mushroom output and consumption are rising in Bangladesh (Sarker *et al.*, 2020). Although Bangladesh's mushroom production has expanded, the demand has not yet been met. It is projected that importing mushrooms would cost the nation BDT 85 to 90 crore annually (Thompson *et al.*, 2013). There are more than 10 million tons of mushrooms

produced worldwide (Martinez *et al.*, 2019). However, only 0.04 million tons come from the nation. More than 25 districts in Bangladesh are home to commercial mushroom farming (Easin *et al.*, 2017). Among Bangladeshi rural farmers, small-scale mushroom farming is expanding using a variety of production methods.

There were observable differences in the monthly production of mushrooms in 10 farms of various sizes. Despite the larger farm's (32 sq ft) size, the difference in management style may be the cause of the smaller farm's (15–30 sq ft) higher output efficiency. The Netherlands now produces 270 million kg of mushrooms annually and supports more than 10,000 jobs, making it the largest mushroom-producing nation in the EU. The Netherlands is third in the market behind the United States and China. With a market share of 70%, China has the highest production of mushrooms among the top ten producing countries, followed by Italy (10.16%) and the United States (5.29%). Around the world, millions of tons of mushrooms are grown annually. However, Poland was the world's top exporter of mushrooms. The Netherlands topped the list of leading mushroom eaters in terms of consumption, with 11.62 kg per person annually. In China, Japan, Poland, and India, the average annual consumption of mushrooms was less than one kilogram (Kakraliya *et al.*, 2020).

2.9 Socio-economic Status of Mushroom Growers

The production of mushrooms is primarily done by local people and even specialists around the world. According to a study on mushroom growers in the Savar upazila, the percentage of women involved in the production of mushrooms is exceptionally high (82.6%). The study also revealed that 31.4% of farmers engaged in mushroom farming as a supplementary employment, while 68.6% of farmers chose it as their major occupation (Ferdousi, 2020; Sarker, 2015). Small-scale mushroom farming was found to need relatively little investment, which resulted in low

daily production, with 80% of farms producing only 1–5 kg and the remaining 20% producing 5–10 kg (Ferdousi *et al.*, 2020).

2.10 Nutritional Benefits of Mushrooms

It is widely known that mushrooms include all the necessary elements of a balanced diet. The truth is that mushrooms are high in easily digested essential amino acids, abundant protein, vitamins, and minerals, but deficient in high-quality unsaturated fat and water-soluble carbohydrates (Marshall *et al.*, 2019). Additionally, it is abundant in other crucial elements like phosphorus, potassium, calcium, copper, iron, and the vitamin B complex (Ferdousi *et al.*, 2020). The mineral composition of various mushroom species also varied. In 2009, Khan *et al.*, 's analysis of the mineral content of various mushroom species revealed that per 100 g of dried mushrooms, there were 15.4-69 mg Fe, 16-275 mg Ca, 11.1-28.8 mg Zn, 14-31.4 mg Mg, and 685-1740 mg P. Different mushroom species have metabolizable energy contents that range from 150 to 300 Kcal per 100g of dry mushroom (Khan *et al.*, 2019). Depending on species, the nutritional index of mushrooms ranges from 6 to 31, indicating a good nutritious value of this meal. Edible mushrooms generally have fewer calories and fat than other plant-based foods, are high in vitamins, minerals, and protein (Alam *et al.*, 2017). Since, mushrooms contain all the nutrients a person needs in the right amounts, they are regarded as a full, healthy diet that is excellent for all age groups, from children to the elderly. Numerous variables, such as the mushroom's species, variety, developmental stage, and ambient conditions, have an impact on its nutritional content. Mushrooms are a good source of vitamins, minerals, dietary fiber, and protein. Dietary and fermentable fibers make up the majority of the carbohydrates; starch and sugars make up a little part of the total carbohydrates. Methionine and cysteine-amino acids that contain sulfur-are in short supply in edible mushrooms, which are rich in threonine and valine-

based proteins but lacking in other amino acids. Another benefit is the low lipid level, which has no cholesterol and a larger percentage of polyunsaturated fatty acids. Ergosterol, which is found in mushrooms, is a precursor for the body's production of vitamin D (Kakraliya *et al.*, 2020).

A mushroom's vitamin D level is influenced by postharvest management, particularly accidental sun exposure. The US Department of Agriculture gave proof that mushrooms that have been exposed to UV light contain significant quantities of vitamin D. Ergosterol in mushrooms, even after harvest, is transformed to vitamin D₂ when exposed to ultraviolet (UV) light. This process is being employed consciously to produce fresh vitamin D mushrooms for the functional food supermarket market. Researchers conducted a thorough analysis of the safety of producing vitamin D in fresh mushrooms, demonstrating that artificial UV light technologies were just as effective at producing vitamin D as mushrooms that were exposed to natural sunlight. UV light has a long history of being used safely to produce vitamin D in food. Mushrooms are also a great source of vitamins C and B (folate, thiamine, riboflavin, and niacin). In addition to other necessary elements (Cu, Zn, and Mg) in small amounts, minerals such as potassium, sodium, and phosphorous are found in larger concentrations in mushroom fruit bodies. However, iron and calcium are generally lacking. Enzymes, alkaloids, sterols, antioxidants, and other unidentified chemical compounds are among the substances that are most important for encouraging growth in mushrooms. (Krebs *et al.*, 2017).

2.8 Medicinal Benefits of Mushrooms

Edible fungus has long been valued for their enormous health advantages and frequently utilized in folk medicine. Particular bioactive substances found in medicinal mushrooms, such as polysaccharides, triterpenoids, low-molecular-weight proteins, glycoproteins, and immune-modulating substances, have therapeutic effects (Kakraliya *et al.*, 2020). Therefore, it has been

demonstrated that mushrooms can strengthen the immune system, improve general health, reduce the risk of cancer, stop the growth of tumors, assist in maintaining blood sugar balance, fight off bacteria, fungi, and viruses, lessen inflammation, and support the body's detoxification processes. Due to its low-fat level, higher proportion of unsaturated fatty acids, and lack of cholesterol, the mushroom diet is excellent for the heart. It has been discovered that mushrooms, which have low sodium and high potassium content, improve human blood circulation and salt balance. They are also beneficial for people with high blood pressure. (Kakraliya *et al.*, 2020).

Due to their low-calorie content, lack of starch, and low sugar content, mushrooms are a preferred diet for diabetic and obese people. As fiber acts as food for helpful microorganisms in the human digestive system, the fermentable dietary fiber in mushrooms enhances the normal operation of the bowel system. Kresin is one of the most often used cancer drugs in the pharmaceutical industry. It is a compound that limits tumor activity. A particular antioxidant, ergothioneine that present in *Flammulina velutipes* and *Agaricus bisporus*, is essential for the health of the eyes, kidneys, bone marrow, liver, and skin, slowing the aging process. The antioxidants found in mushrooms scavenge the body's free radicals and delay cell maturation. Thus, it functions as an anti-aging agent. The human immune system is controlled and strengthened by a variety of polysaccharides (betaglucans) and minerals that were identified from mushrooms (Kakraliya *et al.*, 2020). The therapeutic value of mushrooms is widely acknowledged. Health tonics, tinctures, teas, soups, and herbal formulas can all contain the 6% of edible mushrooms that have been shown to offer medical benefits (Ferdousi *et al.*, 2015). Due to their therapeutic benefits, edible mushrooms have been a key ingredient in the creation of some pharmaceutical products (Cheung *et al.*, 2019). *Shiitake (Lentinula edodes)* and *Reishi (Ganoderma lucidum)* mushrooms are well known for their medical benefits and are thought to

have anti-tumor, antiviral qualities including anti-HIV and anti-hepatitis B, and the ability to eliminate serum cholesterol from the blood stream (Wasser *et al.*, 2014). In Asian locations, they have been used for promoting and sustaining good health as well as the treatment of ailments since ancient times, although in the West, this strategy is much more recent. According to reports, medicinal mushrooms (MMs) have a wide range of pharmacological effects, including prebiotic, antibacterial, anti-inflammatory, immunomodulatory, antidiabetic, cytotoxic, and antioxidant qualities. They also have hepatoprotective, anticancer, antiallergic, and antihyperlipidemic capabilities (Venturella *et al.*, 2021).

Many bioactive metabolites found in the mycelium, but especially in the fruiting body, are responsible for these activities. Their biological effects vary depending on their chemical makeup, and their distribution changes depending on the type of fungus. Due to the increased interest in using natural products, including as adjuvants in conventional therapies, a great lot of research has been done and is being done to identify and characterize mycochemicals as well as to define their actions and mechanisms (Walton *et al.*, 2014). The mushroom of immortality' is the *Reishi* mushroom (Kaul *et al.*, 2011). Natural compounds found in mushrooms have been shown to be particularly beneficial in regulating blood pressure, decreasing blood cholesterol and blood sugar levels, preserving the liver, controlling some types of cancer, increasing the immune system, and therefore promoting overall fitness (Islam *et al.*, 2013). There are a wide variety of fungal compounds that have bioactivity and may be effective in the treatment and prevention of different diseases. The most crucial are polysaccharides, which serve as building blocks for the fungal cell wall. The polysaccharides are highly effective at transporting biological information. They exhibit anticancer, immunomodulatory, antioxidant, anti-inflammatory, antibacterial, and anti-diabetic activities, to be more precise. In truth, some molecular structural characteristics,

such as the weighted degree of branching, backbone linkage, side-chain units, and the kind of constituent monosaccharides, have an impact on the type and modulation of these biological activities. The most prevalent and well-known are α - and β -glucans. The biological activity is also influenced by heteroglycans, peptidoglycans, and polysaccharide-protein complexes (Zhao *et al.*, 2020). They have the ability to bind to particular cell wall receptors and induce particular immunological responses, which makes them principally responsible for immunomodulatory effects. Medicinal mushrooms are frequently utilized in cancer treatments as biological response modifiers (BRMs), which are beneficial for curing cancer, minimizing its side effects, and enhancing the patient's quality of life (Watling *et al.*, 2013).

The terpenes, which are made up of units of five-carbon isoprene atoms and are particularly significant for their bioactivity, are a different family of chemicals. By adding functional groups, terpenes create terpenoids. Besides having anti-inflammatory, antioxidant, and anticancer activities, they affect the immune system by promoting the expression of genes that code for immune response-related proteins. Species of mushrooms in the genus *Ganoderma* P. Karst have high terpenoid concentrations (Zhao *et al.*, 2020). Proteins in mushrooms are abundant and have cytotoxic and anticancer activities. Some of them are well-known for having a distinctive and noticeable immunomodulatory action. These proteins are identified as fungal immunomodulatory proteins (FIPs) with a variety of potential modes of action (Walton *et al.*, 2014). Additionally, proteins have lectins, which have a high selectivity reversible binding to mono and oligosaccharides, detecting and interacting with different carbohydrates and proteoglycans on the cell surface. Their immunomodulatory mechanism varies depending on the origin of the chemical, and they are engaged in a wide range of biological processes, including innate immunity and cell-to-cell communication. Additionally, they have anticancer, antiproliferative,

and immunomodulatory qualities (El-enshasy *et al.*, 2013). Other fungal byproducts that have biological function include phenolic compounds, antioxidants with various modes of action (oxygen scavenging, metal inactivation, free radical suppression, and peroxidase breakdown), laccases (copper-containing oxidases), and fatty acids (Elkhateeb *et al.*, 2020)

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study Area

The study was conducted in Benin City, the capital of Edo State, Nigeria.

3.2 Study Design

This study was designed to assess the effect of chemical preservatives on freshly harvested mushrooms for a period of 6 days.

3.3 Sample Collection

Mushrooms samples was collected from the African Centre for Mushroom Research and Technology Innovation [ACMRTI] Ugbowo, Benin City, the capital of Edo state, Nigeria. The fresh mushrooms were transported in sterile buckets with cover in the laboratory. All samples

were transported to the microbiology laboratory for analysis within 1 hour of collection to avoid sample degradation.

Preparation of chemical preservatives

The preservatives used are hydrogen peroxide and citric acid in concentrations of 0.5%, 1.5%, 2.0% and 2.5%. They were prepared by mixing the appropriate ratio with sterile deionized water. The preservatives concentration were kept in corked bottles and sterilized at 121°C for 15 minutes.

3.3.1. Sample processing

A total of 18 sterile plastic containers with holes bored with their covers were selected for use to accommodate analysis, in duplicates, from 0 to 6 days. Each container has 100g of mushrooms and 200ml of preservatives solutions respectively labeled. The containers were transferred to the refrigerator for storage.

3.3.2. Cultural analysis

3.4. Sterilization of Materials

Materials such as Petri-dishes, pipette, glass wares (conical flask, round bottom flask), test tubes, beakers, conical flasks, Petri-dishes, sterile cotton swabs, sterile gloves, stirring glass rod and measuring cylinders, pipettes and bottles were washed, drained and dried. They were wrapped with aluminum foil and sterilized in a hot-air oven at 160°C for an hour. They were allowed to cool after sterilization before usage. An aseptic working environment was achieved with the use of Bunsen burner flame and disinfection of work surfaces with alcohol.

3.4.1. Preparation of Nutrient agar

Twenty-eight grams (28 g) of nutrient agar was dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in the distilled water in a conical flask. The medium will be then placed in an autoclave to sterilize it for 15 minutes at 121 °C. After sterilization, the flask will be allowed to cool and poured on sterile petri dishes.

3.4.1.2 Preparation of Mannitol Salt agar

111grams of Mannitol Salt agar was suspended in 1000 ml distilled water in a conical flask corked with sterile cotton wool and foil paper and allowed to dissolve in the distilled water in a conical water. The medium was sterilized by autoclaving at 121 °C for 15 mins and then left to cool before dispensing on sterile petri dishes.

3.4.1.3 Preparation and Sterilization of materials

Biochemical test reagents and Gram's staining kit were also sterilized. All glassware which include Petri dishes, test tubes, conical flasks, measuring cylinders and pipettes, were sterilized at 160°C for 1 hour in a hot-air-oven before use. Agar media, agar slant and biochemical reagents were prepared freshly and refrigerated at 3 - 4°C. Aseptic conditions were ensured during inoculation and sub-culturing.

3.5. ISOLATION AND ENUMERATION OF BACTERIA

Upon arriving at the laboratory, a 5g of the freshly preserved mushroom sample was weighed and subjected to serial dilution in sterile peptone water. This method was employed to ensure accurate colony counts and to minimize overcrowding on the plates. The mixture was vortexed to ensure proper homogenization. A series of tenfold dilutions were prepared by transferring 1 ml of the initial solution into a second test tube containing 45 ml of sterile peptone water, thereby producing a 10^{-1} dilution. This process was repeated to create further dilutions (10^{-2} , 10^{-3} , and 10^{-4}).

The pour plate method was used to isolate microorganisms from the diluted samples. For each dilution, the following steps were carried out: 1 ml of each dilution (from 10^{-1} to 10^{-4}) was pipetted aseptically into sterile Petri dishes. Approximately 15-20 ml of molten agar (cooled to about 45°C) was poured into each Petri dish and gently swirled to ensure even distribution of the inoculum. Plates containing Nutrient Agar, were incubated at 37°C for 24-48 hours for the isolation of bacterial colonies. After the incubation period, distinct colonies were counted using a colony counter. The number of colony-forming units (CFUs) per milliliter was calculated based on the dilution factor.

3.5.1 Subculturing of Pure Isolates

After colony counting, well-isolated colonies with distinct morphologies were selected and sub-cultured onto fresh Nutrient Agar and Mannitol salt agar plates to obtain pure cultures. These pure cultures were then subjected to further identification tests, such as biochemical and morphological characterization.

3.6 BACTERIAL IDENTIFICATION

The bacterial isolates were characterized based on colonial morphological characteristics such as colony shape, size, elevation, optical activity, margination and pigmentation on Nutrient agar and Mannitol agar. Biochemical tests were also carried out to further identify the bacterial isolates.

3.6.1 Gram staining

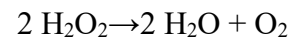
Smears of the bacterial isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Grams' iodine solution for one minute. This was washed off with distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed off with distilled water. The smears were then counter stained with safranin solution for one

minute. Finally, the slides were washed off with distilled water, air dried and observed under oil immersion objective.

3.7. BIOCHEMICAL TEST

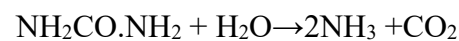
3.7.1 Catalase Test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.



3.7.2 Oxidase Test

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-pphenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and smeared on the wet piece of paper. Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test.



3.7.3 Citrate Utilization Test

This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was incubated at 37°C for 24 - 48 hr. The development of deep blue colour after incubation indicates a positive result.

3.7.4 Indole Test

Indole test is performed to determine the ability of the organism to split tryptophan molecule into indole. This test is performed to help differentiate species of the family enterobacteriaceae. Kovac's reagent which contains hydrochloric acid, dimethyl-aminobenzaldehyde and amyl alcohol is used. The broth was inoculated with the test organism and incubated for 18 hours at 37°C. 5ml of Kovac's reagent was then added down the inner wall of the tube. Development of bright red colour at the interface of the reagent and the broth within seconds after adding the reagent was indicative of the presence of indole and a positive result.

3.7.5. Triple sugar iron (TSI) agar test

The Triple Sugar Iron (TSI) test is an ability to test an organism's capability to ferment sugars and to produce hydrogen sulphide (H₂S) or gas (O₂), or both. The test was used primarily to differentiate members of the *Enterobacteriaceae* family based on their sugar fermentation patterns and from other Gram-negative rods. An agar slant prepared of a TSI agar was used in carrying out this test in a sterile test tube at a slanted angle. The slanted medium was inoculated with TSA pure culture using a straight inoculation needle by stabbing first through the center to the bottom of the tube and streaking the agar slant's surface. After inoculations, the test tubes were covered with foil paper and left at an ambient temperature of 36°C to incubate for 24 hours. Reactions on test tubes were examined, and sugar fermentations were indicated by the production of H₂S, gas and a change in colours from red (alkaline) to yellow (acid). When an alkaline/acid (red top/yellow bottom) slant reaction appeared, it only indicated dextrose (glucose) fermentation. When an acid/acid (yellow top/yellow bottom) slant reaction appeared, it showed the fermentation of dextrose, lactose and/or sucrose. The appearance of an alkaline/alkaline (red top/red bottom) slant reaction represented the absence of sugar fermentation. The blackening of

the medium in the slant indicated H₂S production. Bubbles, cracks, or bottom-raised space in the slanted agar indicated gas production (formation of CO₂ and H₂) (Fawole and Oso, 2007).

3.7.6 Hydrogen Sulphide production

This was determined by using the triple sugar iron (TSI), which is a multi test medium. It tests the ability of bacteria to ferment glucose, sucrose and xylose. The fermentation or production of hydrogen sulphide and carbon (IV) oxide. Colonies were streaked on TSI agar slants and incubated for 72 hours with the corks of the bottles set loosely. Formation of blackening sections in the medium indicated a positive test for hydrogen sulphide.

3.7.7 Vogues Proskeuer test

This test was used to demonstrate bacteria that ferment carbohydrate with the production of acetyl methyl carbinol (CH₃-CO-CHOH.CH₃). This compound is oxidized during the test to diacetyl which reacts with a guanido group under alkaline condition to give a pink colour. The bacteria culture of the test organism was inoculated into 2 ml of sterile glucose phosphate peptone water and incubated at 37°C for 48 hours. 1 ml of 40% KOH and 3 ml of 5% alcoholic alpha-naphthol (Barritts reagent) was then added. It was then shaken and observed for colour formation. A pink colour within 2-5 minutes indicates a positive result.

3.7.8 Sugar fermentation test

Many bacteria species can be differentiated on the basis of the sugars they utilize and ferment. The fermentation medium was prepared by the addition of 0.1 g of peptone, 0.1 g of sodium chloride and 0.1 g of fermentable sugar (glucose, mannitol, lactose, sucrose and mannose) in 10 ml of distilled water. 4 ml of the medium was pipetted into Bijou bottles containing Durham tubes. 1 ml of phenol red indicator was also added to the tubes. The Bijou bottles containing the sugar solution were inoculated with the test bacterial isolates and incubated at 37°C for 24-48

hours. After incubation, a change of color from red to yellow indicates acid production and the presence of gas in the inverted Durham tubes was indicative of gas production (O'Hara *et al.*, 1993).

3.7.9 Methyl red test

This tests how low the pH of the medium has been reduced to. The effective test pH range is 4.5 and below. Methyl red is red in an acidic medium and gold in an alkaline medium. A drop of overnight bacterial broth medium was introduced to a drop of methyl red indicator on a clean, grease-free slide. A positive result was indicated by the mixture turning red while gold /yellow was negative.

3.7.1.1 CO² gas production

Durham tubes were inserted facedown into the glucose fermentation test tubes before sterilization at 121^{0c} for 5 mins. If the bacterium produces gas during fermentation of the sugar, the gas produced displaces the Durham tubes and takes up the vacated space. This is indicated by visible clear space within the Durham tube,

3.8. PATHOGENICITY TEST

3.8.1. Gelatinase production

In the preparation of gelatinase agar, 0.5gram of N.A (agar nutrient) and 0.5 grams of gelatinase were added in 50ml of water and was autoclaved for 121^{0c} for 15 mins and was allowed to cool before aseptically pouring into the petri dish, allowed to solidify , inoculated incubated for 24hrs at 37oc. A clear zone around colonies indicates gelatinase positive.

3.8.3. Lipase production

1egg weighing 25 gram was washed and soaked in 35% ethanol for 30minutes. The yolk were carefully extracted and 20ml was added into 20 ml of sterile and was mixed to form emulsion. 0.5gram of agar powder was added into 50ml of water and was autoclaved for 121^{0c} for 15mins,

allowed to cool before aseptically pouring in petri dish along with the yolk emulsion and was swirled, allowed to solidify before inoculating and thereafter incubated for 48hours at 25^{0c}. A shiny appearance on the medium indicates lipase positive.

3.8.4. Protease production

0.5 grams of N.A (nutrient agar) was added into 50 ml of water and autoclaved for 121^{0c} for 15min allowed to cool , blood was added and swirled to mix before aseptically pouring in petri dish, allowed to solidify and thereafter incubated for 24hrs at 37^{0c}. A clear zone around colonies indicates positive

3.9. Antibiotic Susceptibility test

The identified colonies of bacteria were used to determine the susceptibility and resistance of bacterial isolates, which were subjected to standard antibacterial susceptibility testing (AST) to decipher their resistance or susceptibility to common antibiotics used for treatment within the locality. The standard discs were produced by Oxoid, UK, which was used to execute the disc diffusion method employed in this study. For this assay, a fully grown bacterial culture (from 18-24 hours) was cultured on MHA. The inoculum corresponding to 1.5 x 10⁸ cells/ml McFarland standard was streaked using a sterile loop onto the MHA plates before the introduction of antibiotic discs and were added with extreme care to the plates with the aid of sterile forceps. The susceptibility results were recorded after incubation for 24 hours at 37 °C. Following the standard or rules of AST established in 2017 by CLSI (Clinical Laboratory Standards Institute). The inhibition zone around each disc (measured using a meter rule in diameter) was assessed and interpreted based on the 2020 CLSI standard as Resistant (R), Intermediate resistant (I) and Sensitive (S).

3.9.1. Multiple Antibiotic Resistance (MAR) Index

This index is a good tool which identifies the region where the isolates were obtained. Whether they are from places of high or low risks or from areas where antibiotics are abused. This tool becomes necessary for health risk assessment. According to Davis and Brown (2016), an index of ≥ 0.2 and above is indicative of a 'high-risk' contamination source. In this study the MAR index was determined by employing the methods delineated by Chitanand *et al.* (2010). The formula below was used to decipher MAR index of bacterial isolates. It is a general established rule that MAR index greater than 0.2 is indicative of the fact that the bacterium originates from areas where antibiotics have been abused (or regularly used) or worse still from areas of high-risk source of contamination.

3.10. Statistical Analysis

Tests were conducted in duplicates to obtain mean and standard deviation or errors and were reported as mean \pm standard deviation. Results were processed using Microsoft Excel and SPSS 19.00.

CHAPTER FOUR

RESULTS

Sensory evaluation for aroma, colour, texture and overall appearance was presented below as:

The mushroom sample at refrigerated temperature recorded its highest count (6.6 ± 1.4) after 24 hrs as shown in Table 1, while the mushroom samples at 4°C recorded the lowest count (2.6 ± 1.4) after 24 hrs.

The mushroom sample at refrigerated temperature recorded its highest count (4.6 ± 7.5) after 24 hrs as shown in Table 2, while the mushroom samples at 4°C recorded the lowest count (1.4 ± 2.6) after 24 hrs.

The mushroom sample at refrigerated temperature recorded its highest count (4.6 ± 2.4) after 24 hrs as shown in Table 3, while the mushroom samples at 4°C recorded the lowest count (2.1 ± 1.6) after 24 hrs.

The mushroom sample at refrigerated temperature recorded its highest count (3.9 ± 1.7) after 24 hrs as shown in Table 4, while the mushroom samples at 4°C recorded the lowest count (3.1 ± 1.1) after 24 hrs.

Table 5 shows the total heterotrophic bacterial count for nutrient agar with highest bacterial count recorded at $18.25 \pm 15.35 \times 10^4$ cfu/g. while the lowest count was recorded at $1.28 \pm 0.025 \times 10^4$ cfu/g.

Table 6 shows the total heterotrophic bacterial count for eosin methylene blue agar with highest value recorded at $8.1 \pm 0.4 \times 10^4$ cfu/g. while the lowest count was recorded as $1.3 \pm 8.5 \times 10^4$ cfu/g.

Table 7 shows the characterization and identification of bacterial isolates from samples. The biochemical test conducted includes indole, urase, citrate, lactose, maltose, xylose, glucose, methyl red, mannitol, gas production, H_2S , and KOH test.

Table 8 shows the antibiotic sensitivity test of bacteria isolates. The antimicrobial discs were coated with various antibiotics like streptomycin, cephalosporin, clindamycin, augmentin e.t.c. the bacterial isolates showed responses to the antimicrobial discs by exhibiting some characteristics (sensitive, resistance and intermediate)

Table 9 shows the Virulence factor

Table 1: Aroma evaluation of Mushroom treated with chemical preservatives during storage

Treatment	Storage duration (Day)			
	0	2	4	6
0.5% H ₂ O ₂	6.09±1.56	4.6±1.5	5.0±2.8	3.8±2.4
1.5% H ₂ O ₂	5.8±2.4	5.8±1.6	5.4±3.2	5.0±3.4
2.0% H ₂ O ₂	6.09±1.56	6.6±1.4	5.8±1.6	2.6±1.4
2.5% H ₂ O ₂	5.8±2.4	4.6±1.4	4.2±3.2	3.4±2.0
0.5% citric acid	6.09±1.56	5.0±1.3	3.4±2.9	5.0±3.2
1.5% citric acid	5.8±2.4	6.2±2.7	4.6±3.7	5.0±3.6
2.0% citric acid	6.09±1.56	5.0±2.3	3.0±1.8	4.2±2.7
2.5% citric acid	5.8±2.4	5.0±1.5	3.4±2.9	5.0±3.6
Water (control)	6.09±1.56	6.6±1.4	4.6±3.7	4.2±2.7

Table 2: Colour evaluation of Mushroom treated with chemical preservatives during storage

Treatment	Storage (Day)			
	0	2	4	6
0.5% H_2O_2	3.1±4.2	1.2±5.2	4.1±4.6	3.8±3.9
1.5% H_2O_2	2.6±3.6	2.7±3.7	3.7±3.7	3.5±4.6
2.0% H_2O_2	3.1±4.2	3.6±5.8	3.2±2.2	4.1±2.1
2.5% H_2O_2	2.6±3.6	4.6±7.5	3.4±2.5	4.0±2.6
0.5% citric acid	3.1±4.2	1.4±2.6	3.6±3.1	3.6±4.6
1.5% citric acid	2.6±3.6	3.4±3.6	2.8±3.8	3.0±3.6
2.0% citric acid	3.1±4.2	4.6±2.2	3.3±2.0	3.5±4.6
2.5% citric acid	2.6±3.6	2.5±3.4	2.7±3.7	3.0±4.0
Water (control)	3.1±4.2	3.5±3.5	3.5±2.7	3.5±3.6

Table 3: Texture evaluation of Mushroom treated with chemical preservatives during storage

Treatment	Storage(Days)			
	0	2	4	6
0.5%H ₂ O ₂	2.1±1.6	3.5±1.6	3.4±3.2	3.2±3.9
1.5%H ₂ O ₂	2.3±1.9	2.7±1.8	3.8±3.4	3.9±3.3
2.0%H ₂ O ₂	3.1±2.4	4.2±1.3	3.0±3.4	3.2±3.4
2.5%H ₂ O ₂	2.9±3.9	3.6±1.1	3.8±4.2	3.4±3.5
0.5% citric acid	2.9±4.9	2.9±1.7	4.6±2.4	3.9±3.2
1.5% citric acid	3.9±2.2	3.4±1.3	2.8±3.7	3.5±3.4
2.0% citric acid	2.9±1.9	4.6±2.3	3.8±3.6	3.9±3.2
2.5% citric acid	2.1±3.8	3.8±2.4	3.6±1.7	3.8±3.0
Water (control)	3.5±1.9	3.9±1.7	3.3±1.2	3.1±3.2

Table 4: overall appearance of Mushroom treated with chemical preservatives during storage

Treatment	Storage (Day)			
	0	2	4	6
0.5% H_2O_2	3.1±1.6	3.2±1.1	3.6±1.0	3.2±1.9
1.5% H_2O_2	3.6±1.1	3.5±1.4	3.5±1.3	3.9±1.3
2.0% H_2O_2	3.4±1.2	3.1±1.2	3.1±1.4	3.5±1.2
2.5% H_2O_2	3.9±1.7	3.8±1.5	3.4±1.5	3.2±1.1
0.5% citric acid	3.4±1.5	3.5±1.5	3.6±1.3	3.5±1.4
1.5% citric acid	3.8±1.3	3.1±1.2	3.9±1.6	3.1±1.1
2.0% citric acid	3.9±1.7	3.1±1.4	3.8±1.2	3.9±1.2
2.5% citric acid	3.2±1.4	3.2±1.2	3.4±3.1	3.8±1.4
Water (control)	3.2±1.1	3.4±1.1	3.2±1.1	3.2±1.2

Table 5: Total heterotrophic bacterial count of Mushroom treated with chemical preservatives during storage

Treatment	Storage (Day)			
	0	2	4	6
0.5% H_2O_2	$5.32 \pm 4.17 \times 10^1$	$1.9 \pm 0.5 \times 10^2$	ND	$1.5 \pm 0.3 \times 10^4$
1.5% H_2O_2	$1.725 \pm 0.275 \times 10^1$	$2.55 \pm 0.55 \times 10^2$	ND	$5.18 \pm 4.10 \times 10^4$
2.0% H_2O_2	$5.32 \pm 4.17 \times 10^1$	$6.7 \pm 0.9 \times 10^2$	ND	$5.1 \pm 3.9 \times 10^4$
2.5% H_2O_2	$1.725 \pm 0.275 \times 10^1$	$1.38 \pm 0.03 \times 10^2$	ND	ND
0.5% citric acid	$5.32 \pm 4.17 \times 10^1$	$4.85 \pm 3.65 \times 10^2$	ND	$2.01 \pm 0.18 \times 10^4$
1.5% citric acid	$1.725 \pm 0.275 \times 10^1$	$6.3 \pm 0.3 \times 10^2$	$1.3 \pm 0.02 \times 10^3$	$1.49 \pm 0.27 \times 10^4$
2.0% citric acid	$5.32 \pm 4.17 \times 10^1$	$5.5 \pm 0.5 \times 10^2$	ND	$18.25 \pm 15.35 \times 10^4$

2.5% citric acid	$1.725 \pm 0.275 \times 10^1$	$2.0 \pm 0.5 \times 10^2$	ND	$1.28 \pm 0.025 \times 10^4$
Water (control)	$5.32 \pm 4.17 \times 10^1$	$8.1 \pm 0.5 \times 10^2$	ND	$1.28 \pm 0.02 \times 10^4$

ND-Not determined

Table 6: *Escherichia* spp counts of Mushroom treated with chemical preservatives during storage

Treatment	Storage (Day)			
	0	2	4	6
0.5% H ₂ O ₂	$3.25 \pm 0.25 \times 10^1$	$5.5 \pm 2.5 \times 10^2$	ND	$5.5 \pm 0.9 \times 10^4$

1.5% H_2O_2	$6.5 \pm 1.5 \times 10^1$	$2.3 \pm 1.2 \times 10^2$	ND	$2.9 \pm 2.6 \times 10^4$
2.0% H_2O_2	$3.25 \pm 0.25 \times 10^1$	$0.6 \pm 0.6 \times 10^2$	ND	$6.0 \pm 1.4 \times 10^4$
2.5% H_2O_2	$6.5 \pm 1.5 \times 10^1$	$7.5 \pm 1.8 \times 10^2$	ND	$7.5 \pm 0.7 \times 10^4$
0.5% citric acid	$3.25 \pm 0.25 \times 10^1$	$3.3 \pm 1.6 \times 10^2$	ND	$8.1 \pm 0.4 \times 10^4$
1.5% citric acid	$6.5 \pm 1.5 \times 10^1$	$1.3 \pm 8.5 \times 10^2$	ND	$5.10 \pm 5.24 \times 10^4$
2.0% citric acid	$3.25 \pm 0.25 \times 10^1$	$2.78 \pm 1.7 \times 10^2$	ND	$4.3 \pm 4.10 \times 10^4$
2.5% citric acid	$6.5 \pm 1.5 \times 10^1$	$4.45 \pm 4.3 \times 10^2$	ND	$3.25 \pm 4.59 \times 10^4$
Water (control)	$3.25 \pm 0.25 \times 10^1$	$2.32 \pm 1.52 \times 10^2$	ND	$2.0 \pm 0.21 \times 10^4$

Table 7: Characterization and identification of bacterial isolates from Mushroom samples treated with chemical preservatives during storage

Cultural	A	B	C	D	E	F	G	H	I	J	K
Characterization											
Colour	Light yellow	Creamy	Milky	Creamy	Golden yellow	Colourless	Colourless	Creamy	Dark green	Very dark green	Peach
Margin	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Rough	Rough	Rough	Smooth	Smooth
Shape	Oval	Oval	Oval	Oval	Rough	Dual	Irregular	Irregular	Irregular	Spot	Oval
Size	5mm	3 mm	3 mm	4 mm	1.5 mm	5 mm	4 mm	7 mm	4 mm	1 mm	3.5 mm
Elevation	Flat	Flat	Flat	Flat	Elevated	Flat	Elevated	Elevated	Elevated	Flat	Elevated
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Transparent	Transparent	Opaque	Opaque	Opaque	Opaque
Wetness/Dryness	Wet	Dry	Wet	Wet	Dry	Dry	Wet	Wet	Wet	Dry	Wet
Shining/dull	Shiny	Shiny	Dull	Dull	Shiny	Dull	Dull	Dull	Dull	Shiny	Shiny
Morphological Characterization											
Gram's Reaction	Curve	Curve	Curve	Curve	Curve	Curve	Curve	Curve	Curve	Curve	Curve
Gram's morphology	Cocci	Cocci	Rods	Bacilli	Rods in cluster	Mods in clusters		Bacilli		Rods	Cocci
Biochemical Characterization											
VP	+	+	+	+	+	-	-	-	+	+	-

KOH	-	+	-	+	-	+	-	+	-	-	+
Methyl red	-	-	+	-	-	-	-	+	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	-	-	+	-	+	-	+	-
Glucose	+	+	+	+	+	+	+	+	-	+	+
Lactose	+	+	+	+	+	-	+	+	-	+	+
Maltose	+	+	-	+	+	+	+	+	+	+	+
D-mannitol	+	+	-	+	+	+	+	+	+	-	+
Indole	-	-	-	-	-	-	+	-	-	+	+
Xylose	+	+	-	+	+	+	+	+	-	+	+
Citrate	+	+	+	+	+	+	+	+	+	+	+
Urase	+	-	-	-	-	-	-	-	-	+	-
H ₂ S	-	-	-	-	-	-	-	-	-	-	-
Gas	+	+	+	+	+	+	+	+	-	+	+
Possible Identity	<i>Klebsiella pneumoniae</i>	<i>Moraxella catarrhalis</i>	<i>Pantoea agglomerans</i>	<i>Bacillus subtilis</i>	<i>Cronobacter sakazakii</i>	<i>Bacillus cereus</i>	<i>Escherichia coli</i>	<i>Corynebacterium jeikeium</i>	<i>Serratia marcescens</i>	<i>Aeromonas hydrophila</i>	<i>Enterococcus faecalis</i>

Table 8. Percentage frequency of Occurrence for bacteria isolates

Isolates	Percentage %
<i>Klebsiella pneumonia</i>	7
<i>Moraxella catarrhalis</i>	9
<i>Pantoea agglomerans</i>	8
<i>Bacillus subtilis</i>	11
<i>Cronobacter sakazalei</i>	8
<i>Bacillus cereus</i>	8
<i>Escherichia coli</i>	10
<i>Corynebacterium jeikeium</i>	9
<i>Serratia marcescens</i>	8
<i>Aeromonas hydrophilia</i>	11
<i>Enterococcus faecalis</i>	11

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Table 9: Antibiotic Sensitivity Test of bacterial isolates

Gram Positive	RD(20µg)	CIZ(30µg)	S(30µg)	AZN(10µg)	AMX(30µg)	CPX(10µg)	E(30µg)	LEV(30µg)	CN(10µg)	CEF(30µg)
<i>B. subtilis</i>	R	S	R	R	R	S	I	I	R	R
<i>B. cereus</i>	R	R	S	S	S	S	I	I	R	R
<i>C. jeikeium</i>	R	S	R	S	S	S	I	I	R	R
<i>E. faecalis</i>	R	R	R	S	S	S	I	I	R	R
Gram Negative	OFX(10µg)	AU(30µg)	PEF(10µg)	CTZ(30µg)	CN(10µg)	CFX(30µg)	CEP(10µg)	TRX(20µg)	S(30µg)	CEF(30µg)
<i>K. pneumonia</i>	S	R	S	R	R	S	R	R	R	R
<i>M. catarrhalis</i>	S	R	S	R	R	S	R	R	R	R
<i>P. agglomerans</i>	S	R	S	R	R	S	R	R	R	R
<i>C. sakazalei</i>	R	R	S	R	R	S	S	R	R	R
<i>E. coli</i>	R	R	R	R	R	S	R	R	R	R
<i>Serratia marcescens</i>	S	R	S	R	R	S	R	R	R	R
<i>Aeromonas hydrophilia</i>	S	R	R	R	R	S	R	R	R	R

Key: S = Sensitive R = Resistant I = Intermediate

GN: Gentamycin, RD: Rifampicin, AMX: Amoxil, CPX: Ciprofloxacin, AZM: Azithromycin, AU: Augmentin, LEV: Levofloxacin, E: Erythromycin, PEF: Perflacin, CTZ: Ceftazidime

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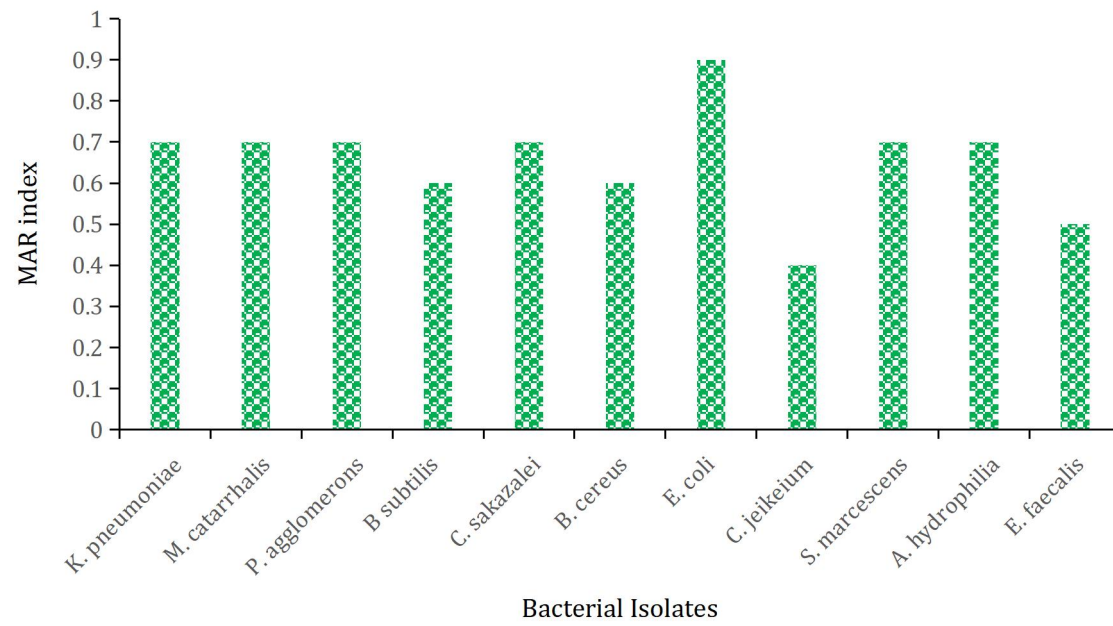


Figure 1: Multiple antibiotic resistance index

CHAPTER FIVE

DISCUSSION

This study was carried out to evaluate the mushroom preservation using chemical preservatives to extend their shelf life over an extended period of time. Preservatives significantly slow down microbial growth, reducing spoilage and extending the storage period. Among the tested chemicals, hydrogen peroxide showed the best preservation effect by inhibiting browning and microbial contamination. Some preservatives, particularly citric acid, help retain vitamin C and antioxidant properties, maintaining nutritional value. However, prolonged exposure to certain preservatives, such as sodium benzoate, led to minor nutrient degradation over time. Mushrooms treated with preservatives maintain better texture, color, and odor compared to untreated samples. High concentrations of preservatives, however, sometimes resulted in off-flavors or slight discoloration. Hence, the results obtained from this study show that the preservatives of mushrooms using hydrogen peroxide and citric acid at 0.5, 1.5, 2.0 and 2.5% concentration at day 2 have shown to be better in texture, smell and color than preserved mushrooms in the same concentration at day 4. The results obtained from the present study are summarized here under: oyster mushrooms of uniform size, intact veil and free from mechanical damage were selected. The most successful strategies aimed to prevent browning occurring on fresh-cut fruit and vegetables are based on treatments with reducing agents, acidifying agents, chelating substances and calcium solutions (Martin-Diana *et al.*, 2014). All the treatments were effective in keeping the veil intact up to the 4th day of storage. Among the various treatments used citric acid (2.5%) was the most effective in delaying browning. However, the mushrooms treated with 2.5% citric acid recorded the lowest (1.19) veil opening (i.e. veil intact) whereas the highest maturity index of 0.9

was observed in on 4th day of storage. Weight loss in mushrooms is a common phenomenon which occurs mainly due to moisture loss and loss of carbon reserves due to respiration. Treatment of mushrooms with various chemicals had a significant effect in controlling weight loss and it might be because these treatments helped in reducing the rate of respiration and transpiration. Use of citric acid and hydrogen peroxide was found to be effective in reducing weight loss as compared to EDTA. Bayoumi et al., 2017] also reported that H₂O₂ treatment significantly reduced fresh weight loss of fruits during storage under room temperature and fridge conditions. The antimicrobial effect of citric acid and H₂O₂ helped in controlling the bacterial growth. Among the various treatments citric acid was found to be most effective in inhibiting microbial growth. The significant effect of citric acid in limiting the development of food spoilage and pathogenic microorganisms is widely accepted.

5.1 CONCLUSION

To maintain the quality and to extend its acceptability, the treatment of mushrooms with various chemicals like hydrogen peroxide and citric acid were effective in retaining the quality parameters of mushrooms. From the observations it was observed that citric acid is the most effective in controlling weight loss, maturity index and microbial growth. Citric acid showed slightly antibacterial potential but induced a slight yellowness on mushroom surface. It was also observed that higher concentration of citric acid led to more yellowness on the surface of mushrooms as but browning was significantly reduced. Hydrogen peroxide is more effective in maintaining the whiteness of mushrooms and was found to be at par with citric acid. It had showed the senescence inhibition of oyster mushrooms by the combined effect of chemical treatment and MAP, resulting in maintenance of tissue firmness and sensory quality, inhibition of lipid peroxidation and better retention of phenolics and antioxidant ability as compared to the

control samples which is water. These results suggested that a combination of chemical treatment and MAP had good promise in maintaining oyster mushroom quality and extending their postharvest life up to 25 days when stored at 4°C. Another important observation made was that the use of chemical treatment alone was more effective than the use of MAP alone. High weight loss with visible evidence of deterioration and senescence were the main effects observed in the chemically untreated samples.

REFERENCES

- Abatenh, E. and Gizaw, B. (2018). Mushroom preservation protocol. *Journal of Microbiology and Biotechnology*, 3(1): 000127.
- Adams, L. S., Chen, S., Phung, S., Wu, X. and Ki, L. (2008). White button mushroom (*Agaricus bisporus*) exhibits antiproliferative and proapoptotic properties and inhibits prostate tumor growth in athymic mice. *Nutrition and Cancer*, 60(6): 744-756.
- Ahmed, O. M., Ebaid, H., El-Nahass, E. S., Ragab, M. and Alhazza, I. M. (2020). Nephroprotective effect of *Pleurotus ostreatus* and *Agaricus bisporus* extracts and carvedilol on ethylene glycol-induced urolithiasis: Roles of NF- κ B, p53, bcl-2, bax and bak. *Biomolecules*, 10(9): 1317.
- Alam, N., Khan, A., Hossain, M. S., Amin, S. R. and Khan, L. A. (2007). Nutritional analysis of dietary mushroom *Pleurotus florida* Eger and *Pleurotus sajor-caju* (Fr.) Singer. *Bangladesh Journal of Mushroom*, 1(2): 1-7.
- Ali, N. A., Mothana, R. A. A., Lesnau, A., Pilgrim, H. and Lindequist, U. (2003). Antiviral activity of *Inonotus hispidus*. *Fitoterapia*, 74, 483-485.
- Alonso, E. N., Ferronato, M. J., Fermento, M. E., Gandini, N. A., Romero, A. L., Guevara, J. A., ... & Curino, A. C. (2018). Antitumoral and antimetastatic activity of Maitake D-Fraction in triple-negative breast cancer cells. *Oncotarget*, 9(34): 23396-23412.
- Alonso, E. N., Ferronato, M. J., Gandini, N. A., Fermento, M. E., Obiol, D. J., Lopez Romero, A. and Curino, A. C. (2017). Antitumoral effects of D-fraction from *Grifola frondosa* (maitake) mushroom in breast cancer. *Nutrition and Cancer*, 69(1): 29-43.
- Alonso, E. N., Orozco, M., Nieto, A. E. and Balogh, G. A. (2013). Genes related to suppression of malignant phenotype induced by Maitake D-Fraction in breast cancer cells. *Journal of Medicinal Food*, 16(7): 602-617.
- Alves, M. J., Ferreira, I. C., Dias, J., Teixeira, V., Martins, A. and Pintado, M. (2012). A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. *Planta Medica*, 78(16): 1707-1718.
- American Mushroom Institute. (2018). The economic and fiscal impacts of the mushroom industry. American Mushroom Institute, 1284 Gap Newport Pike, Avondale, PA 19311.

- Atila, F., Owaid, M. N. and Shariati, M. A. (2021). The nutritional and medical benefits of *Agaricus bisporus*: A review. *Journal of Microbiology, Biotechnology and Food Sciences*, 2021, 281-286.
- Banglapedia. (2019). National Encyclopedia of Bangladesh. Retrieved from <http://en.banglapedia.org/index.php?title=Mushroom>.
- Bano, Z., Shashirekha, M. N. and Rajarathnam, S. (1993). Improvement of the bioconversion and biotransformation efficiencies of the oyster mushroom (*Pleurotus sajor-caju*) by supplementation of its rice straw with oil seed cakes. *Enzyme and Microbial Technology*, 15(11): 985-993.
- Bazee, T. J. and Janardhanan, K. K. (2007). Indian medicinal mushrooms as a source of antioxidant and antitumor agents. *Journal of Clinical Biochemistry and Nutrition*, 40(2): 157-162.
- Blumfield, M., Abbott, K., Duve, E., Cassettari, T., Marshall, S. and Fayet-Moore, F. (2020). Examining the health effects and bioactive components in *Agaricus bisporus* mushrooms: A scoping review. *The Journal of Nutritional Biochemistry*, 84:108-453.
- Boa, E. R. (2004). *Wild edible fungi: A global overview of their use and importance to people* (No. 17). Food and Agriculture Organization of the United Nations (FAO): Rome.
- Chang, S. T. and Miles, P. G. (1992). Mushroom biology: A new discipline. *Mycologist*, 6(2): 64-65.
- Cheung, L. M. and Cheung, P. C. K. (2005). Mushroom extracts with antioxidant activity against lipid peroxidation. *Food Chemistry*, 89, 403-409.
- Chong, P. S., Fung, M. L., Wong, K. H. and Lim, L. W. (2019). Therapeutic potential of *Hericium erinaceus* for depressive disorder. *International Journal of Molecular Sciences*, 21(1): 1-63.
- Cui, F., Zan, X., Li, Y., Sun, W., Yang, Y. and Ping, L. (2016). *Grifola frondosa* glycoprotein GFG-3a arrests S phase, alters proteome, and induces apoptosis in human gastric cancer cells. *Nutrition and Cancer*, 68(2): 267-279.
- Das, A. K., Nanda, P. K. and Dandapat, P. (2021). Edible mushrooms as functional ingredients for development of healthier and more sustainable muscle foods: A flexitarian approach. *Molecules*, 26(9): 24:63.
- Dhamodharan, G. and Mirunalini, S. (2010). A novel medicinal characterization of *Agaricus bisporus* (white button mushroom). *Pharmacologyonline*, 2:456-463.
- E TIMES. Agarwal, S. (2017). Know all about mushrooms.

- Easin, M. N., Ahmed, R., Alam, M. S., Reza, M. S. and Ahmed, K. U. (2017). Mushroom cultivation as a small-scale family enterprise for the alternative income generation in rural Bangladesh. *International Journal of Agriculture, Forestry and Fisheries*, 5(1): 1-8.
- El Enshasy, H. A. and Hatti-Kaul, R. (2013). Mushroom immunomodulators: Unique molecules with unlimited applications. *Trends in Biotechnology*, 31:668-677.
- Elkhateeb, W. A. (2020). What medicinal mushroom can do? *Chemical Research Journal*, 5, 106-118.
- Elsayed, E. A., Enshasy, H. E. and Wadaan, M. A. M. (2014). Mushrooms: A potential natural source of anti-inflammatory compounds for medical applications. *Mediators of Inflammation*, 2014, 1-15.
- Fasidi, I. O. and Ekuere, U. U. (1993). Studies on *Pleurotus tuber-regium* (Fries) Singer: Cultivation, proximate composition and mineral contents of sclerotia. *Food Chemistry*, 48(3): 255-258.
- Ferdousi, J., Riyadh, Z. A., Hossain, I., Saha, S. R. and Zakaria, M. (2020). Mushroom production benefits, status, challenges and opportunities in Bangladesh: A review. *Annual Research & Review in Biology*, 4(6): 1-13.
- Fischer, M. W. and Money, N. P. (2010). Why mushrooms form gills: Efficiency of the lamellate morphology. *Fungal Biology*, 114(1): 57-63.
- Food and Agriculture Organization (FAO). (2017). FAOSTAT: Crops. Retrieved from <http://www.fao.org/faostat/en/#data/QC/visualize>.
- Guillamón, S., García-Lafuente, A., Lozano, M., et al. (2010). Edible mushrooms: Role in the prevention of cardiovascular diseases. *Fitoterapia*, 81(7): 715-723.
- Hall, I. R., Stephenson, S. L. and Buchanan, P. K. (2003). *Edible and poisonous mushrooms of the world*. Cambridge, UK: Timber Press.
- Hawksworth, D. L. (2001). Mushrooms: The extent of the unexplored potential. *International Journal of Medicinal Mushrooms*, 3(4): 333-340.
- ICAR – Directorate of Mushroom Research, National Research Centre for Mushroom. Rai, R. D. and Arumuganathan, T. (2008). *Post Harvest Technology of Mushrooms*.
- ICAR – Directorate of Mushroom Research. Ahlawat, O. P., Kumar, S., Arumuganathan, T. and Tewari, R. P. (2008). *25 Years of AICRP (Mushroom): All India Coordinated Research Project on Mushroom*.

- Imtiaj, A. and Rahman, S. A. (2008). Economic viability of mushrooms cultivation to poverty reduction in Bangladesh. *Tropical and Subtropical Agroecosystems*, 8, 93-99.
- Iqbal, M. S. H., Rauf, A and Sheikh, I. M. (2005). Yield performance of oyster mushroom on different substrates. *International Journal of Agriculture and Biology*, 7, 900-903.
- Islam, M. K., Khan, M. M. H. and Islam, M. N. (2013). An unrealized manner for poverty alleviation: The mushroom industry. *Dhaka University Journal of Marketing*, 16(1): 43-56.
- Islam, M. Z., Rahman, M. H. and Hafiz, F. (2009). Cultivation of oyster mushroom (*Pleurotus flabellatus*) on different substrates. *International Journal of Sustainable Crop Production*, 4(1): 45-48.
- Jacinto-Azevedo, B., Valderrama, N. and Henríquez, K. (2021). Nutritional value and biological properties of Chilean wild and commercial edible mushrooms. *Food Chemistry*, 356, 129-651.
- Jedinak, A. and Sliva, D. (2008). *Pleurotus ostreatus* inhibits proliferation of human breast and colon cancer cells through p53-dependent as well as p53-independent pathway. *International Journal of Oncology*, 33(6): 1307-1313.
- Jedinak, A., Dudhgaonkar, S., Wu, Q. L., Simon, J. and Sliva, D. (2011). Anti-inflammatory activity of edible oyster mushroom is mediated through the inhibition of NF- κ B and AP-1 signaling. *Nutrition Journal*, 10(1): 1-10.
- Jiang, Q., Zhang, M. and Mujumdar, A. S. (2020). UV induced conversion during drying of ergosterol to vitamin D in various mushrooms: Effect of different drying conditions. *Trends in Food Science & Technology*, 105, 200-210.
- Kakraliya, S. S. (2020). Economic importance of mushroom and their uses. *Just Agriculture-The Future of Agri Innovation*. Sher-e-Kashmir University of Agricultural Science and Technology of Jammu-Jammu. ARTICLE ID: 025.
- Kaul, T. N. (2001). *Biology and conservation of mushrooms*. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India.
- Kawagishi, H., Zhuang, C. and Yunoki, R. (2008). Compounds for dementia from *Hericium erinaceum*. *Drugs of the Future*, 33(2): 1-49.
- Khan, M. A., Khan, L. A., Hossain, M. S., Tania, M. and Uddin, M. N. (2009). Investigation on the nutritional composition of the common edible and medicinal mushrooms cultivated in Bangladesh. *Bangladesh Journal of Mushroom*, 3(1): 21-28.

- Li, I., Lee, L. Y., Tzeng, T. T., Chen, W. P., Chen, Y. P., Shiao, Y. J. and Chen, C. C. (2018). Neurohealth properties of *Hericium erinaceus* mycelia enriched with erinacines. *Behavioural Neurology*, 2018.
- Liang, Z., Wu, C., Shieh, Z. and Cheng, S. (2009). Utilization of grass plants for cultivation of *Pleurotus citrinopeleatus*. *International Biodeterioration & Biodegradation*, 63(5): 509-514.
- Liu, Q., Zhu, M., Geng, X., Wang, H. and Ng, T. B. (2017). Characterization of polysaccharides with antioxidant and hepatoprotective activities from the edible mushroom *Oudemansiella radicata*. *Molecules*, 22(2): 2-34.
- Marshall, E. and Nair, N. G. (2009). *Make money by growing mushrooms*. Food and Agriculture Organization of the United Nations (FAO): Rome.
- Martínez-Ibarra, E., Gómez-Martín, M. B. and Armesto-López, X. A. (2019). Climatic and socioeconomic aspects of mushrooms: The case of Spain. *Sustainability*, 11(4): 10-30.
- Mattila, P., Konko, K., Euroola, M., Pihlava, J. M. and Astola, J. (2001). Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *Journal of Agricultural and Food Chemistry*, 49(5).
- Mohan, D. (2009, April 21). Mushroom cultivating methods. *Technology for Mushroom Cultivation*.
- National Horticulture Board. *Button Mushroom*.
- Nisar, J., Mustafa, I., Anwar, H., Sohail, M. U., Hussain, G., Ullah, M. I., Faisal, M. N., Bukhari, S. A. and Basit, A. (2017). Shiitake culinary-medicinal mushroom, *Lentinus edodes* (Agaricomycetes): A species with antioxidant, immunomodulatory, and hepatoprotective activities in hypercholesterolemic rats. *International Journal of Medicinal Mushrooms*, 19, 981-990.
- Nitha, B., Fijesh, P. V. and Janardhanan, K. K. (2013). Hepatoprotective activity of cultured mycelium of Morel mushroom, *Morchella esculenta*. *Experimental and Toxicologic Pathology*, 65(1-2): 105-112.
- Obodai, M., Sawyerr, L. C. B. and Johnson, P. N. T. (2002). Yield of seven strains of oyster mushrooms (*Pleurotus* spp.) grown on composted sawdust of *Triplochiton scleroxylon*. *Tropical Science*, 40(2): 95-99.
- Obodai, M., Sawyerr, L. C. B. and Johnson, P. N. T. (2002). Yield of seven strains of oyster mushrooms (*Pleurotus* spp.) grown on composted sawdust of *Triplochiton scleroxylon*. *Tropical Science*, 40, 95-99.

- Onuoha, C. I., Oyibo, G. and Judith, E. (2009). Cultivation of straw mushroom (*Volvariella volvacea*) using some agro-waste material. *Journal of American Science*, 5, 135-138.
- Patra, A. K. and Pani, B. K. (1995). Yield response of different species of oyster mushroom (*Pleurotus*) to paddy straw. *Current Agriculture Research*, 8, 11-14.
- Pennstate Extension. Beyer, D. M. (2016, June 27). Six steps to mushroom farming.
- R R Mushroom Sales & Marketing. (2013, August 18). Types of edible mushroom in India & their medicinal facts.
- Rahi, D. K. and Malik, D. (2016). Diversity of mushrooms and their metabolites of nutraceutical and therapeutic significance. *Journal of Mycology*, 7654123, 1-18.
- Rahman, M. (2018). Problems and prospects of quality mushroom supply for domestic market. *Ms Thesis, Department of Agribusiness and Marketing, Sher-e-Bangla Agricultural University, Dhaka.*
- Reis, F. S., Barros, L., Martins, A. and Ferreira, I. C. (2012). Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An inter-species comparative study. *Food and Chemical Toxicology*, 50(2): 191-197.
- Ribeiro, B., Valentão, P., Baptista, P., et al. (2007). Phenolic compounds, organic acids profiles and antioxidative properties of beefsteak fungus (*Fistulina hepatica*). *Food and Chemical Toxicology*, 45(10): 1805-1813.
- Sanchez, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Applied Microbiology and Biotechnology*, 85, 1321-1337.
- Sarang, I., Ghosh, D., Bhutia, S. K., Mallick, S. K. and Maiti, T. K. (2006). Anti-tumor and immunomodulating effects of *Pleurotus ostreatus* mycelia-derived proteoglycans. *International Immunopharmacology*, 6(8): 1287-1297.
- Sarker, N. C. (2020). Agricultural development: Revolutionary tools for mushroom production in Bangladesh. Retrieved from <https://dailyasianage.com/news/215401/revolutionary-tools-for-mushroom-production-in-bangladesh>.
- Sarker, N. C., Kakon, A. J., Amin, R. and Choudhury, M. B. K. (2015). Microbiological assessment of mushroom capsules as food supplement produced by Bangladesh mushroom traders. *Bangladesh Journal of Mushroom*, 9(1): 43-47.
- Sher, H. (2006). Ecological and economic evaluation of some Morels Mushroom (*Morchella* Sp). *Wild Mushrooms*, 33, 23-44.

- Simon, A. E., Gonzalez-Fandos, E. and Tobar, V. (2005). *Journal of Food Science and Technology*, 40(1-10).
- Spelman, K., Sutherland, E. and Bagade, A. (2017). Neurological activity of Lion's mane (*Herichium erinaceus*). *Journal of Restorative Medicine*, 6(1): 19-26.
- Thompson, W. and Rayner, A. D. M. (1983). Extent, development and function of mycelial cord systems in soil. *Transactions of the British Mycological Society*, 81, 333-345.
- TIME IS Technology Innovation Management & Entrepreneurship Information Service, Department of Science and Technology FICCI. (2010, September). *Mushroom Cultivation & Processing*.
- Ultimate Mushroom. (2021). Edible mushroom- *Agaricus bisporus*. [Image]. Retrieved from <https://ultimate-mushroom.com/edible/10-agaricus-bisporus.html>.
- Valverde, M. E., Hernández-Pérez, T. and Paredes-López, O. (2015). Edible mushrooms: Improving human health and promoting quality life. *International Journal of Microbiology*, 2015, 376387.
- Valverde, M. E., Hernández-Pérez, T. and Paredes-López, O. (2015). Edible mushrooms: Improving human health and promoting quality life. *International Journal of Microbiology*, 2015, 376387.
- Vamanu, E., Ndungutse, V., Mereddy, R. and Sultanbawa, Y. (2015). Bioactive properties of mushroom (*Agaricus bisporus*) stipe extracts. *Journal of Food Processing and Preservation*, 39(6): 2225-2233.
- Venturella, G., Ferraro, V., Cirlincione, F. and Gargano, M. L. (2021). Medicinal mushrooms: Bioactive compounds, use, and clinical trials. *International Journal of Molecular Sciences*, 22(2): 634.
- Venturella, G., Ferraro, V., Cirlincione, F. and Gargano, M. L. (2021). Medicinal mushrooms: Bioactive compounds, use, and clinical trials. *International Journal of Molecular Sciences*, 22(2): 634.
- Vetayasuporn, S. (2006). Oyster mushroom cultivation on different cellulosic substrates. *Research Journal of Agriculture and Biological Sciences*, 2(6): 548-551.
- Walton, E. (2014). Buried treasure: Unlocking the secrets of medicinal mushrooms. *Biomed Journal*, 37, 339-342.
- Wani, B. A., Bodha, R. H. and Wani, A. H. (2010). Nutritional and medicinal importance of mushrooms. *Journal of Medicinal Plants Research*, 4(24): 2598-2604.

- Wani, B. L., Bodha, R. H. and Wani, A. H. (2010). Nutritional and medicinal importance of mushrooms. *Journal of Medicinal Plant Research*, 4(24): 2598-2604.
- Wasser, S. P. (2002). Review of medicinal mushrooms advances: Good news from old allies. *Herbal Gram*, 56, 28-33.
- Wasser, S. P. (2014). Medicinal mushroom science: Current perspectives, advances, evidences, and challenges. *Biomed Journal*, 37, 345-356.
- Watling, J. (2003). *Fungi*. The Natural History Museum, London.
- Wikipedia, The Free Encyclopedia. (2018, February 14). *Volvariella volvacea*.
- Wikipedia, The Free Encyclopedia. (2018, May 29). *Pleurotus*.
- Wikipedia, The Free Encyclopedia. (2018, November 8). *Agaricus bisporus*.
- Wikipedia, The Free Encyclopedia. (2018, October 14). *Shiitake*.
- Wu, X., Zeng, J., Hu, J., Liao, Q., Zhou, R., Zhang, P. and Chen, Z. (2013). Hepatoprotective effects of aqueous extract from lingzhi or reishi medicinal mushroom *Ganoderma lucidum* (higher basidiomycetes) on α -Amanitin- induced liver injury in mice. *International Journal of Medicinal Mushrooms*, 15(4).
- Xiao, C., Wu, Q., Xie, Y., Zhang, J. and Tan, J. (2015). Hypoglycemic effects of *Grifola frondosa* (Maitake) polysaccharides F2 and F3 through improvement of insulin resistance in diabetic rats. *Food & Function*, 6(11): 3567-3575.
- Zhang, Y., Geng, W., Shen, Y., Wang, Y. and Dai, Y. C. (2014). Edible mushroom cultivation for food security and rural development in China: Bio-innovation, technological dissemination and marketing. *Sustainability*, 6(5): 2961-2973.
- Zhao, S., Gao, Q., Rong, C., Wang, S., Zhao, Z., Liu, Y. and Xu, J. (2020). Immunomodulatory effects of edible and medicinal mushrooms and their bioactive immunoregulatory products. *Journal of Fungi*, 6, 269. [CrossRef].