Post harvest preservation for sustainable utilization of indigenous mushrooms and downstream processing of spoiled mushrooms to produce biodegradable plastic.

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Abstract

Indigenous mushrooms have been enjoyed by local people since ancient times. These mushrooms grow naturally in the wild during the rainy season and during this time people scout for them and sell them on the informal market. Since the shelf life of the mushrooms is short, these informal marketers usually resort to drying the indigenous mushrooms to prolong their shelf life. However, there are other diverse methods which can be used to enhance their sustainable utilization. On the other hand sometimes cultivated mushrooms meant for sale in their fresh state get spoiled before being completely sold out. Therefore, this research aimed to preserve indigenous mushrooms for their sustainable utilization as well as to process spoiled mushrooms to make biodegradable plastic.

Firstly, a questionnaire was used to conduct a survey to access market availability of indigenous mushrooms and the rate of spoilage of cultivated mushrooms. Responses were obtained from 26 branches of 3 types of modern markets. Results of the survey showed that none of these modern markets had indigenous mushrooms available, neither in their fresh or preserved state. The only types of mushrooms which were available were oyster mushrooms and button mushrooms, in their fresh state. It was also noted that about 35.37% of these cultivated mushrooms would spoil on the shelves with time. For sustainable utilization of both cultivated and indigenous mushrooms preservation techniques were explored. Preservation methods which were used on *Amanita zambiana, Cantharellus spp* and *Cantharellus miomboensis* included oven drying, pickling, steeping and freezing. Assessments of changes in the proximate nutritional composition, microbial load and sensory quality of the preserved mushrooms were done over a period of nine months. Results of the nutritional analysis showed that protein values of the mushrooms significantly decreased with time after preservation. It was also noted that the nutritional contents of the mushroom samples changed after the preservation procedures. The initial protein content of the preserved mushroom samples ranged from 12.17±0.05% to 14.27 ± 0.06% whereas after the last month of preservation they had significantly decreased to a range of 5.39±0.03% to 9.55±0.02%. Pickled samples had a significantly increased content of lipids from an initial range between 9.34±0.02% and 12.42±0.02% to a final range of 14.91±0.02% to 19.27±0.02%. The ash content of the samples decreased from an initial range of 9.22±0.01% to 12.74±0.03%, to a final rage of 3.02±0.01% to 6.87±0.04±%. The crude fibre content of pickled

and steeped samples also decreased from an initial range of 4.23±0.03% to 6.76±0.02% to a final range of 2.87±0.01 to 5.07±0.02.

Results showed that the initial range of the microbial counts of the fresh samples was 4.95±0.12 log cfu/ml to 4.98±0.05 log cfu/ml respectively whereas the preserved samples had microbial loads which ranged from 4.42±0.03 log cfu/ml to 4.95±0.11 log cfu/ml. Hedonic scales were used to access the aroma, colour, texture overall acceptability of the preserved indigenous mushrooms and in general pickled mushrooms had higher sensorial scores ranging from 7.9 to 8.5.Spoiled *Pleurotus ostreatus* mushroom was used to produce a bioplastic and it was noted that it`s weight decreased by an average of 5.5±0.23% and 2.2±0.02% under soil and laboratory degradation respectively, showing that the plastic was biologically degradable. The overall conclusion from the study was that preservation methods can be used to enhance utilization of indigenous mushrooms and spoiled mushrooms can be processed to make biodegradable plastic. Out of this study it was also concluded that even after the rainy season ends, indigenous mushrooms can still be found in the market in the form of whole steeped mushrooms, pickled mushrooms, frozen mushrooms or dried mushrooms. On the other hand spoiled mushrooms can be added to the other sources of biodegradable plastic which are already available. More research on preservation of other various types of underutilized mushroom types is recommended and other hand research on starch extraction methods which result in high yields of starch is also advisable.

Dedication

This thesis is dedicated to my husband, my daughter, and the rest of my family for supporting me throughout this journey.

Declaration

This thesis represents the author's (Ncube Tashian Silibaziso, C21147753P) original work and has never been previously submitted nor will it be submitted to any university for any award of degree, diploma, or certificate other than Chinhoyi University of Technology. I have appropriately acknowledged all help that I received in my research work and in the preparation of the thesis itself. I also certify that all information sources and literature are appropriately referenced and acknowledged in the thesis.

Ncube

Date 8/1/24

Ncube Tashian Silibaziso (Candidate)

Approval

I certify that Tashian Silibaziso Ncube was under my supervision. I further certify that I have sufficiently supervised her and that she has fulfilled all requirements set before her. In my judgment, the thesis entitled, "Post harvest preservation for sustainable utilization of indigenous mushrooms and downstream processing of spoiled mushrooms to produce biodegradable plastic" is of sufficiently high standard to meet the standards of the Master of Philosophy degree awarded by Chinhoyi University of Technology.

Approved by:

Name of Supervisor (Dr. T.J Chisango)

Signature..... Date..... 8/7/24

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Chapter 1

1.1 Introduction

Edible mushrooms are a category of mushrooms which have been appreciated since long time back for their good taste and nutrition (Marcal et al., 2021). Nutritional components of these mushrooms include proteins, carbohydrates, lipids, starch, fibre and minerals (Marcal et al., 2021; Ayodele et al., 2011). Generally there are edible mushrooms which are cultivated through use of spawn and others which are found naturally in the wild, normally referred to as edible non timber forest products (Marcal et al., 2021;Manjula & Kumar, 2024; Boa, 2004). In Zimbabwe cultivated mushrooms include *Pleurotus ostreatus*and *Agaricus bisporous* whereas wild indigenous mushrooms include *Amanita zambiana* , *Lactarius kabansus* , *Cantharellus miomboensis, Cantharellus spp.,Clavurina cristata, Lactarius spp and Termitomyces clypeatus,*(Reid et al., 2016; Gleaner, 2023).

Mushrooms which are cultivated in Zimbabwe are found on both the formal and informal markets all year round (Mutema et al., 2018). Even though these mushrooms are found all year round there has been a problem of spoilage of these mushrooms before they are all completely sold to consumers because they have a short shelf life (Mutema et al., 2018). Wild indigenous mushrooms are found on the informal markets and road sides during the rainy season and they are appreciated as a source of income as well in communities where they sprout during this time (Mlambo & Maphosa, 2017; Mutsaka, 2023). They are mostly found during the rainy season because they need high humidity to sprout in undisturbed environments (Gleaner et al., 2023). Researches on availability of indigenous mushrooms on formal Zimbabwean markets are not well documented.

Individuals who scout these indigenous mushrooms usually dry or freeze them off to prolong their shelf lives because even before they stop sprouting after the rainy season ends, they also tend to quickly spoil if they are in their fresh state, (Reid et al., 2016). There is a need to use various methods of preservation on these indigenous mushrooms so as to enhance and prolong their shelve lives so as to make them available in various forms to the consumers even after the rainy season stops.

Preservation methods have long been used to enhance the shelve lives of mushrooms (Dawadi et al., 2022; Shonte et al., 2024). Various types of preservation methods which have been researched on include pickling, brining, caning, use of coatings, different drying methods such as freeze drying, solar drying, sun drying and oven drying, freezing as well as use of chemicals (Marcal et al., 2021; Zhang et al., 2018; Akata et al., 2015; Reid et al., 2016). These methods mainly work through reduction of moisture content and microbial as well as enzymatic activities (Agomuo et al., 2022, Berry et al., 2009; Marcal et al., 2021). Considering the market unavailability of indigenous mushrooms in Zimbabwe after fall of the rainy season, these preservation methods could be possibly used to enhance their shelf lives making them available for longer periods. Research which has been documented on the preservation of indigenous mushrooms in Zimbabwe is limited which therefore means that more still needs to be done in this area.

In addition there could be a way of utilizing the spoiled mushrooms, instead of discarding them, considering the current challenges being faced in the Zimbabwe. One of these challenges is the pollution of the environment by non-degradable plastics. According to Moyo (2021), the Environmental Management Agency (EMA) went on a campaign to reduce plastic pollution through phasing out the use of synthetic plastics, which are non-degradable and remain in the environment forever thereby polluting the land and the aquatic areas. However, this plan was not very successful due to lesser availability of alternatives. Spoiled mushrooms can therefore be factored in as additional sources of the available alternatives which have been researched on and used to produce biodegradable plastic.

1.2 Problem Statement

As much as mushroom farming is becoming a common practice in Zimbabwe, growing and cultivation of indigenous mushrooms is not yet being practiced therefore people wait for the natural growth of these mushrooms in the environment during the rainy season when they sprout, (Reid et al., 2016). This is the only time when consumers get supplies of indigenous mushrooms. The main problem is that edible indigenous mushrooms are characterized by seasonal availability and short time abundant supply as they are only found during the rainy season, (Gleaner, 2023). This deprives the consumers of access to these indigenous mushrooms during other seasons when rain will not be available. In general, indigenous mushrooms have a short fresh state shelf

life which therefore means after the rainy season lapses they cannot be stored in their fresh state for a long time (Mlambo & Maphosa, 2017). There is still less documented research on preservation of indigenous mushrooms in Zimbabwe. This also extends to literature information on effects of various preservation methods on the nutritional contents, microbial loads and sensorial attributes of the indigenous mushrooms.

On the other hand, as much cultivation of the exotic mushrooms in Zimbabwe is also increasing and it has been noted that some of these exotic mushrooms intended for sell in their fresh state end up getting spoiled before they are finished off the shelves. There have been reports from other mushroom famers who also reported losses due to spoilage of mushrooms before they were all supplied (Mutema et al., 2018). This problem is mainly due lack of proper cold storage facilities and unreliable power supplies (Mutema et al., 2018). This problem of postharvest spoilage of mushrooms is not only being faced in Zimbabwe but also in other parts of the world such as China, Japan and America where mushroom farming is also being widely practiced,(USDA, 2019; Zhao et al., 2020; Nakano et al., 2020). As it stands, there is less tangible documented information on utilization of spoiled mushrooms. According to Nissa et al. (2022), mushrooms contain starch. Considering the research documentation that has been done on use of starch from biological materials such as cassava and potatoes to produce biodegradable plastic, spoiled mushrooms could also be possibly taken in as a supplementary source of bioplastics.

There has been a rising problem of synthetic plastic pollution problem, not only in Zimbabwe only but around the world at large. Synthetic plastics are non-degradable and therefore they remain in the environment forever. They therefore end up reaching the aquatic environments thereby polluting both the land and water bodies. In water bodies, plastics cause harm to the aquatic life and they also clog drainage systems. Conventional plastic production also contributes to increases in release of greenhouse gases such as carbon dioxide and methane which contribute to global warming.

The bio plastics currently in production internationally at industrial scale are prohibitively expensive for most of the companies that need them. This is because they get the bulk of their starch from cereal grains such as wheat and corn and from tubers like potatoes, arrowroot or from the pith of the sago plant. Since there is a general food shortage especially in the third

world countries in Asia and Africa, the use of cereals and tubers for commercial scale bio plastic production means worsening of the food shortage problem. Therefore this study was aimed at exploring various preservation methods for mushrooms and to also investigate the possibility of using spoiled mushrooms in biodegradable plastic production

1.3 Justification

The main reason why this research study is worth doing is because it is going to cover preservation of indigenous mushrooms thereby providing other methods that can be used to enhance their availability and utilization for longer periods other than the rainy season only. In another way this study will contribute towards efforts to ensure food availability and security in the country as mushrooms are highly nutritious and their availability to consumers for longer periods will be a benefit. This study will also provide practical information on how preservation methods affect the overall properties of mushrooms including nutritional composition, sensorial attributes and microbial content thereby giving an insight on different methods which result in more retention of nutrients by the different indigenous mushrooms.

On the other hand, the production of plastics from spoiled mushrooms reduces the dependency of production using petroleum resources. This will also benefit the mushroom farmers since they will not incur complete losses from the spoiled mushrooms by simply discarding them off. The use of mushrooms that are spoiled and no longer safe for human consumption, as a source of starch for bioplastic production instead of edible cereals and tubers, reduces the competition for food in the third world countries. Utilization of inedible, spoiled mushrooms in bioplastic production also reduces the general cost of the bioplastics.

More production of bioplastics will also reduce utilization of non degradabe polymers. This will also lead to less pollution of the land and aquatic enviroment. Emmition of greenhouse gases from production of synthetic plastics will also be reduced.

In overall this reearch will provide positive steps towards enhancing availablity of indigenous mushrooms to consumers for longer periods. On the other handthe research will also provide a posssible supplementary solution to the problems caused by non degradable plastics through utilization of spoiled mushrooms to produce biodegradable plastics.

1.4 Objectives and hypotheses

1.4.1 Hypothesis

H_0 –Postharvest preservation has no effect on the nutritional, sensorial and microbial attributes of indigenous mushrooms

H_1 –Postharvest preservation has an effect on the nutritional, sensorial and microbial attributes of indigenous mushrooms

H_0– Biodegradable plastic cannot be produced from spoiled mushrooms

H_1- Biodegradable plastic can be produced from spoiled mushrooms

1.4.2 Main objective

To explore various preservation methods for Indigenous mushrooms so as to enhance their long term availability and utilization and to utilize spoiled mushrooms to make biodegradable plastics.

1.4.3 Specific objectives

1. To determine the market availability of fresh as well as preserved cultivated and indigenous mushrooms
2. To determine the effect of various preservation methods (oven drying, brining, pickling and freezing) on nutritional, sensorial and microbial attributes of indigenous mushrooms
3. To develop and characterize bio plastic films obtained from the spoiled mushroom starch.

1.5 Overview of the thesis

Chapter 1 of the thesis is presents the overall background of the thesis. It provides information of what the research will be on likewise the preservation of indigenous mushrooms to make them available for longer periods after the rainy season as well as the downstream processing of fresh mushrooms intended for sale which get spoiled whilst on the market shelves. The literature review is in **Chapter 2** where literature searches on researches which have already been documented involving preservation of mushrooms and downstream processing of spoiled mushrooms was done. **Chapter 3** includes highlights of a market survey on which was done to document the general availability of cultivated mushrooms, indigenous mushrooms and preserved mushrooms in formal markets. It also covers the preservation of indigenous

mushrooms through oven drying, freezing, pickling and steeping in brine solution. Impact of these methods on nutritional content of the mushrooms was also done. In **Chapter 4**, the effect of preservation methods on microbial content and sensorial quality was covered. This chapter also includes the use of spoiled mushrooms to make biodegradable plastic. In **Chapter 5** the overall conclusion of the research was given.

CHAPTER 2

A review on the impact of postharvest preservation on the nutritional, microbiological as well as sensorial attributes of mushrooms and downstream processing of spoiled mushrooms

Abstract

Edible mushrooms are macrofungi which are considered to be nutritious, possessing components such as proteins, lipids, starch, fibre and minerals. They are also considered as tasty food possessing other good sensorial attributes such as good aroma and tender texture when properly cooked. In Zimbabwe, mushrooms are taken as part of consumers` diet. Some of these mushrooms are found naturally in the wild whereas some are cultivated in-house. Both these types of edible mushrooms have a short shelf life and quickly get spoiled. Besides quick spoilage, wild indigenous mushrooms are also generally seasonally available and when the rainy season ends, their availability seizes. Post-harvest preservation methods are therefore needed to extend the shelf lifes of these indigenous mushrooms. On the other hand, some of the cultivated mushrooms intended for sale in markets, get spoiled before they are sold out and therefore there is also need for a procedure to utilize these spoiled mushrooms. The main aim of this review was to critically research and gather information existing information on the effects of postharvest preservation methods on the nutritional, microbial and sensorial attributes of mushrooms so as to get overall knowledge on more methods that could be researched on in regards to Zimbabwean indigenous mushrooms. Drying methods, especially those which include use of higher temperatures to reduce microbial content can lead to the degradation of lipids and proteins as well as changes in texture and flavor. Freezing is considered one of the of the long-term best methods used to preserve mushrooms through inactivating pathogens but it also causes loss of proteins and vitamins but no significant changes on the aroma of the mushrooms. Pickling in salts and acids reduces microbial content and also causes loss of crude fibre, proteins and vitamins but also increases lipid content and mineral content depending on the pickling medium used. On the other hand the other goal of the review was to gather information on rate of spoilage of mushrooms intended for sale in their fresh state in different parts of the world in a bid to come up with ways to utilize them. It has been noted that these mushrooms contain starch which can be possibly utilized to produce biodegradable plastic. This review presents methods

that may be used to preserve mushrooms and their impact on their microbial, sensorial and nutritional attributes as well as a possible method to utilize spoiled mushrooms.

2.1 Introduction

Mushrooms are macrofungi which have a distinctive fruiting body which can be found either above the ground or underground (Wandati et al., 2013). They are classified under a kingdom called Mycota and division Eumycota (Kuo, 2003). Most edible fungi are found in the phylum Basidiomycota with Cantherelles, Hymenochaetales, Phallales, Boletales and Agaricales which stand out as the most important orders (Mutukwa, 2014). Some types of mushrooms are cultivated (Obdai, 2001; Kakon et al., 2012) whilst others are found naturally in the wild especially during the rainy season (Qwarse et al., 2021).

Nwanze et al. (2006) reported that mushrooms are mainly cultivated for their good nutritional attributes, sensory properties and industrial utilization. Tanimola et al. (2022) observed that wild mushrooms are considered to be nutritionally beneficial but are underutilized in Africa due to seasonal availability, lack of use of preservation methods and misconception about fungi as well as due to their short fresh state shelf life. A particular study by Kalu et al. (2013) on utilization of mushrooms by Nigerians showed that, indigenous mushrooms had not been fully embraced due to mysterious beliefs as well as seasonal scarcity and unavailability. Nakalambe et al. (2015) also observed that the usage and nutritional potential of wild and indigenous mushrooms have been overshadowed by the well-known cultivated mushrooms which include *A. bisprous, L. edodes* and *Pleurotus* species.

According to Mlambo et al. (2017), wild mushrooms in Zimbabwe are largely ignored and underutilized as a source of income and food for rural communities due to seasonal availability and fear of poisoning. The issue of seasonal availability of wild mushrooms is also supported by Nakalembe et al. (2015) who reported that the rural people of Uganda collect wild mushrooms during the rainy season to use as a food source as they consider them to be of high nutritional value. Another factor which contributes to the underutilization of mushrooms is that they have a very short fresh state shelf life due to the fact that they are highly perishable, (Castellanos-Reyes et al., 2021).This perishability is mainly caused by the fact that they lack an outer protective cuticle, they have a high rate of respiration, a high level of enzymatic activity, high presence of microflora and high percentage of moisture content (Zhang et al., 2018 & Fernandes et al.,

2012). They are therefore more prone to microbial attack, mechanical damage, weight loss and enzymatic browning (Donglu et al., 2016; Cheung, 2010).

Findings from a research by Diamantopoulou et al. (2015) on the shelf life of *A.bisporous, P. ostreatus, L. edodes* showed that mushrooms can reach only one to three days under ambient temperatures (20-25^{0}C) and five to seven days under refrigeration at 4^{o}C for *A.bisporous* and *P. ostreatus* mushrooms. Whilst *L. edodes* mushrooms reach only up to five days under refrigeration as they have greater perishability owing to high respiratory rates (Xiao et al., 2011). To maintain the bioactive compounds of mushrooms and to increase their shelf lives appropriate preservation methods should be used (Jarwoska et al., 2014).

Although it is common knowledge that mushrooms are highly perishable, the shelf lives of wild mushrooms including various Zimbabwean indigenous mushrooms are not well documented. The researcher also noted that comprehensive research on the preservation of indigenous mushrooms has not been thoroughly done yet as there is still less documentation available on them in the literature. In terms of availability of fresh mushrooms on the Zimbabwean market, cultivated mushrooms are found on both the formal and informal whereas for Indigenous mushrooms, there is documentation of their availability on informal markets and no proper documentation of their availability on the formal markets.

On the other hand, there are mushrooms intended for sale in their fresh state which inevitably get spoiled in the markets (Mutema et al., 2018). Vast documentation of the utilization of spoiled mushrooms is also not available in literature. This paper is therefore is set to gather information on different researches which involve preservation of mushrooms that have been done so far as well as researches on how starch from other sources has been used to produce biodegradable plastic. This is taken as an initial step towards covering the mentioned gaps.

2.2 Market availability and utilization of mushrooms in Zimbabwe

In different parts of the world, both wild and cultivated mushrooms are found on formal and informal markets either in fresh or preserved forms (Ruan-Soto et al., 2017). Generally, in Zimbabwe there are some mushrooms found naturally in the wild during the rainy season, (Gleaner, 2023). These mushrooms include *A. zambiana, L. kabansus ,C. miomboensis, Cantharellus spp.,C. cristata, Lactarius spp and T. clypeatus,* (Reid et al., 2016; Gleaner, 2023).

According to Mlambo & Maphosa (2017), these indigenous mushrooms are usually found on the informal markets and roadsides. Their availability on the modern and formal markets is not well documented, literatures search of this information did yield valid conclusions. There are also some exotic species are cultivated and are found available all year round for food and commercial purposes which include *P. ostreatus* and *A. bisporous*, (Pamire & Pawarira, 2018). These mushrooms are found on both the informal and formal markets all year round (Mutema et al., 2018). The availability of either preserved indigenous mushrooms or preserved cultivated mushrooms in the markets of Zimbabwe is not well documented.

According to Gudeta et al. (2023) and Mlambo & Maphosa (2017), wild mushrooms found in Africa, with Zimbabwe included are largely underutilized as seen by the fact that their numbers and their nutritional factors are not well documented. Documentation of their availability on different forms of markets is also not yet vast. More research still needs to be done to increase their utilization.

2.3 Nutritional quality of mushrooms

Mushrooms in general are considered as a source of healthier and more nutritional food with good taste (Khatua et al., 2013). Various research studies have shown that mushrooms are a good source of nutrients compared to common vegetables, cereals, meats, and dairy products in terms of nutritional content (Nakalembe et al., 2015; Mshandete & Cuff, 2007; Sing et al., 2016; Enas et al., 2016; Girma & Tasisa, 2018). Experimental findings from research by Nissa et al., (2022) on the nutritional content of *A. bisporous* and *P. ostreatus* also back up the fact that mushrooms have high nutritional content. Opige (2006) also reported that wild mushrooms have high nutritional quality after their study on five edible mushrooms from Uganda namely *P.tenucullus*, *T. tyleranus*, *T. clypeatus*, *V. speciosa* and *T. microcarpus.* Proximate nutritional content results of the nutritional compositions of these mushrooms showed that they had high contents of protein, dry matter, ash and total carbohydrates ranging between 11.56 - 27.54%, 82.34 -99.76%, 10.79-16.87% and 37.12-61.05% respectively, (Opige, 2006).

A general over view of different studies on different types of wild and cultivated mushrooms are actually considered to be good sources of proteins by vegetarians as they contain 20 to 25% protein on a dry basis (Valverde et al., 2015; Del Toro et al., 2006; Pelkonon, 2008; Kalac, 2013). The high protein content of mushrooms is due to the availability of several essential

amino acids which include methionine, valine, glutamine, glutamic acid, aspartic acid and arginine (Akata, 2015; Rathore et al., 2017; Valverde et al., 2015). On the other hand, rationally they have lower levels of cholesterol and saturated fat content (Wani et al., 2010). Kalac (2013) cited that mushrooms contain 2 to 3% fat content which are linoleic acids and oleic acids. Mushrooms also contain dietary fibre, phenolic compounds with antioxidant activity as well as energetic density (Marcal et al., 2021; Cheung, 2010; Manzi et al., 2005).

According to Kalac (2013), fresh mushrooms have a high percentage of water and dry matter between 8 and 14%. Rathore et al. (2017) also cited that mushrooms usually have 50 to 65% carbohydrate content which mainly represents their dry matter consisting of sugars and polysaccharides. Mushrooms also have vitamins such as thiamine(B1), cobalamin(B12), niacin (B3), folate(B9), riboflavin(B12) as well as fat-soluble vitamins (A, E and D) (Rathore et al., 2017; Valverde., 2015). They are considered as the only non-animal source of vitamin D (Marcal et al., 2021). Guillamon et al. (2010) also noted that mushrooms have different types of minerals, depending on the type of the species and included potassium, phosphorous, magnesium, manganese, selenium, iron, copper as well as sodium.

It has been noted that the nutritional contents of different mushrooms vary. In the case cultivated mushrooms these variations are mainly attributed to the cultivation methods and growing conditions likewise the substrate that would have been used to grow the mushrooms, temperature as well as light exposure (Hoa et al., 2015). On the other hand, in wild mushrooms the environments in which the mushrooms would have matured in which would have supported them with different amounts of nutrients cause these variations, (Gleaner, 2023). The maturity stage and time of harvesting also affects the nutritional contents in both cultivated and wild mushrooms with more matured mushrooms having more nutrients (Barros et al., 2007). Species-specific characteristics of mushrooms also differ; likewise different mushrooms have different nutritional profiles because of differences in their genetic makeup (Enas et al., 2016). Likewise *L.edodes* are generally well known for their high contents of vitamin D contents and *P.ostreatus* mushrooms have more protein content compared to other types of cultivated mushrooms.

The nutritional values that have been found to be available in mushrooms prove that they can be used to add on to sources of food and nutrition especially in vulnerable societies of developing countries where malnutrition is still on the high side (Hamidu et al., 2003; Nakalembe et al.,

2021).In some societies problems of malnutrition problems associated with poor weaning practices in children have been on the rise (Emmanuel et al., 2016). Due to these problems, the Food and Agricultural organization actually supported the use of mushrooms as food supplements for protein deficient societies in developing countries (Nissa et al., 2022). Tanimola et al. (2022) highlighted the importance of preserving and processing them so as to increase their time of availability and utilization as this will enable the low income-society, the elderly and the vegetarians to have longer time availability of a variety of sources of high protein content.

2.4 Post-harvest preservation of mushrooms

Commercialization of mushrooms in their fresh state is a challenge because they are highly perishable and lose quality quickly after being harvested with an average shelf life of 1 to 3 days at room temperature (Zhang et al., 2018). Various methods of methods to preserve mushrooms have been researched on (Marcal et al., 2021). These methods include sun drying, oven drying, freeze drying, canning, pickling**,** freezing, use of chemicals, and steeping in brine solution or oils (Macal et al., 2021, Zhang et al., 2018). In Zimbabwe out of all these methods drying is the one that is mostly used by the local rural people to preserve their excess harvests of indigenous mushrooms before they get spoiled and also freezing to a lesser extent, (Reid et al., 2016). In other parts of the world likewise China, Mexico, India, and Japan where wild mushroom scouting and farming is vastly done, all these methods are used to preserve both wild and cultivated mushrooms for both formal and informal market selling (Castellanos-Reyes, 2021; Dawadi et al., 2022; Marcal et al., 2021)

2.4.1 Drying methods

Drying in general is considered a post-harvest technology which ensures lengthening of shelf lives of food products by reducing water activity to a microbial safe level and it is also taken as an essential step for the processing of value added products (Argyropoulus et al., 2011). In Zimbabwe, people who harvest indigenous mushrooms usually dry their excess harvests using the sun. However, research on the effects of this method of preservation on the overall nutritional contents and microbiological content on these indigenous mushrooms has not been conducted. Methods for drying mushrooms which are edible include solar drying, natural air drying, hot air drying, thin layer drying, freeze drying, microwave drying, drying with chemicals and sometimes a combination of some of these methods to improve the quality of the final preserved mushrooms (Hu et al., 2020; Zhang et al., 2018).Upon comparison with fresh mushrooms, dried mushrooms

have been found to have an intense flavor as well as altered contents of nutrients (Fan et al., 2012; Pei et al., 2016).

i. Natural sun drying

Natural sun drying is a drying method which is achieved by cutting mushrooms into thin layers and spreading them on a flat surface and directly exposing them to sun and wind (Muller & Muhlbauer, 2011). This method is commonly used in the Mediterranean, tropical and subtropical countries (Nolle et al., 2017) as well as in African countries (Ayodele et al., 2022). If this method of drying is done correctly it results in mushrooms with intensified flavor without significant loss of colour and texture (Argyropoulos et al., 2011) and it has also been shown to enhance the content of vitamin D2 in mushrooms due to the presence of ultra violet light which is provided naturally by sunlight during the drying procedure (Kristensen et al., 2012; Phillips & Rasor 2013; Simon et al., 2011). Findings from a research by Ayodele et al. (2017) who carried out a comparative study on the effects of sun, smoke and oven drying methods on four Nigerian wild edible mushrooms which included *L. squarrosulus*, *P. atroumbonata, C. micaceus* and *R. aprile*, showed that the different drying methods have different effects on the nutritional content of the mushrooms. Sun-dried samples had more nutritional contents afterwards compared to the other two methods because the temperatures used for sun drying are generally lower, not exceeding 40^0C therefore many nutritional components are not denatured in the process, (Ayodele al., 2023). On the other hand sundried samples retained more moisture content than samples which were dried using other methods, (Ayodele et al., 2017), and the structures of nutritional components are not altered very much and that is why the nutritional contents remain higher (Akpaja et al., 2003). Since lower natural temperatures are utilized, lesser moisture content is also lost that is why sun dried samples retain more moisture content (Nwachukwu et al., 2007).

In a study involving sun drying and solar drying of *P. saju caju* mushrooms which was carried out by Thi et al. (2020), results showed that the mushrooms which were sun dried had higher percentages of proteins, carbohydrates, flavonoids and B-glucan with percentage contents of 16.22%, 24.84%, 5.82% and 0.41% respectively (Thi et al., 2003). On the other hand, sun drying usually results in deterioration of the quality of the mushroom samples due to insufficient drying especially if it is carried out during seasons when there will be lesser availability of day sunlight

(Nolle et al., 2017). Furthermore, samples are put at more risk of contamination by microbes and foreign materials such as dust and insects, since it is also carried out in the open (Kalaras et al., 2012). According to Muller & Muhlbaer (2011), to avoid problems of natural sun drying, some researchers developed solar dryers which use energy from the sun to more efficiently dry the mushrooms.

i. Solar drying

Solar drying is a method of drying in which a solar dryer that utilizes the hot air convection principle for moisture release in samples is used (Sukkanta et al., 2023; Bala et al., 2009; Jentada et al., 2018).This method is considered an advancement of the natural sun drying method and in a research by Sukkanta et al. (2023), it was found that *P. ostreatus* mushrooms which were dried using the solar drying method had a better colour quality as well as lesser moisture and it took lesser time and lesser energy to complete the drying process compared to sun drying. It was reported that solar-dried samples of *A. bisporous* mushrooms had higher concentrations of vitamin D2 (39µg/g) compared to the sun-dried sample which had (36µg/g), (Nolle et al., 2017) and this is due to the higher ultraviolet radiation which is absorbed in the solar dryer because of the presence of the aluminum foil (Urbain et al., 2011).

ii. Hot air drying

One of the methods of drying which has been widely researched on is oven drying which is also called hot air drying and it is a method which involves use of an oven set at a temperature (Marcal et al., 2021).Hot air drying is said to be the first method which was used on mushrooms and it is also the one which is commonly used (Fernandes., et al., 2013; Zhang et al., 2018). In this preservation procedure, moisture content is reduced to a very low percentage around 13% so as to prevent growth of microbes, activities of enzymes as well as morphological damages (Piskov et al., 2020; Shishir et al, 2019; Xu et al., 2019). Xu et al. (2019) cited that drying changes the qualities of the mushrooms which include colour, moisture content, flavor, nutritional quality and texture.

It has its own disadvantages which include high dehydration times and high temperatures as in accordance to (Wu et al., 2019). High temperatures and high dehydration times result in browning, increased hardness and chewing as well as decreased elasticity (Qi et al., 2014; Zhang

et al., 2018).This therefore means that hot air dying affects the overall sensorial attributes of the mushrooms. According to Gasecka et al. (2020), use of the high temperatures during drying also decreases the contents of polysaccharides in the mushrooms due to conversion of polysaccharies to oligosaccharides as well as the Malliard reactions. Hot air drying also results in high level of free amino acids, decrease in contents of certain types of minerals which include calcium, potassium, phosphorous, magnesium as well as sodium and increased total soluble sugars content, total soluble phenolics content, and increased starch content of the mushrooms due to removal of moisture content which increases the concentrations of soluble components of these nutrients in the mushrooms (Nissa et al., 2022; Gasecka et al., 2020; Tian et al., 2016). Tian et al. (2016) also reported an increase of Vitamin B12 and free amino acids content in *L. edodes* samples after hot air drying treatment at a temperature of 70^0C. An increase in nutritional components after hot air drying at 30^0C in an oven is also reported by (Fernandes et al., 2013). Their research findings showed an increase in total saturated fat content and total tocopherol content of *M. pocera* mushroom samples after oven drying.

Effects of hot air drying vary across different species of mushrooms, likewise findings on researches on other species show a decrease in certain nutritional components, other findings show an increase in certain nutritional contents whilst others show no significant changes. Likewise, in accordance to Hu et al. (2020), results which were obtained after hot air drying *S. annulata* samples did not significantly change in protein content. A decrease in nutritional content which was noted was on the contents of crude fibre, carbohydrates and total soluble sugars. This is in contrast with the aforementioned results which were found after hot air drying of *P. ostreatus* and *A.bisporous*. Slawinska et al. (2016) also reported that no significant change in vitamin D contents was observed in an experiment which involved use of hot air drying on *A. bisporous*, *P. ostreatus* and *L.edodes*.

iii. Microwave drying

Microwave drying involves use of electromagnetic waves of the frequency 300 MHz to 300Mgz, (Sun, 2012). Microwave drying results in change of texture as well as flavor of the mushrooms, (Peiet al., 2016). After a comparison of this method and hot air drying, Sun (2012) actually found out that microwave drying was a quicker method to use to dehydrate and preserve mushrooms,

avoiding protein denaturation and causing an increase in the content of vitamin B12 in *L. edodes* (Tian et al., 2016).

On the other hand results from this research by Tian et al. (2016) also showed that the content of vitamin D in the microwave-dried samples of *L. edodes* decreased by 15.11% but the contents of other nutritional components such as proteins and uronic acid did not significantly change. In a research by Wu et al. (2015), in which *H. mamoreus* samples were dried using microwave drying, hot air drying and vacuum drying, it was found that microwave drying resulted in the highest retention of the total sugar content. Microwave drying also decreases microbial content, (Wu et al., 2015). Sensorial characteristics such as colour, texture, taste and aroma of the mushrooms also changed, (Wu et al., 2015).

iv. Freeze drying

Freeze drying is also another method that can be utilized to dry mushrooms. According to Zhang et al. (2018), this method involves utilization of a procedure called water sublimation. In this technique free water present in the mushroom samples is firstly frozen then directly converted to the vapour phase without passing through the liquid phase first (Fellows, 2009). Findings from research by Pei et al. (2014) of freeze drying on *A. bisporous* mushrooms showed that this method results in samples which have a higher nutritional quality and since it is done at a low temperature, heat sensitive properties of the mushrooms such as vitamins are maintained at a higher quantity. Xu et al. (2021) also made a report on a comparison between freeze drying and air drying in which it is stated that freeze drying was found out to be a more efficient method to use for drying and it resulted in higher quality finished products.

Findings from research by Shams et al. (2022) also support the fact that freeze-drying results in more retention of nutrients as well as removal of more moisture content. In this study effects of freeze drying were compared to effects of cabinet drying. Results obtained showed that the crude protein (%), crude fibre(%), crude fat(%), ash(%), carbohydrate(%) and energy (Kcal/100g) of freeze dried button mushroom samples were significantly higher, ($p<0.05$) with 0.533, 25.34, 12.79%, 2.03%, 6.88%, 60.67%, and 362.31Kcal/100g respectively whereas cabinet dried samples showed significantly lower values ($p<0.05$) of 0.674%, 24.26%, 12.65%, 1.89%, 6.75%, 59.50%, and 352Kcal/100g respectively. Lower moisture content from freeze-dried samples of

5.09% as compared to the cabinet-dried samples which had 7.6% is due to the fact that in freeze drying moisture is turned straight to ice and lost off straight as vapour skipping off the liquid phase altogether, (Jaworska et al., 2014).

Huang et al. (2015) considered freeze drying to be an expensive drying method to use since the machine which is used for this procedure consumes a lot of energy and the rate at which the drying procedure is undertaken will be very low. Freeze drying is usually used in the pharmaceutical industries and its use in the food industries is limited (Menlik et al., 2009; Nireesha et al., 2013).

According to Nicksic et al. (2015), the texture of mushrooms after drying can be easily retained by soaking in water before use which means the matter of mushrooms becoming harder after preservation is reversed after the soaking procedure. It can be noted that different drying methods have different effects on the overall end properties of the mushrooms. The decision of which method to use is usually mainly based on the availability of equipment to be used, costs to be incurred as well as intended end quality of mushroom required (Piskov et al., 2020). All these factors will therefore need to be looked at before one decides on the method to use for drying.

Table 2. 1 showing effects of drying methods on different mushroom`s nutritional quality, sensorial quality and microbial content

Drying Method	Nutritional Content	Sensorial Quality	Microbial Content	References
Hot air drying	Retains moderate levels of nutrients, protein content can be preserved but may lose some vitamins.	Can negatively impact color and flavor, texture may become tougher.	Effective in reducing microbial load but may not eliminate all pathogens.	(Piskov et al., 2020, Fernandes et al., 2012)
Sun Drying	Can possibly enhance vitamin D content due to UV exposure, may lead to loss of some vitamins	Often results in a darker color and altered texture, flavor may be intensified.	Reduction of microbial load varies and depends on the environmental conditions.	(Kalaras et al., 2012, Ayodele et al., 2017)
Oven Drying	Nutrient retention varies, can lead to loss	May result in a dry, less appealing	Effective in reducing microbial load,	(Tian et al., 2016, Peiet al., 2016)

	of volatile compounds affecting flavor.	texture; color may darken	but may not be as effective as freeze drying.	
Microwave Drying	Can preserve nutrients effectively, however, uneven heating may lead to nutrient loss.	May result in a less desirable texture, flavor can be altered due to rapid heating.	Effective in reducing microbial content; rapid drying minimizes microbial growth.	(Sun, 2012, Wu et al., 2015)
Freeze Drying	Best method for retaining nutritional quality, preserves vitamins and bioactive compounds.	Maintains original color, flavor, and texture, often results in a superior product.	Highly effective in reducing microbial load, ensuring safety and shelf life.	(Zhang et al., 2018, Fellows, 2009)

2.4.2 Freezing

Freezing is a method of preservation which involves storing samples in a refrigerator at temperatures below 0^0C and it allows for preservation of the characteristics of the mushrooms such as colour, aroma, taste and texture (Reis et al., 2000). According to (Bernas & Jaworkska., 2010) freezing is considered one of the best methods to use to preserve nutritional contents of mushrooms for a longer period. In comparison to other preservation methods such as drying methods, freezing results in better preservation of various sensorial qualities of mushrooms such as colour, texture, taste and aroma (Fernandes et al., 2013). The other benefit of using freezing as a preservation method for mushrooms is that it greatly reduces microbial growth as well as chemical and cellular reactions of the mushroom samples. According to Kexin et al. (2018) freezing is a good method that can be used to extend the shelf life of mushrooms. Diamantopoulou & Philippoussis, (2015) also noted that at 0^0C the respiration rate of mushrooms becomes 10 times lower than at 10^0C. Fresh mushrooms taken straight from harvest are the ones that are usually ideal for freezing (Bernas` et al., 2006).

Findings from a research by Fernandes et al. (2015) which involved freezing *M.porcera* samples showed that nutritional quantities of carbohydrates, fats and total sugars reduced over time but the quantities of ash and proteins did not significantly change. Fat content decreased due to a decrease in the amounts of monosaturated and saturated fatty acids (Fernandes et al., 2013). On

the other hand (Bernas et al., 2016) carried out a research of the effects of freezing on the vitamin content of *A Bisporus* and their findings showed that the quantities of vitamins were reduced over twelve months. Assessment of mushroom samples of the species *P. ostreatus* after twelve months of freezing at -25^{0}C also showed that the contents of dry matter, protein and total amino acids had reduced compared to the quantities that had been observed in the fresh samples (Jaworska et al., 2011). In another report by (Bernas & Jaworska, 2016), after a study on *A.bisporous* mushroom samples that were stored at -20^{0}C for 0,6 and 12 months, it was stated that the total amounts of carbohydrates, protein, fat and ash in the samples did not significantly change. Significant reductions in amounts were noted in the amounts of vitamins BA, B2, B6, ascorbic acid, α-tocopherol, β-carotene and lycopene and higher losses were noted in the last six months (Bernas & Jaworska, 2016). Bernas & Jaworska, (2016) proceed to explain that very low freezing temperatures and more time of preservation result in more reduction of the contents of the nutrients available in the mushroom samples as well as reduced microbial activity.

According to Reid et al. (2016), freezing is also used for preservation of indigenous mushrooms in Zimbabwe. However, reports of the effects of freezing on the indigenous mushrooms are not well documented. From this review on freezing it can be noted that as much nutritional contents of the frozen samples decrease over time for some samples it can also be adopted as a convenient method to use for preservation of indigenous mushrooms in areas where the resources required for freezing will be available.

2.4.3 Pickling

Pickling is a method is often used on foods such as vegetables, fruits, meats and eggs (Holban et al., 2017). In accordance to Jablonska et al. (2019) pickling can also be used to preserve mushrooms. This procedure does not require special equipment and it is sometimes combined with other preservation methods such as fermenting, canning or just refrigerating (Barret, 2023). According to Lee (2004), there are two methods of pickling which include chemical pickling and fermentation pickling. Chemical pickling involves immersing the food sample in an edible liquid which reduces action and growth of microorganisms and these edible liquids include brine, oil, alcohol or vinegar (Lee, 2004). On the other hand, in fermentation pickling, the food sample itself causes its own preservation by producing a preservative agent that inhibits growth of microorganisms mostly through production of lactic acid (Lee, 2004).

Perreira et al. (2015) also support the fact that pickling is a method which reduces activity and growth of pathogens which cause spoilage of mushrooms. This inhibition is caused by the acidic conditions which will be created by the salty and acidic pickling solutions. The acidic environment of pickling causes a reduction in total aerobic count, yeast and mold counts as well as occurrence of coliform bacteria in mushrooms, (Beaulieu & D'Aprano, 2003; Tham et al., 2020). These acidic solutions also cause an impact on the sensorial attributes of the mushrooms and it has been observed that after pickling mushrooms usually end up having a tart flavour, firm texture and they become more translucent, (Beaulieu & D'Aprano, 2003; Pereira et al., 2015)

Pickling also changes some of the nutritional contents of the mushrooms likewise according to a report which was compiled after research by Kumar et al., (2008) on the pickling of *A bisporous* mushrooms using sesame oil and mustard oil, no significant change which was observed in the quantities of ash, protein and fat in the samples for a storage period of up to one year.A decrease in ascorbic acid could however not be explained (Kumar et al., 2008). No changes were observed in the organoleptic scores of the preserved button mushrooms and as much as the microbial count of the samples increased, it did not surpass the acceptable limit of 2 log cycles. Conclusions from the research were that picking was considered a good long term preservation procedure which could be used to store button mushrooms for more than a year.

Tamimola et al. (2022) also carried out a research on effects of pickling treatments on *P. ostreatus* and *P. pulmonarius* in Nigeria. Their results showed that after the preservation procedures the mushroom samples still remained with considerably high amounts of proteins and they concluded that this method of preservation was simple and could be used to preserve nutritional quality of mushrooms. Rai et al. (2008) also reported pickling using vinegar as a long-term preservation method for mushrooms, which can keep mushrooms preserved for up to six months. Various spices which can be included in the pickling liquid include turmeric powder, black mustard seed powder, black pepper, red chili powder, fennel seed powder and mustard oil (Rai et al., 2008). Permitted quantities of sodium benzoate and acetic acid should also be added to aid longer preservation time, (Rai et al., 2008)

Pickling is a procedure that presents a chance for long-term storage mushrooms. This method can also be implemented on indigenous mushrooms.

2.4.4 Steeping in salts or acids

Steeping is also a type of preservation which is similar to pickling as it involves preserving samples in solutions of salt, acids, or a combination of both (Ratnoo et al., 2013). Smolenski et al. 2004 observed that steeped mushrooms were becoming high in demand in the European markets, particularly in the Netherlands due to their longer storage shelf life and lesser production costs. According to Sethi et al. (2000), mushrooms can be preserved using this method for a longer period of time if the steeping solution is also mixed with permissible preservatives. Salts and acids used in the steeping solution inhibit quick growth of microbes thereby preserving the mushrooms (Sethi et al., 2000). Rai & Arumuganathan (2008) reported that blanching mushroom samples before steeping them also helped to maintain their color and to stop microbial growth. This means that it is essential to blanch mushroom samples before steeping them. The taste and aroma of steeped mushrooms are usually altered from the original qualities of the fresh mushrooms (Rai & Arumuganathan, 2008).

Gupta et al. (2015) researched on effects of steeping solutions on the quality of *A. bisporous* mushrooms preserved for 80 days. They used different concentrations of steeping solutions which included 2% sodium chloride mixed with 0.1% potassium metabulshite, 0.1% citric acid and .1% tartaric acid, second solution was 2.5% sodium chloride mixed with 0.1% ascorbic acid,0.2% citric acid, 0.1% sodium hydrocarbonate and 0.1% potassium metabulshite, the third steeping solution contained 2% sodium chloride mixed with 2% sugar, 0.3% citric acid, 0.1% potassium metabisulphite and 1% ascorbic acid, the fourth solution had 2.5% sodium chloride mixed with 0.1% acetic acid, 0.2% citric acid, 0.1% sodium bicarbonate and 0.1% potassium metabisulphite. The fifth solution was a control consisting of only water. Their results showed that mushrooms which were steeped in water got spoiled after 20 days whilst after the 80 days samples which were preserved in lower percentage sodium chloride solutions had the highest amounts of microbial spoilage. The findings from this research showed that steeping helps to prevent microbial growth in samples above the acceptable limit of 1000c.f.u/ml in accordance to the Food Safety and Standard Regulation, (2010).

Another report on steeping mushrooms was made by Jarwoska et al., (2003). Their results showed that samples preserved in high brine concentrations of 15% to 20% remained edible for up to 12 months. This conclusion came up after research on the preservation of *A. bisporous, C.*

cibarius and *B. edulis* by Yang et al. (2007) who carried out a research which showed that the nutritive value of the mushrooms decreased over lapse of period of 6 months but microbial spoilage was highly reduced. Horubala et al. (2008) reported that steeped mushrooms need to be desalted first before preparation for consumption since they would have taken up a lot of sodium chloride concentration from the steeping solution. The sensorial qualities of the mushrooms such as colour and texture where maintained and the taste were altered due to the salty solutions (Horubala et al., 2008).

From these researches one can note that steeping is also another which is ideal for mushroom preservation. Reports on use of the steeping solutions on indigenous mushrooms are not vastly documented and this is a method which can also be researched for use to enhance the shelf lives of indigenous mushrooms.

2.5 Downstream processing of spoiled mushrooms

As much as preservation methods exist which can be employed to increase shelf life of mushrooms, others will still get spoiled since they will be available in their fresh states especially in the markets awaiting retail. According to Mutema et al. (2018), mushroom cultivation is on the rise in Zimbabwe which therefore means that the amounts of mushrooms which will possibly get spoiled before they are sold out are also likely to increase. After a survey by Mutema et al. (2018) on profitability of mushroom farming in Harare, it was noted that 6.6% of participants mentioned the problem of perishability of the mushrooms before they reached the consumers. A total of 40% of the respondents also mentioned the problem of spoilage of the mushrooms by fungal diseases and flying insects (Mutema et al., 2018). This problem of spoilage of mushrooms before they reach consumers is also being faced in other larger mushroom cultivating countries like Japan, America and China (USDA, 2020; Zhao et al., 2020; Nakano et al., 2020).China is the highest global mushroom producing country, accounting for over 70% of the world mushroom harvests an estimate of up to 30% of the harvested mushrooms is lost due to spoilage (Zhao et al., 2020). In America records of spoilage of mushrooms range from 10% to 40% and in Japan recorded estimates range from 10 to 20% (Zhao et al., 2020; Nakano et al., 2020).

Considering the studies the challenges being caused by synthetic plastics that are being faced around the world and also in Zimbabwe, spoiled mushrooms could be factored in as a supplementary source of starch to produce bio degradable plastics. Starch is a polysaccharide

that is found in plants as well as in mushrooms. Research on the production of biodegradable plastic from sources like bananas, potatoes as well as cassava have been successfully done before (Mohapatra et al., 2010; Gustafsan et al., 2015). Considering that these are food grade materials, researches on other potential sources is still important so as to increase the variety of the biodegradable plastic sources.

In a research by Shibata & Diamiate (2016) it was found that the starch content of *A. sylvaticus* mushrooms was 36.21%. Results from studies on eleven species of *L. edodes* by Brauer et al. (2011) showed that mushrooms had starch concentrations that ranged from 20 to 100mg/g In a research by Nissa et al. (2022) it was observed that the estimated starch content of *A. bisporous* mushrooms was 29.7mg/g of *P. ostreatus* was found to be 4.03mg/g. The lesser amounts of starch which were yielded in this research could be attributed to the method of extraction that was used.

Conditions that increase the purity and yield of starch production from mushrooms also include the extraction parameters, such as antioxidant concentration, extraction time, and temperature (Nissa et al., 2022). The method and solvent used in the homogenization of the starch extraction process in mushrooms also affect the final yield (Nissa et al., 2022)

2.5.1 Starch content of mushrooms

In a research by Dermiate et al. (2023) it was found that the starch content of *A. sylvaticus* mushrooms was 36.21%. Results from studies on eleven species of *L. edodes* by Brauer et al. (2011) showed that mushrooms had starch concentrations which ranged from 20 to 100mg/g In a research by Nissa et al. (2022) it was observed that the estimated starch content of *A. bisporous* mushrooms was (29.7)mg/g of *P. ostreatus* was found to be (4.03)mg/g. Manzi et al., (2004) conducted a study on the starch content of mushrooms which included *A.bisporous*, *P.ostreatus* and *L.edodes* and concluded that mushrooms can be a significant source of starch. Results of total starch contents which were found in these mushrooms ranged from 5% to 20% dry weight. Different studies have also showed that mushrooms contain a significant amount of starch ranging from 5% to 30% (Kalac, 2009; Wani et al., 2010; Valverde et al., 2015, Reis et al., 2012). One can note that different studies result report different starch yields and this caused by the fact that extraction methods of starch extraction result in different amount of starch yield, (Reid et al., 2012).

Conditions that increase the purity and yield of starch production from mushroom also include the extraction parameters, such as antioxidant concentration, extraction time, and temperature. (Liu & Han, 2006). A study by Liu & Han, (2006) also showed that the method used in the homogenization of the starch extraction process in mushrooms affects the yield. The solvent used in the homogenization also has a significant effect on the starch yield, (Liu & Han, 2006).

2.5.2 Plastic Usage in Zimbabwe

According to the country's Environmental Management Agency (EMA), Zimbabwe generates about 300,000 tons of synthetic plastic waste per year. A significant proportion of that waste is dumped in the streets or open areas, rather than being recycled (Moyo, 2021).Government effort to regulate the use of flimsy plastic carrier bags in 2010 ran into consumer resistance and failed, (Moyo, 2021). Efforts were made in the past years to reduce pollution through the ban on thin plastic with a diameter of less than 30 micrometers and the ban was later widened to the inclusion of polystyrene through the statutory Instrument 98 of 2011 and 84 of 2012 respectively (Tsiko, 2022). Regardless of these bans however, plastic pollution is still on the high and the 5th session of the United Nations Environmental Assembly (UNEA 5.2) romped in Zimbabwe to reduce plastic pollution through the production of alternative packaging (Moyo, 2021). Biodegradable plastic can therefore be factored in as part of the alternative sources

2.5.3 Bio-plastics

A material can be defined as a bio plastic if it is either bio-based, biodegradable, or features both properties (European Bioplastics Facts and Figures, 2020). The term bio-based refers to materials that are completely or partly derived from renewable resources (biomass); thus the petrochemical resin typical of common plastics is replaced by vegetable or animal polymers and the compounds like glass or carbon fiber or talc are replaced by natural fibers (wood fibers, hemp, flax, sisal, jute), (Kumar et al., 2017). For those concerning biodegradability, a material could be defined as biodegradable if it undergoes decomposition by biological processes, during composting to yield carbon dioxide, water, inorganic compounds, and biomass at a rate consistent with those of other known, compostable materials and leaves no other distinguishable or toxic residue, (Kumar al., 2017).

In the synthesis of bio-plastics, plasticizers can be incorporated to increase the durability of the bio-plastic and these plasticizers are defined as low-volatile molecules added to bio-polymeric

materials to ensure an increase in their extensibility, dispensability, flexibility, and elasticity (Wypych, 2017). Ideal plasticizers are said to be miscible and compatible in all proportions with plastic components, and they may be added to polymers in solution using the dispersion technique or after solvents have been removed using the absorption technique (Wypych, 2017).

Various studies on use of starch from various materials to produce bioplastics have been carried out and documented but literature searches on studies of starch based on spoiled mushroom starch did not yield much credible information on this subject.

2.6 Conclusion

The use of postharvest preservation methods to increase the shelf life of mushrooms is important especially on the ones which are found seasonally in the wild. On the other hand those mushrooms that become spoiled whilst on the market can be possibly processed to provide a supplementary option to the already available sources of bio plastics so as to aid to the reduction of synthetic plastic pollution being faced in the country and around the world at large.

CHAPTER 3

Availability of indigenous mushrooms in modern markets and an evaluation of the preservation impact on their nutritional contents

Abstract

Edible mushrooms are considered as a nutritious source of food possessing core components such as protein, lipids, carbohydrates, starch, moisture, vitamins and dietary fibre. There are indigenous and cultivated types of edible mushroom and both of them are found in Zimbabwe. Cultivated mushrooms are found all year round and indigenous mushrooms are found during the rainy season. Results of a market survey showed that there was 0% availability of indigenous mushrooms in the formal modern markets (walk in supermarkets) and 100% availability of cultivated mushrooms. There was also 0% availability of preserved forms of mushrooms in these modern markets. The main goal of the research was to preserve indigenous mushrooms through oven drying, pickling in vinegar and spices, steeping in a brine solution, and freezing. Mushrooms that were preserved included *C. miombensis*, *Cantharellus spp* and *A. zambiana*. Determination of the effect of preservation methods on compositions of crude protein, moisture, crude lipid, crude fibre and ash was done through proximate analysis over a period of nine months. At a significance level of $p<0.05$ it was found that the different methods had varying impacts on the different mushrooms. The initial protein content of the preserved mushroom samples ranged from 12.17±0.05% to 14.27 ± 0.06% whereas after the last month of preservation they had significantly decreased to a range of 5.39±0.03% to 9.55±0.02%. Pickled samples had a significantly increased content of lipids from an initial range between 9.34±0.02% and 12.42±0.02% to a final range of 14.91±0.02% to 19.27±0.02%. The ash content of the samples decreased from an initial range of 9.22±0.01% to 12.74±0.03%, to a final rage of 3.02±0.01% to 6.87±0.04±%. The crude fibre content of pickled and steeped samples also decreased from an initial range of 4.23±0.03% to 6.76±0.02% to a final range of 2.87±0.01% to 5.07±0.02%.In conclusion, preservation affects the nutritional contents of the indigenous mushrooms. It can be noted that, if more research is done to establish the safety of the preserved indigenous mushrooms, the formal markets could appreciate them for sale thereby also enhancing their availability to the consumers.

3.1 Introduction

Wild edible mushrooms have been included in human diet since ancient times, mainly for their good taste (Khatua, 2013; Reid, et al., 2016). They have also been valued since a long time back for their favorable contents of nutrients (Kalac, 2009; Pereira et al., 2012). The nutritional components found in wild mushrooms include protein, lipids, carbohydrates, starch, moisture, vitamins and dietary fibre (Sharif et al., 2017; Mshandete & Cuff, 2007; Singh, 2016, Enas, 2016, Girma & Tasisa, 2018).These facts have led to their commercialization and according to Kalac (2013), there are about 200 species of wild mushrooms which are actually commercialized in different parts of the world. Commercialization and consumption of wild mushrooms around the world has escalated due to continuous ongoing research which is continuously proving that they possess good nutritional benefits, (Khatua, 2013).

In Zimbabwe, a variety of edible indigenous mushrooms sprout naturally in forests during the rainy season (Gleaner, 2023). People forage for these mushrooms for sale on informal markets as well as for consumption at their homes (Gleaner, 2023). Once the rainy season lapses, the availability of these indigenous mushrooms, in the forests as well as on the informal markets ceases (Mlambo & Maphosa, 2017). There is little information on the availability of indigenous mushrooms on the formal markets of Zimbabwe either in fresh or preserved form, during the rainy season and after the rainy season in literature.

According to Gill, (2011) the main reason why mushrooms stop sprouting after the rainy season ends is because fungi require moisture to produce mushrooms which are their fruiting bodies therefore they cannot be produced in the absence of rain. In general, Indigenous mushrooms are underutilized because they are not found readily available throughout the year (Nakalembe et al., 2015; Mlambo & Maphosa, 2017). Other factors that contribute to the underutilization of wild mushrooms include fear of poisoning and their very short fresh state shelf life (Mlambo & Maphosa, 2017; Castellanos-Reyes et al., 2021).

The short shelf life of mushrooms is mainly caused by the high percentage of moisture content which the mushrooms generally possess (Castellanos-Reyes et al., 2021). Other factors include lack an outer protective cuticle, high rate of respiration, high level of enzymatic activity and high presence of micro flora (Zhang et al., 2018; Fernandes et al., 2012). They are therefore more

prone to microbial attack, mechanical damage, weight loss as well as enzymatic browning (Donglu, 2016).

Post-harvest preservation methods can be used as a solution to the short shelf life of variety of food samples that quickly get spoiled, (Shudel et al., 2023; Siti-Nuramira et al., 2022). By definition, post-harvest preservation methods are technologies, be it complicated or simple which are used to increase the shelf life of a food sample by reducing enzyme activity as well as reducing chances of quick deterioration due to microbial attack (Desrosier & Singh, 2024). These methods include old methods such as refrigeration and fermentation and modern methods such as canning, pasteurization, freezing, irradiation, and use of chemicals (Desrosier & Singh, 2024). Preservation methods that have been used on mushrooms include different dying methods such as freeze drying, microwave drying, sun drying, and solar drying as well as other methods such as canning, pickling, freezing, use of chemicals (Zhang et al., 2018; Argyropoulus et al., 2011; Marcal et al., 2021; Ruan-Soto et al., 2017). From these facts it can also be noted that postharvest preservation can be possibly used to increase utilization of indigenous mushrooms as well as to contribute to their availability for longer periods surpassing the rainy season only.

Of the aforementioned preservation methods, people who harvest the indigenous mushrooms usually preserve them through sun drying and also freezing on a lower scale due to the unavailability of power resources in some parts of rural areas (Reid et al., 2016). This sun drying method as well as other methods which have been researched on using other mushrooms has been found to have different effects on the nutritional quality of the mushrooms during the preservation period (Marcal et al., 2021; Zhang et al., 2018; Argyropoulus et al., 2011).

The main aim of this research was therefore to firstly conduct a market survey so as to establish the state of availability of fresh and preserved (indigenous and cultivated) mushrooms in various markets of Zimbabwe to justify the importance of usage of the preservation methods to enhance their market availability. The other aim was to determine the effects of preservation methods on the nutritional content of the mushrooms through proximate analysis.

3.2 Materials and methods

3.2.1 Market Survey

The market survey was carried out across 26 branches of three different types of modern markets located in Mashonaland Central (Harare) and Mashonaland West (Chinhoyi and Karoi) named A (11 branches), B, (8 branches) and C (7 branches) in this research. It was carried out between the period of October 2021 and December 2021. Modern markets involved were basically formal walk in supermarkets in which the consumer gets to select products for themselves and pay at a till point. Respondents who participated were the store personnel. The survey had questions on types of mushrooms sold by the market (both fresh and preserved), shelf life of the mushrooms, and estimated percentage of mushrooms which spoil whilst on the shelves as well as how they got rid of the spoiled mushrooms. The questionnaire is attached as an appendix.

3.2.2 Sample Collection

Mushroom samples which included *A.zambiana*, *C.miombensis*, and *Canthrellus spp* were bought from the Chinhoyi informal Market (from mushroom vendors on the store pavements). Identification of the mushroom samples by the researcher was archived through comparing them with the photographical, morphological and anatomical characteristics descriptions given in the manuals on identifications of Southern African mushrooms (Sharp, 2011; Ryvarden et al., 1994).

3.2.3 Preservation procedures

i. Control Samples

Control samples for oven drying and freezing were left on the bench top at ambient temperatures without any treatments and control samples for pickling and brining were immersed in distilled water

i. Preliminary processes

Washing under running water to remove grit was done before the implication of all the preservation processes. All samples were blanched in 2% brine solution at 95^0C for five minutes first before being preserved (Rai et al., 2008).

ii. Oven drying

Oven drying was achieved through a method by Cancler, (2012). The oven was preheated to 60^0C. The mushrooms were dried for 3 hours and left to cool at ambient temperatures. Dried mushrooms were stored in plastic packaging for analysis.

iii. Steeping in brine solution

Steeping was done through following a method by Wabali& Simeon, (2013). Steeping solutions were prepared. Samples of mushrooms weighing 100 grams were transferred into jars containing 100mls of 15% and 18% and 20% sodium chloride concentrations respectively. Samples were stored for analysis.

iv. Pickling in vinegar and spices

The method by Rai et al. (2008) was followed.Distilled water (500mls) was mixed with 250 ml of olive oil, 250 ml of white vinegar, 5 grams of black peppercorns, 10g chili powder, 20g of turmeric powder,and 5g of pickling salt. Glass jars were sterilized then equal amounts of the hot mushroom (100g mushrooms mixed with 100ms of the pickling ingredients) mixtures were added up to a 20 millimeter headspace of the jars. The mixtures were evenly distributed in the jars. The rims of the jars were cleaned with vinegar soaked towels. The jars of the pickled marinated mushrooms were then processed in boiling water for twenty minutes and stored for analysis.

v. Freezing

The method by Cancler, (2012) was followed. Blanched samples weighing 100g were packed into packaging plastics and were then transferred into a Capri upright laboratory refrigerator which was set at 22^0C and stored for analysis.

3.2.4 Proximate nutritional analysis

Samples from each of the preservation techniques were analyzed independently and in triplicate.

i. Crude protein determination

Samples were ground using a mortar and pestle in a tris buffer. The protein content in the preserved mushroom samples was determined using the Bradford method (Bradford, 1976). Before the assay Bovine Serum Albumin stock solution, Bradford reagent, and series of dilutions of the Bovine Serum Albumin protein standard were prepared. Test samples for the Bradford

protein assay were prepared in falcon tubes by adding 50ml of the test sample followed by 200ml of the bradford reagent. The samples were incubated at room temperature for ten minutes. The absorbencies of the samples were measured using a Shimadzu UV-19001 spectrophotometer. The absorbance results were used to create a standard curve using Microsoft Excel. The samples for the Bradford protein assay were then prepared whereby in the falcon tubes, 50ml of the ground mushroom sample followed by 200ml of Bradford reagent were added. The absorbance of each protein sample was measured using a Shimadzu UV-19001 spectrophotometer at 595nm. The protein concentrations were calculated from the equation of the standard curve. The equation of the standard curve was y=0.00049 + 0.028236.

ii. Crude fibre determination

A method by Krishanu, (2017) was used to determine the crude fibre content. Each preserved mushroom sample (M_3) was weighed (3g) and transferred into a round bottomed flask. This sample was digested through boiling under reflux in 200mls of 1.25% sulphuric acid at 100^0C using a heating mantle and washed with distilled water, sample was then digested again by boiling in 200mls of 1.25% sodium hydroxide on a hot plate. The sample was then filtered and the residue left was washed with distilled water. The residue was then boiled up to 110^0C for about two hours and it was weighed afterwards (M_1). After weighing the residue was heated in an incinerator at 350^0C for 3 hours. After heating the residue was then cooled again and weighed again (M_2). Total crude fibre will be expressed in a percentage as follows;

$$\text{Crude Fibre} = \frac{M_1 - M_2}{M_3} \times 100$$

Whereby M_1 – Mass of residue and crucible

M_2- Mass of residue and crucible after heating

M_3 – Mass of sample

iii. Crude ash content determination

Total ash was determined according to a method by AOAC (1995). Empty crucibles were weighed first (M_1). Each preserved mushroom sample weighing 3g was put into the crucible and

the weight of the crucible containing the sample was noted (M_2). The samples were heated to ash in an incinerator at 550^0C for 6 hours. After heating the crucibles containing the samples were taken out of the incinerator using tongs and cooled in a desiccator. The crucibles containing the ash were weighed (M_3). Total ash content was expressed as a percentage on dry basis as follows;

$$\text{Total Ash \%} = \frac{M_2 - M_1}{M_3 - M_1} \times 100$$

Whereby - $M_2 - M_1$ - Sample mass in grams on dry basis

- $M_3 - M_1$ - Mass of ash in grams

iv. Moisture content determination

The percentage of the moisture content in the samples was measured using a Shimadzu moisture analyzer. 1g of each sample was weighed and the moisture analyzer was set to determine total moisture content of the sample.

v. Crude lipid content determination

An adjustment method by Folch (1976) was used. In the method by Folch, the explanation on how the chloroform was evaporated to obtain the lipid residue was not clarified therefore in this research evaporation of the chloroform was done in a water bath at 50^0C.

Each preserved mushroom sample (5g), was ground using a mortar and pestle and suspended in 100 milliliters of chloroform and methanol which was mixed at a ratio of 2:1 volume to volume respectively. Sample was thoroughly mixed and left to stand for 3 minutes. The solution was filtered and centrifuged at 1000×g. The upper layer of methanol was removed by a Pasteur pipette and remaining mixture of chloroform and lipids was transferred into a beaker which had been pre weighed (M2). The beaker containing the chloroform and the lipid mixture was put in a water bath set at a temperature of 50^0C causing the chloroform to evaporate. After the chloroform had evaporated, the beaker containing the remaining crude lipid sample was weighed (M3). The percentage of the crude lipid content was calculated using the following equation,

$$\text{Total Lipid \%} = \frac{M_2 - M_3}{M_1} \times 100$$

Whereby - M_1 – Sample mass in grams

M_2 – Mass of beaker in grams

M_3 –Mass of crude lipid and beaker in grams

3.3 Data analysis

All experiment were performed thrice (n=3). The Microsoft Excel tool was used for data analysis. The mean and standards deviations (SD) were calculated using Microsoft Excel. Statistical differences between the monthly results were noted using a t test at $p<0.05$. Analysis of Variance at $P<0.05$ was also conducted to test for statistically significant differences in overall.

3.4 Results

3.4.1 Results for mushroom market survey

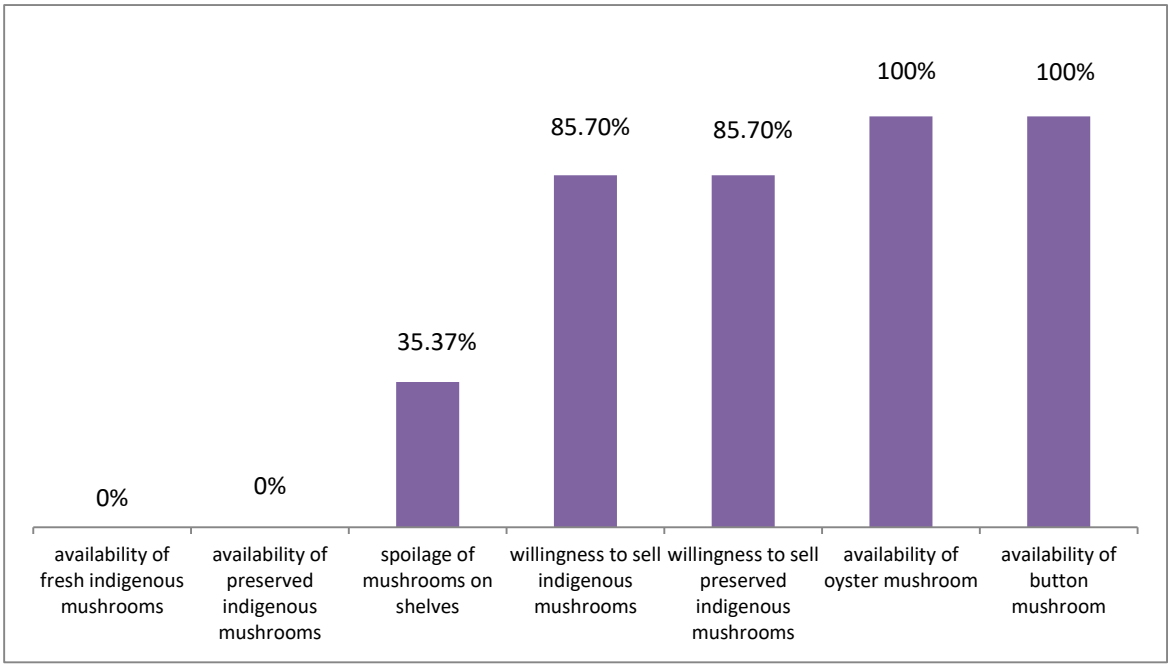

Figure 3. 1 Averages of responses from different participants involved in the market survey

As shown in Figure 3.1, all the branches sold both oyster and button mushrooms on their shelves. However, none of them sold indigenous mushrooms or any preserved types of mushrooms. It was also noted from the responses that on average 35.37% of the oyster and button mushrooms

that would have been supplied for sale got spoiled whilst on the shelves after a range of 3 to 5 days.

3.4.2 Proximate composition of the preserved Indigenous mushrooms

i. Results for fresh samples

Table 3.1proximate nutritional analysis results for fresh samples

Mushroom type	Crude Protein %	Crude Lipid%	Crude fibre%	Moisture%	Crude Ash%
CM	15.22±0.03[a]	12.42±0.02[b]	6.76±0.02[c]	89.23±0.03[a]	12.74±0.03[a]
CS	12.17±0.05[b]	9.34±0.02[c]	4.23±0.03[a]	88.67±0.02[c]	9.22±0.01[b]
AZ	14.27±0.06[c]	11.33±0.04[a]	5.86±0.03[b]	90.87±0.02[c]	12.33±0.03[c]

CM- Cantharellus miombensis, CS- Cantharellus spp, AZ-amananita zambiana, (n=3), results shown as mean ± standard deviation, mean values with different superscripts down the column are statistically different at $p<0.05$

As shown in Table 3.1, there was a significant difference ($p<0.05$) which was noticed in the proximate nutritional components of the different types of the fresh mushrooms. The highest moisture content of 90.87±0.02% was recorded in *A. Zambiana* followed by 89.23±0.03 in *C. Miombensis* and 88.67±0.02 in *Cantharellus spp.* samples respectively.

i. Crude lipid content results

Table 3. 2lipid content results of the preserved mushrooms

	Dried			Frozen			Pickled			Brine 15%			Brine 18%			Brine 20%		
M	CM	AZ	CS	CM	AZ	CS	CM	AZ	CS	CM	AZ	CS	CM	AZ	CS	CM	AZ	CS

1	6.84±0.01[a]	5.68±0.08[a]	4.21±0.02[a]	10.89±0.03[a]	9.23±0.01[a]	5.73±0.03[a]	14.52±0.01[a]	13.25±0.06[a]	11.95±0.02[a]	6.75±0.01[a]	5.93±0.01[a]	5.35±0.04[a]	6.23±0.01[a]	5.82±0.01[a]	5.35±0.02[a]	6.13±0.01[a]	5.23±0.03[a]	4.93±0.02[a]
2	6.85±0.01[a]	5.66±0.02[a]	4.19±0.02[a]	10.87±0.03[a]	9.21±0.03[a]	5.72±0.02[a]	16.55±0.01[b]	15.21±0.01[b]	12.92±0.01[b]	6.73±0.01[a]	5.89±0.02[a]	4.25±0.01[b]	6.17±0.01[a]	5.76±0.03[a]	4.25±0.01[b]	6.08±0.01[b]	5.12±0.02[b]	3.86±0.02[b]
3	6.83±0.02[a]	5.65±0.11[a]	4.17±0.02[a]	10.71±0.07[a]	9.19±0.02[a]	5.69±0.05[a]	18.51±0.01[c]	15.28±0.07[b]	14.93±0.02[c]	6.57±0.02[b]	5.62±0.02[b]	4.18±0.01[c]	6.05±0.09[b]	5.53±0.13[b]	4.18±0.01[b]	5.95±0.02[c]	4.93±0.02[c]	3.71±0.02[c]
5	6.33±0.01[b]	5.02±0.12[b]	4.04±0.01[b]	10.28±0.01[c]	9.15±0.00[b]	5.46±0.03[b]	18.61±0.03[c]	19.28±0.01[c]	14.90±0.02[c]	6.22±0.02[c]	5.09±0.03[d]	3.91±0.02[d]	5.94±0.05[b]	4.87±0.02[b]	3.91±0.02[c]	5.77±0.02[d]	4.48±0.06[d]	3.67±0.02[d]
7	6.29±0.01[b]	4.97±0.03[c]	4.06±0.02[b]	10.17±0.01[d]	9.21±0.02[b]	5.41±0.02[b]	18.62±0.02[c]	19.23±0.03[c]	14.81±0.01[c]	6.02±0.02[d]	4.91±0.01[e]	3.88±0.03[d]	5.76±0.03[c]	4.18±0.08[c]	3.88±0.03[c]	4.33±0.01[e]	3.87±0.07[e]	3.43±0.02[e]
9	6.22±0.02[c]	4.92±0.02[c]	3.99±0.01[b]	10.11±0.02[e]	9.17±0.14[b]	5.19±0.02[c]	18.61±0.01[d]	19.27±0.02[d]	14.91±0.02[c]	5.86±0.02[e]	4.73±0.01[f]	3.74±0.02[e]	5.72±0.02[c]	4.04±0.04[d]	3.74±0.02[d]	4.13±0.05[f]	3.49±0.02[f]	3.16±0.02[f]

M- Month, *CM- C. Miombensis, CS- Cantharellus spp, AZ- A. Zambiana,* (n=3), results shown as mean ± standard deviation, mean values with different superscripts down the columns are statistically different at a significance level of $p<0.05$

As shown in Table 3.2, the different preservation procedures had different effects on the overall lipid content of the mushrooms. The lipid contents of the mushrooms which were recorded in their fresh state were different from the ones which were recorded after preservation. In overall the highest lipid contents were recorded in pickled samples whereas the lowest lipid contents were recorded in samples steeped in 20% brine solution.

i. Crude protein content results

Table 3. 3crude protein content results of the preserved mushrooms

	Frozen			**Dried**			**Pickled**			**Brine 15%**			**Brine 18%**			**Brine 20%**		
M	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**
1	12.42±0.01[a]	9.83±0.03[a]	11.23±0.05[a]	9.97±0.01[a]	6.21±0.04[a]	6.21±0.01[a]	12.54±0.01[a]	11.27±0.07[a]	10.65±0.04[a]	12.84±0.03[a]	11.16±0.02[a]	9.86±0.01[a]	12.57±0.02[a]	10.67±0.03[a]	9.53±0.01[a]	10.95±0.02[a]	10.18±0.01[a]	9.17±0.01[a]

2	12.17±0.04[b]	9.79±0.02[a]	10.28±0.02[b]	8.41±0.02[b]	6.19±0.05[a]	6.19±0.05[a]	12.53±0.13[a]	11.10±0.02[a]	10.44±0.06[b]	12.67±0.02[b]	11.14±0.03[a]	9.62±0.02[b]	12.42±0.03[b]	10.44±0.01[b]	9.26±0.02[b]	10.02±0.01[b]	10.08±0.01[a]	9.14±0.01[a]
3	11.62±0.11[c]	9.67±0.03[b]	10.00±0.02[c]	8.34±0.01[c]	5.86±0.08[b]	5.96±0.02[b]	12.55±0.05[a]	10.92±0.01[b]	10.21±0.02[c]	12.58±0.02[c]	11.01±0.07[b]	9.21±0.02[c]	12.22±0.02[b]	10.02±0.02[b]	9.24±0.04[b]	9.72±0.05[c]	9.75±0.09[b]	7.87±0.02[b]
5	9.54±0.09[d]	9.22±0.01[c]	10.01±0.05[c]	7.92±0.02[d]	4.87±0.03[c]	4.87±0.03[c]	10.21±0.01[b]	10.76±0.03[c]	9.65±0.02[d]	11.97±0.01[d]	10.85±0.01[c]	9.18±0.02[c]	10.84±0.01[c]	9.73±0.02[c]	8.13±0.01[c]	8.05±0.03[d]	8.55±0.01[c]	6.78±0.02[c]
7	8.66±0.02[e]	8.67±0.13[d]	9.77±0.01[d]	7.78±0.04[e]	3.48±0.1	3.48±0.02[d]	9.17±0.08[c]	9.56±0.02[d]	9.27±0.02[e]	10.44±0.02[d]	9.15±0.02[d]	9.15±0.05[c]	9.13±0.01[d]	8.23±0.06[d]	7.68±0.05[d]	7.47±0.02[e]	6.94±0.14[d]	6.06±0.02[d]
9	8.58±0.01[f]	8.37±0.02[e]	9.22±0.01[e]	7.44±0.01[f]	3.41±0.02[e]	3.41±0.04[e]	9.08±0.03[c]	9.49±0.02[d]	9.24±0.04[e]	9.23±0.02[e]	8.01±0.02[e]	7.09±0.02[d]	7.63±0.03[e]	7.14±0.02[e]	6.26±0.02[e]	6.67±0.02[f]	6.03±0.05[e]	5.68±0.01[e]

M- Month, *CM- C. miombensis, CS- Cantharellus spp, AZ- A. zambiana,* (n=3), results shown as mean ± standard deviation, mean values with different superscripts down the columns are statistically different at a significance level of $p<0.05$

As shown in Table 3.3, the different preservation procedures had different effects on the overall protein content of the mushrooms. The protein contents of the mushrooms which were recorded in their fresh state were different from the ones which were recorded after preservation. In overall the highest protein contents were recorded in frozen samples whereas the lowest protein contents were recorded in samples steeped in 20% brine solution

i. Crude ash content results

Table 3. 4crude ash content results of the preserved mushrooms

	Frozen			**Dried**			**Pickled**			**Brine 15%**			**Brine 18%**			**Brine 20%**		
M	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**
1	4.54±0.02[a]	4.38±0.01[a]	3.79±0.00[a]	6.77±0.02[a]	4.072±0.0[a]	3.20±0.02[a]	5.82±0.02[a]	4.87±0.02[a]	3.02±0.01[a]	4.18±0.01[a]	3.23±0.02[a]	3.23±0.02[a]	5.53±0.02[a]	5.77±0.02[a]	4.18±0.01[a]	6.43±0.04[a]	3.98±0.02[a]	6.19±0.02[a]
2	4.51±0.03[a]	4.37±0.02[a]	3.75±0.06[a]	6.73±0.05[a]	4.052±0.0[a]	3.20±0.02[a]	6.02±0.02[b]	4.91±0.04[a]	3.05±0.05[a]	4.31±0.02[b]	3.78±0.02[b]	3.78±0.02[b]	5.69±0.02[b]	6.38±0.01[b]	5.39±0.02[b]	6.47±0.01[a]	4.81±0.01[b]	6.51±0.02[b]

3	4.55±0.05[a]	4.36±0.04[a]	3.77±0.04[a]	6.72±0.02[a]	4.04±0.03[a]	3.21±0.01[a]	6.23±0.01[c]	5.33±0.02[b]	4.22±0.02[b]	4.54±0.04[c]	3.81±0.03[c]	3.81±0.03[b]	5.71±0.02[b]	6.41±0.01[b]	5.41±0.01[b]	6.59±0.03[c]	4.91±0.02[b]	6.89±0.02[c]
5	4.53±0.02[a]	4.39±0.05[a]	3.75±0.05[a]	6.74±0.03[a]	4.02±0.04[a]	3.19±0.04[a]	7.02±0.01[d]	6.43±0.01[c]	4.59±0.02[c]	4.88±0.01[d]	3.96±0.01[d]	3.96±0.01[b]	5.79±0.02[b]	6.66±0.02[c]	6.45±0.01[c]	6.75±0.01[d]	5.15±0.02[c]	7.88±0.01[d]
7	4.52±0.02[a]	4.36±0.04[a]	3.85±0.05[a]	6.75±0.06[a]	4.07±0.06[a]	3.23±0.05[a]	7.15±0.01[e]	6.71±0.02[d]	4.67±0.02[c]	5.23±0.02[e]	4.22±0.02[e]	4.12±0.02[c]	5.96±0.01[c]	6.69±0.02[c]	7.02±0.02[d]	6.91±0.01[e]	5.75±0.02[d]	8.13±0.02[e]
9	4.57±0.05[a]	4.41±0.08[a]	3.81±0.04[a]	6.79±0.04[a]	4.05±0.03[a]	3.22±0.01[a]	7.22±0.01[f]	6.78±0.01[d]	4.74±0.02[d]	5.22±0.01[e]	4.28±0.01[e]	4.16±0.01[c]	6.28±0.01[d]	6.91±0.02[d]	7.17±0.02[d]	7.12±0.02[f]	6.02±0.02[e]	8.57±0.02[f]

M- Month, *CM- C. miombensis, CS- Cantharellus spp, AZ- A. zambiana,* (n=3), results shown as mean ± standard deviation, mean values with different superscripts down the columns are statistically different at a significance level of $p<0.05$

As shown in Table 3.4, the different preservation procedures had different effects on the overall crude ash content of the mushrooms. The crude ash contents of the mushrooms which were recorded in their fresh state were different from the ones which were recorded after preservation. In overall the highest crude ash contents were recorded in pickled samples whereas the lowest lipid contents were recorded in dried samples.

i. Crude fibre content results

Table 3. 5 crude fibre content results of the preserved mushrooms

	Frozen			**Dried**			**Pickled**			**Brine 15%**			**Brine 18%**			**Brine 20%**		
M	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**
1	5.21±0.03[a]	4.82±0.02[a]	3.69±0.02[a]	7.05±0.04[a]	6.92±0.06[a]	5.76±0.02[a]	5.02±0.01[a]	4.94±0.02[a]	4.21±0.02[a]	4.26±0.01[a]	4.04±0.01[a]	3.18±0.02[a]	5.56±0.02[a]	5.84±0.02[a]	3.63±0.02[a]	5.72±0.01[a]	4.69±0.02[a]	3.74±0.02[a]
2	5.24±0.04[a]	4.81±0.03[a]	3.66±0.04[a]	7.08±0.03[a]	6.61±0.01[a]	5.74±0.03[a]	4.93±0.01[a]	4.75±0.01[b]	4.12±0.01[b]	4.09±0.02[b]	3.87±0.01[b]	3.07±0.01[a]	5.32±0.02[b]	5.74±0.03[a]	3.57±0.03[a]	5.57±0.04[b]	4.56±0.02[a]	3.68±0.01[a]
3	5.25±0.02[a]	4.84±0.04[a]	3.68±0.03[a]	7.06±0.06[a]	6.69±0.01[a]	5.74±0.02[a]	4.88±0.06[a]	4.53±0.02[c]	4.05±0.11[c]	3.82±0.02[c]	3.72±0.31[c]	3.00±0.10[c]	5.03±0.02[c]	5.31±0.02[b]	3.48±0.04[ab]	5.26±0.03[c]	4.28±0.02[b]	3.65±0.01[a]

5	5.22 ±0.04[a]	4.85 ±0.05[a]	3.70 ±0.02[a]	7.04±0.05[a]	7.03 ±0.02[a]	5.73±0.04[a]	4.61±0.03[b]	3.96±0.02[d]	3.78±0.01[d]	3.46±0.01[d]	3.69 ±0.02[c]	2.97±0.02[c]	4.83±0.02[d]	5.26±0.02[b]	3.23±0.01[c]	5.11±0.02[d]	4.71 ±0.02[c]	3.53±0.02[b]
7	5.23 ±0.04[a]	4.83 ±0.03[a]	3.69 ±0.02[a]	7.08±0.06[a]	7.67 ±0.01[d]	5.72±0.02[a]	3.92±0.09[c]	3.81±0.14[d]	3.53±0.01[e]	3.34±0.02[e]	3.62 ±0.02[cc]	2.57±0.02[d]	4.63±0.01[e]	4.26±0.03[c]	3.14±0.05[c]	5.09±0.01[d]	4.68 ±0.01[c]	3.31±0.02[c]
9	5.24 ±0.03[a]	4.80 ±0.06[a]	3.68 ±0.02[a]	7.06±0.04[a]	7.04 ±0.03[e]	5.73±0.03[a]	3.82±0.05[c]	3.64±0.02[f]	2.87±0.01[f]	3.04±0.07[f]	3.03 ±0.02[d]	2.44±0.04[e]	4.37±0.02[f]	4.15±0.03[c]	3.09±0.02[c]	5.07±0.02[d]	4.57 ±0.02[c]	3.28±0.02[c]

M- Month, *CM- C. miombensis, CS- Cantharellus spp, AZ- A. zambiana,* (n=3), results shown as mean ± standard deviation, mean values with different superscripts down the columns are statistically different at a significance level of $p<0.05$

As shown in Table 3.5, the different preservation procedures had different effects on the overall crude fibre content of the mushrooms. The lipid contents of the mushrooms which were recorded in their fresh state were different from the ones which were recorded after preservation. In overall the highest crude fibre contents were recorded in dried samples whereas the lowest lipid contents were recorded in samples steeped in 15% brine solution

i. Moisture content results

Table 3. 6moisture content results of the preserved mushrooms

	Frozen			**Dried**			**Pickled**			**Brine 15%**			**Brine 18%**			**Brine 20%**		
M	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**	**CM**	**AZ**	**CS**
1	16.21±0.01[a]	21.37±0.02[a]	14.31±0.01[a]	4.21±0.01[a]	5.61±0.108[a]	3.44±0.06[a]	17.36±0.11[a]	21.55±0.02[a]	13.34±0.03[a]	16.72±0.01[a]	24.69±0.05[a]	13.84±0.02[a]	15.56±0.02[a]	22.84±0.02[a]	13.63±0.07[a]	14.94±0.04[a]	21.26±0.01[a]	12.98±0.06[a]
2	16.19±0.03[a]	21.35±0.05[a]	14.30±0.02[a]	4.23±0.01[a]	5.6±0.01[a]	3.44±0.02[a]	17.25±0.02[a]	21.53±0.14[a]	13.28±0.01[b]	16.57±0.02[b]	24.56±0.06[b]	13.78±0.01[a]	15.32±0.02[b]	22.74±0.03[a]	13.57±0.09[a]	14.87±0.01[a]	19.19±0.04[b]	12.84±0.01[b]
3	16.20±0.02[a]	21.33±0.01[a]	14.33±0.03[a]	4.23±0.01[a]	5.59±0.02[a]	3.42±0.01[a]	17.21±0.03[ab]	21.17±0.02[b]	13.21±0.02[b]	16.26±0.03[c]	24.28±0.02[c]	13.70±0.05[ab]	15.03±0.02[c]	22.44±0.05[b]	13.38±0.05[b]	14.22±0.01[b]	19.16±0.04[b]	12.48±0.01[c]
5	16.08±0.03[b]	21.27±0.03[b]	14.26±0.02[b]	4.21±0.01[a]	5.61±0.02[a]	3.45±0.02[a]	17.12±0.01[b]	20.06±0.03[c]	13.00±0.04[c]	15.61±0.02[d]	23.71±0.02[d]	12.49±0.02[c]	14.73±0.06[d]	22.26±0.03[c]	12.83±0.01[c]	13.69±0.00[c]	18.32±0.01[c]	11.97±0.02[d]

7	16.13±0.01[b]	21.29±0.01[b]	14.24±0.01[b]	4.23±0.02[a]	5.59±0.03[a]	3.43±0.01[a]	16.25±0.02[c]	19.08±0.03[d]	11.52±0.02[d]	14.95±0.01[e]	23.28±0.01[e]	12.21±0.08[d]	14.43±0.03[e]	21.36±0.03[d]	12.11±0.02[d]	12.78±0.11[d]	18.14±0.02[d]	11.57±0.07[e]
9	15.58±0.02[c]	21.23±0.02[c]	14.21±0.02[c]	4.23±0.03[a]	5.62±0.01[a]	3.40±0.01[a]	16.13±0.02[d]	18.94±0.02[d]	11.44±0.02[e]	13.73±0.02[f]	22.57±0.02[f]	11.92±0.02[e]	13.37±0.02[f]	19.15±0.03[e]	11.44±0.02[e]	12.13±0.06[d]	18.04±0.02[e]	11.23±0.04[f]

M- Month, *CM- C. miombensis, CS- Cantharellus spp, AZ- A. zambiana*, (n=3), results shown as mean ± standard deviation, mean values with different superscripts down the columns are statistically different at a significance level of $p<0.05$

As shown in Table 3.6, the different preservation procedures had different effects on the overall moisture content of the mushrooms. The moisture contents of the mushrooms which were recorded in their fresh state were different from the ones which were recorded after preservation. In overall the highest moisture contents were recorded in pickled samples whereas the lowest lipid contents were recorded in dried samples.

Discussion

In overall it was noted that out of all the branches all of them (100%) sold both oyster and button mushrooms on their shelves (Figure 3.1). None of them (0%) sold indigenous mushrooms or any preserved types of mushrooms. It was also noted from the responses that on average 35.37% of the mushrooms that would have been supplied for sale got spoiled whilst on the shelves after a range of 3 to 5 days. All respondents reported that their respective city councils collected them as part of all the other spoiled vegetables and discarded them off according to their respective regulations. It was also noted that these markets got their mushrooms supplies from local farmers. Respondents were also asked if they would appreciate getting supplies of regulated fresh and preserved indigenous mushrooms for sale and 85.7% gave positive responses (Yes) with the main reason being it would add on to variety of products to sale in their markets, whilst the remaining 14.3% were skeptical about selling them indicating factors such as mushroom phobia and limited supplies. Out of the 85.7% who responded positively towards selling indigenous mushrooms, some of them gave the opinion that both the fresh and indigenous mushrooms had to go through analysis of nutritional contents, microbial contents, sensory evaluation and toxicity tests as well as approval by a regulatory board for them to be fully accepted a safe for sale and consumption. All the respondents (100%) gave positive responses (Yes) upon being asked if they would also appreciate supplies of preserved oyster and button

mushrooms. An overall conclusion which was made out of the responses was that preservation of indigenous mushrooms is important so as to make them available for consumers on the shelves of formal markets.

3.4.3 Proximate nutritional analysis results

Results for fresh samples

As noted from Table 3.1, there was a significant difference ($p<0.05$) which was noticed in the proximate nutritional components of the different types of the fresh mushrooms, and this has also been reported by Redzic et al. (2010) who noted that out of the twenty five types of wild edible mushrooms they analyzed, all of them had different nutritional values. This is mainly due to the differences in the environments in which they would have sprouted which would have supplied them with different amounts of biochemicals, (Redzic et al., 2010). *C. Miombensis* samples had the highest amounts of protein, fibre, lipid and ash content followed by *A. Zambiana* and *Cantharellus spp* respectively. The highest moisture content of 90.87±0.02% was recorded in *A. Zambiana* followed by 89.23±0.03 in *C. Miombensis* and 88.67±0.02 in *Cantharellus spp.* samples respectively.

Results for the preserved mushroom samples

All the mushroom samples were first washed under running water and blanched before being preserved. The main reason for washing the mushrooms was to remove grit and dirt since they had been picked from forest environments, (Baum, 2020). Blanching is taken as an essential step before preserving mushrooms and the purposes of blanching is to inactivate the enzymes of the mushrooms thereby preventing reactions that could cause a deterioration of their overall quality, (Deylami et al., 2016; Draghici et al., 2023)

A comparison of the lipid content of fresh samples (shown in Table 3.1) to that of preserved samples after the first month (shown in Table 3.2) shows that the oven-dried samples, frozen samples and steeped samples had lipid contents which were significantly lower than those of fresh samples at a significance level of ($p<0.05$). One of the factors that contributed to these decreases is that the mushroom samples were blanched before being preserved and this process of blanching involves boiling at a higher temperature, in accordance to Zhang et al., (2018), this

high blanching temperature increases lipase activity thereby causing the breakdown of lipid components present in the mushroom samples

It was noted that at a significance level of $p<0.05$, the lipid content of pickled samples, shown in Table 3.2 was higher that of fresh mushrooms shown in Table 3.1. The fresh mushrooms had a lipid content ranging from 9.34±0.02%, 11.33±0.04%and 12.42±0.02% for *Cantharellus spp, A. zambiana* and *C. miombensis*, respectively whereas the pickled samples` lipid contents were 14.91±0.02%, 18.66±0.01 and 19.27±0.02 respectively after nine months. The same scenario for pickled samples was observed by Devi & Sunita, (2020) who observed an increase in lipid content in *Pleurotus Spp* after pickling. Lipid content increased due to the presence of the olive oil in the pickling sample which thereby increases the lipid content in the mushroom sample, (Devi & Sunita, 2020). Lowering of crude lipid content which was observed after the first month in the mushroom samples after drying was also reported by Reid et al. (2010) and Barros et al. (2007). Barros et al. (2007) explains that drying mushrooms results in lowering of their lipid content due to a rise in lipase activity and denaturation of the lipid components caused by the high preservation temperature.

As observed in Table 3.2, no significant changes at $p>0.05$ were noted in the lipid content of dried samples after nine months from the first month. After the first month the lipid content for *C. miombensis, Cantharellus spp* and *A. zambiana* samples were 6.84±0.01%, 4.21±0.02% and 5.68±0.02% respectively and after the ninth month they were 6.22±0.02%, 3.99±0.01% and 4.92±0.02% respectively. The reason why no changes occurred could be because no continuous degradation of the lipids occurred as the samples were stored under ambient temperatures.

Concerning Table 3.2, decreases in amounts of lipid contents, at $p<0.05$ after freezing was also noted from 11.95±0.02% , 9.23±0.01% and 10.89±0.03% to 5.39±0.02%, 8.95±0.04%and 10.11±0.02% for *Cantharellus spp, A. zambiana* and *C. miombensis* samples respectively. According to Fernandes, et al. (2013), who noted a decrease in the lipid content of *M. procera* mushroom samples over a period of twelve months, contents of monosaturated and saturated continue to decrease under low temperatures thereby causing a drop in the lipid content of the samples.

After the nine months of preservation the lipid content of mushrooms preserved through steeping in brine solution also had significantly decreased ($p<0.05$). In general, across the samples the highest level of decrease ($p<0.05$) was noted in samples of *Cantharellus spp* preserved in 20% brine solution which had a lipid quantity of 3.16±0.02% after nine months. This trend was reported from other researches which involved use of brine solution to preserve meat, fish and seafood, (Mariutti & Bragagnolo, 2017; Rahman et al., 2019). Sodium chloride causes lipid oxidation so that is why samples preserved in brine solution continuously lose their lipid content, (Mariutti & Bragagnolo, 2017). According to Mozuraityte et al. (2016), lipid oxidation is a process in which a reaction of free radicals involving fatty acids and oxygen occurs resulting in oxidative degradation of lipids which is also termed rancidity. Sodium chloride accelerates this reaction and it was also observed that samples which were preserved in 20% brine solution lost more lipid content compared to those which were preserved in 18% and 15% respectively.

It was observed that at a significance level of $p<0.05$ the protein quantities of the preserved samples (table 3c) were lower than those of the fresh samples, (Table 3.1). In comparison to the protein content of fresh *C. miombensis* samples, *Cantharellus spp* and *A. zambiana* of 15.22±0.03%, 12.17±0.05% and 14.27 ± 0.06% respectively, dried samples had decreased quantities from 9.97±0.01% to 7.44±0.01%, 6.21±0.01% to 3.41±0,04% and 8.78±0.01% to 5.39±0.03% respectively, pickled samples had decreased amounts from 12.54±0.01 to 10.87±0.03%,10.65±0.01% to 9.24±0.04% and 11.27±0.01% to 9.55±0.02% respectively, frozen samples had decreases of 12.42±0.01 to 9.48±0.01, 9.83±0.01 to 8.37±0.02 and 11.23±0.01 to 9.22±0.01 respectively. In overall for the samples preserved in brine solution, highest decreases at $p<0.05$ were observed in samples preserved in 20% brine solution likewise the protein contents in 15%, 18%, and 20% *C. miombensis* samples reduced from 12.84±0.03%,12.57±0.02% and 10.95±0.02% to 9.23± 0.02%, 7.63±0.03% and 6.67±0.02% respectively, for *Cantharellus spp* samples reductions noted were from 9.86±0.01%, 9.53±0.01% and 9.17±0.01% to 7.09±0.02, 6.2±0.02 and 5.68±0.01 respectively, lastly for *A. zambiana* contents reduced from 11.16±0.02 to 8.01±0.02%, 10.64±0.03% to 7.14±0.02% and 10.12± 0.01% to 6.03±0.02% respectively.

According Galoburda et al. (2015), who reported that the protein content of *C. cibarius* mushrooms decreased after blanching at temperatures of 70^0C, 80^0C, 90^0C and 100^0C, blanching

causes loss of proteins due to denaturation of the protein structures and diffusion of the soluble protein components into the blanching medium, and these reasons explain one of the causes of the reductions in protein contents which were noticed in this research.

Besides the blanching procedure, the preservation procedures themselves resulted in a decrease in the protein content. In overall, a significant decrease at $p<0.05$ in protein content of dried, steeped and pickled samples was noted, (Table 3.3). Tian et al. (2016) reported that the protein content of *L.edodes* mushroom samples decreased after oven drying due to the denaturation of the protein components caused by the high oven temperatures. The reason for the continued drop in protein content is explained by Marcal et al. (2021), who reported that over the continued preservation period of dried samples, protein components continue to degrade slowly which is why the protein content continues to reduce.

Jarwoska et al. (2011) reported that protein components of *P.ostreatus* mushroom samples which were frozen and analyzed over a twelve-month period decreased, which is the same as what was noticed in this research. According to Jarwoska et al. (2011), the decrease in protein content is mainly caused by Malliard reactions. Malliard reactions are non-enzymatic reactions which occur even at lower temperatures and thy result in degradation of amino acids thereby reducing the protein contents of the mushroom samples, (Jarwoska et al., 2011; Provost, 2019)

Decreases in protein contents of samples preserved in brine solution and those which were pickled were also observed in a research by Lungu, et al. (2015), who reported that *A. Bisporous* and *P. Ostreatus* mushroom samples which they preserved in brine solution had a significantly decreased content of proteins compared to the fresh samples. A greater percentage decrease of the protein content was recorded in samples which were preserved in brine solutions which contained a higher percentage of sodium chloride of 2.5%, (Lungu et al., 2015). The reason why the protein content of mushroom samples preserved in brine solution decreases is because some of the hydro soluble protein components continuously diffuse from the mushroom samples into the brine solution medium and higher brine concentration result in higher diffusion rates and this is also the same scenario which occurs in pickled samples, (Lungu et al., 2015). This is therefore the reason which explains the decrease in the protein content of the samples which were preserved in brine solution and those which were picked in this research.

An overall observation which was made from the protein content was that, pickled samples and frozen samples retained more proteins compared to samples steeped in brine solution and those which were dried at a statistic value of $p<0.05$.

The ash content of mushroom samples mainly consist of minerals and inorganic components (Sadli et al, 2021). During the blanching procedure, these minerals and ions diffuse into the blanching medium, (Jangchud et al., 2003; Ndangui et al., 2014). This is the main reason for the negative difference at $p<0.05$ which was observed between the fresh samples, (Table 3.1) and the preserved samples after one month after preservation (table 3d). Fresh samples of *C. miombensis, Cantharellus spp, and A. zambiana* had ash content of 12.74±0.03%, 9.22±0.01% and 12.33±0.03% respectively as noted from Table 3.1 whereas across all the preserved samples the ash content after one month ranged from 3.02±0.01% to 6.87±0.04±% a noted from table 3c. Galoburda & Clava, (2017) made similar observation in their research in which they noted that the ash content of *Chanterelle* mushroom samples decreased after blanching. Muyanja et al. (2014) also recorded that the ash content of *P. ostreatus* mushrooms decreased after blanching.

At a statistic level of $p>0.05$, there were no significant changes which were noticed in the ash content of frozen samples from the first month up to after the nine-month period likewise the mean ash content of *C. miombensis, Cantharellus spp* and *A. zambiana* after month 1 to after month nine ranged from 4.54±0.02% to 4.57±0.05, 3.79±0.00% to 3.81±0.04 and 4.38±0.01% to 4.33±0.05 respectively, (table 3.4). This observation tallies with that of Fernandes et al. (2013) who reported that the ash content of *M.porcera* mushroom samples did not significantly change over a preservation period of six months. Bernas & Jarworska, (2016) also reported that in their analysis of frozen *A. bisporous* at monthly intervals over twelve months, no changes in the amounts of ash content were noted. The main reason why the ash content does not change is not well documented in the scientific literature but Bernas & Jarworska, (2016) mention that lower temperatures do not cause any effect on neither the structure nor the content of minerals and ions of mushrooms.

As noted from Table 3.4, no significant changes were also observed in the ash content of dried samples after month one to after month nine, ($p>0.05$) likewise the ash content of *C. miombensis* samples ranged from 6.77±0.02% to 6.79±0.04%, *Cantharellus spp*`sash content ranged from 3.20±0.02% to 3.22±0.03%and ash content of *A. zambiana* samples ranged from 4.07±0.02% to

4.05±0.04% as shown in table 3d. According to Bernas & Jarworska (2016), ambient temperatures have no significant effect on the mineral contents of the mushrooms. Even though no significant were noted in both frozen and dried samples, differences in ash contents of these samples were noted which shows that the methods had an initial impact on the mineral contents of the mushroom samples.

At $p<0.05$, it was noted that the ash content of the pickled mushrooms had significantly increased after the nine months as noted from table 3d. Likewise the ash content of *C. miombensis, Cantharellus spp* and *A. zambiana* increased from 5.82±0.02%, 3.02± 0.01% and 4.87± 0.02% respectively to 6.72± 0.01%, 4.74± 0.02% and 5.78± 0.01% respectively. This observation resonates with that of Tanimola et al. (2022) who reported an increase in the ash content of *A. bisporous* and *P. ostreatus* mushroom samples which were pickled in vinegar and spices. This increase in the ash content is mainly due to diffusion of minerals from the pickling salts and spices into the mushroom samples, Tanimola et al. (2022).

Ash content of samples which were preserved in brine solution also increased over the preservation period, ($p<0.05$), (Table 3.4). Ash contents of *C. miombensis* samples preserved in 15%, 18% and 20% brine solution increased from, 4.18± 0.01%, 5.53±0.02% and 6.43±0.04% to 5.22±0.01%, 6.28±0.01% and 7.12±0.02% respectively. Ash content of *Cantharellusspp* samples preserved in 15%, 18% and 20% increased from 3.23±0.02% to 4.86±0.05, 4.18±0.01% to 5.94±0.03% and 5.98±0.02% to 6.02± 0.02% respectively. For *A. zambiana* samples, it was observed that samples preserved in 15%, 18% and 20% brine solution had an increase in ash content from 4.65±0.02, 5.77±0.05 and 6.19±0.03 to 5.90±0.02, 6.91±0.06 and 8.57±0.02% respectively. It was also noted that *A. zambiana* samples preserved in 20% brine solution and this might have been attributed by the fact that *A. zambiana* generally has soft tissue making it more permeable to the minerals.

More increase, at a significance level of $p<0.05$ was recorded for samples which were preserved in 20% brine solution followed by those which were preserved in 18% and lastly those which were preserved in 15% brine solution. This trend was also reported by Pogon et al. (2016) who reported that *B. edulis* and *S. luteus* mushroom samples which were preserved in brine solution for 12 months had increased levels of ash content. They recorded that *B. edulis* preserved in 10% and 30% brine solution had 0.8±0.02 % and 0.9±0.02 respectively compared to the 0.7±0.03%

for the fresh samples, *S. luteus* ash contents were 1±0.11% in mild brine and 1.1±0.1% in strong brine compared to fresh samples which had 0.9±0.12. According to Pogon et al. (2016), this increase is a result of diffusion of mineral salts from the brine solution into the mushroom samples.

The crude fibre content of mushrooms is mainly made up of partially digestible polysaccharides as well as chitin, (Chambaghat, 2017). There was a significant increase at $p<0.05$, in the crude fibre content of the dried mushrooms, (table 3e) after month one compared to the fresh samples, (table 3a). It was noted that fresh samples of *C. miombensis*, *Cantharellus spp* and *A. zambiana* had crude fibre contents of 6.76±0.02%, 4.23±0.03% and 5.86±0.03% respectively, (table 3a) whilst the dried samples of *C. miombensis*, *Cantharellus spp* and *A. zambiana* after 1month had crude fibre contents of 7.05±0.04%, 5.76±0.02% and 6.62±0.01% respectively, (table 3.5). According to Agomuo et al., (2022), due to evaporation of moisture content during drying, the density of the soluble fibre which was present in the moisture of the sample increases as it becomes concentrated. The combination of this concentrated soluble fibre percentage and the insoluble fibre percentage content results in a higher content of the total crude fibre after drying (Agomuo et al., 2022). Results similar to these ones were recorded in a research by Aishah & Rosli (2013) who recorded that the crude fibre content of oven-dried samples of *P. sajor-caju*samples was found to be higher in oven dried samples (37.5±0.04%) and low in fresh samples (33±1.53%).

After the nine months, it was noted that the crude fibre content of dried samples had not significantly changed ($p>0.05$). After month 1 the crude fibre contents of *C. miombensis*, *A. zambiana* and *Cantharellus spp* samples were 7.05±0.04%, 6.92±0.06 and 5.76±0.01% respectively whilst after month 9 they were 7.06±0.04, 7.04±0.03 and 5.73±0.03% respectively.

The crude fibre content of the frozen samples did not significantly change, ($p<0.05$) over the nine months of analysis as shown in Table 3.5. After month 1 the samples of *C. Miombensis*, *Cantharellus spp* and *A. zambiana* had crude fibre contents of 5.21±0.03%, 3.69±0.02% and 4.82±0.02 respectively and after nine months` records of 5.24±0.03%, 3.68±0.02% and 4.80±0.06 were noted. These results resonate with that from research by Bernas & Jarwoska, (2016), who reported that analysis of the nutrient content of frozen *A. Bisporous* mushrooms at 0, 6 and 12-month intervals showed no significant change in the amounts of crude fibre. The reason

for the non-changes that were observed is given by Olsen, (2020) who explains that the low freezing temperatures do not cause any changes in neither the structures of the contents of polysaccharide and chitin and that is why the crude fibre content of frozen mushrooms samples does not change.

The crude fibre content of samples which were preserved in brine solution had significantly decreased after the nine months, ($p<0.05$) with higher decrease at $p<0.05$ being noted in the samples which were preserved in 15% followed by those which were preserved in 18% and lastly the ones which were preserved in 20% because generally diffusion occurs more in less concentrated solutions, (Table 3.5). Crude fibre content of *C. Miombensis* samples preserved in 15%, 18% and 20% brine solutionsignificantly decreased ($p<0.05$) from 4.04±0.01%, 5.56±0.02% and 5.72±0.01% respectively to 3.03±0.02%, 4.37±0.02% and 5.07±0.02% respectively. Crude fibre content of *Cantharellus spp* samples preserved in 15%, 18% and 20% brine solution significantly decreased ($p<0.05$) from 3.18±0.02%, 3.63±0.02% and 3.74±0.02% respectively to 2.44±0.04%, 3.09±0.02% and 3.59±0.02% respectively. Crude fibre content of *A. zambiana* samples preserved in 15%, 18% and 20% brine solution significantly decreased, ($p<0.05$) from 4.26±0.01%, 5.84±0.02% and 6.69±0.02% to 3.04±0.07%, 4.15±0.03% and 5.57±0.02% respectively.

The same trend was noted in samples which were preserved through pickling in vinegar and spices as noted from Table 3.5. Crude fibre of pickled *C. Miombensis, Cantharellus spp,* and *A. zambiana* samples significantly decreased, ($p<0.05$) from 5.02±0.01%, 4.21±0.02 and 4.94±0.02 respectively to 3.82±0.01%, 2.87±0.01% and 3.64±0.02% respectively. These results are similar to the ones which were reported by Tanimola et al. (2021) after a research in which they preserved *Pleurotus spp* mushrooms in vinegar and spices as well as in brine solution. The crude fibre content of the pickled and steeped mushroom samples decreased after processing. According to Sun (2016) decrease in crude protein of food or vegetable samples that would have been preserved through use of methods such as pickling or brining is mainly caused by the leaching of the soluble fibre into the liquid medium thereby decreasing the total percentage of the total crude fibre of the sample. This was therefore noted as the main reason why the crude fibre percentage of the mushroom samples decreased.

In proximate analysis, moisture content mainly refers to the amount or percentage of water available in a food sample. The first procedure of blanching in 2% brine solution which was done in this research before processing the mushrooms has an impact of reducing the moisture content of the mushrooms due to the diffusion of moisture from the samples into the brine solution, (Elbah et al., 2017). This fact therefore contributes to the significant drop in moisture content, ($p<0.05$) that was observed after the first month of moisture analysis across all the preserved samples (table 3.6). Fresh samples of *C. miombensis, Cantharellus spp,* and *A. zambiana* had moisture contents of 89.23±0.03%, 88.67±0.02% and 90.87±0.02 as shown in Table 3.6 respectively whereas across all the preservation procedures the moisture contents after month1 ranged from 3.44±0.01% to 21.55±0.02%.

At $p>0.05$, no significant changes in moisture were noted for dried samples, (table 3.6). The moisture contents of *C. miombensis, Cantharellus spp,* and *A. zambiana* samples after month one were 4.21±0.01%, 3.44±0.01% and 5.61±0.01% respectively and after the nine months they were 4.23±0.03%, 3.40±0.01% and 5.62±0.01% respectively, (Table 3.6). This observation is in contrast with that of Bhatti et al. (2018) who reported that the moisture content of *P. ostreatus*, decreased from 6.4% to 5.1% in storage at ambient temperatures, however in this research the samples were stored in open trays. The reason why the moisture content in this research did not change over the nine months could be because maximum moisture content had been evaporated from the samples and also because the samples were stored in plastic packaging inhibiting continuous loss of moisture from the samples.

It was noted that at $p<0.05$, minimal losses of moisture content were observed on the frozen samples. Moisture content of *C. miombensis, Cantharellus spp,* and *A. zambiana* samples after month one were 16.21±0.01%, 14.31±0.01% and 21.37±0.02% respectively and after month nine they were 15.98±0.02%, 14.21±0.02 and 21.23±0.02 respectively. This observation is explained by Schafer & Driessen (2021) who reported that during freezing, there is minimal moisture loss which occurs due to evaporation of water from the surfaces of the frozen food sample. In scenarios where a food sample is frozen whilst it will be unpackaged, more moisture content is lost, (Briley, 2002).

A significant decrease in the amount of moisture in samples pickled in vinegar and spices was noted after the nine months of preservation at $p<0.05$, (Table 3.6). Moisture content of *C.*

miombensis, Cantharellusspp, and *A. zambiana* samples after month one were 17.36±0.01%, 13.34±0.02% and 21.55±0.02 and after month nine they were 16.13±0.02%, 11.44±0.02% and 18.94±0.02 respectively, (table 3f). This same trend was observed after researches on pickling other food samples which include fish, cucumbers and cabbage (Koyano et al., 2021; Rahman et al., 2019). The cause for this result is explained by to Sawada et al, (2021) who postulated that salts, vinegar and spices cause a dehydration effect the pickled food sample.

From Table 3.6 it can be noticed that decreases in moisture contents of samples which were preserved in brine solution were also noted over the nine months at a significance level of $p<0.05$. Moisture content of *C. miombensis* samples preserved in 15%, 18% and 20% brine solution decreased from 16.72±0.01% to 13.73±0.02%, 15.56±0.02% to 13.37±0.02% and 14.04±0.04% to 12.13±0.06% respectively. The moisture content of *Cantharellus spp* samples preserved in 15%, 18% and 20% brine solution decreased from 13.84±0.02% to 11.92±0.02%, 13.63±0.07% to 11.44±0.02 and 12.98±0.06 to 11.23±0.04 respectively. The moisture content of *A. zambiana* preserved in 15%, 18% and 20% brine solution decreased from 24.69±0.02% to 22.57±0.02%, 22.84±0.02% to 20.15±0.03% and 19.26±0.01% to 18.04±0.02% respectively. A similar observation was recorded by Nketia et al. (2020) during their research on the effects of storing oyster mushrooms in brine solution. The reason behind loss of moisture content in samples preserved in brine solution is that diffusion of water from the mushroom samples into the concentrated brine solution occurs thereby resulting in reduction of the moisture content of the samples.

3.5 Conclusion

In overall, it is necessary to preserve indigenous mushrooms so as to improve their time of availability. If these indigenous mushrooms are properly preserved and regulated as safe to consume, the formal markets can take them onto their shelves thereby making them available to consumers for periods.

The results of the research showed that preservation methods had different impacts on indigenous mushroom. All the preserved mushrooms retained different amounts of nutrients. It was observed that blanching possibly caused significant changes on amounts of the nutrients at a statistic value of $p<0.05$. It can be noted that across all the mushroom types and preservation methods, the nutrient contents of fresh samples were significantly different from the ones of

preserved samples. In overall it was observed that after the nine months of preservation high lipid contents were noted in pickled samples, frozen samples, dried samples and lastly in steeped samples respectively. At a significance level of $p<0.05$, it was observed that high protein contents were noted in pickled samples, frozen samples, steeped samples and lastly dried samples, high ash contents were observed in pickled samples followed by steeped samples, dried samples and lastly frozen samples respectively, high fiber contents were noted in dried samples followed by frozen samples, steeped samples and lastly in pickled samples. It was also noted that across all the preserved samples, at a statistic level of $p<0.05$, *A. zambiana* had more moisture content compared to the other two mushrooms. It was also generally noticed that in terms of retaining more nutrients, *C. miombensis* samples had more nutrients followed by *A. zambiana* and lastly *Cantharellus spp.* According to Reid et al. (2016), proper preservation of food is essential to make it available in all seasons, however the preservation methods need to be appropriate to retain nutritional content of the food.

CHAPTER 4

Impact of preservation on microbial and sensorial quality of Indigenous mushrooms and downstream processing of spoiled mushrooms to produce biodegradable plastic

Abstract

Mushrooms have been known as beneficial food source since a long time back. There are types of mushrooms which are cultivated and some which are found naturally in the wild which are commonly termed indigenous mushrooms. Generally the indigenous mushrooms which are found in Zimbabwe are being underutilized since they are only found during the rainy season. They cannot be stored for a long time in their fresh state because they are highly perishable and more prone to microbial attack. Preservation methods are therefore necessary to extend their shelf lives. In this study *C. miombensis*, *A. zambiana* and *Cantharellus spp*. mushrooms were

preserved through drying, pickling vinegar and spices, steeping in 15%, 18% and 20% brine solutions as well as freezing. One of the goals of this study was to evaluate the impact of the preservation methods on the microbiological and sensorial properties of the mushrooms. On the other hand, some cultivated mushrooms that are meant for sale in their fresh state get spoiled before they are sold out to consumers. This study was also aimed at processing these spoiled mushrooms to make bio degradable plastic. Microbial evaluation was done through the use of the plate count method over a period of nine months and sensorial evaluations were carried out using a nine-point hedonic scale. Microbiological analysis results showed that the initial range of the microbial counts of the fresh samples was 4.95±0.12 log cfu/ml to 4.98±0.05 log cfu/ml respectively whereas the preserved samples had microbial loads which ranged from 4.42±0.03 log cfu/ml to 4.95±0.11 log cfu/ml. Hedonic scales were used to access the aroma, colour, texture overall acceptability of the preserved indigenous mushrooms and in general pickled mushrooms had higher sensorial scores ranging from 7.9 to 8.5.Spoiled *Pleurotus ostreatus* mushroom was used to produce a bioplastic and it was noted that it`s weight decreased by an average of 5.5±0.23% and 2.2±0.02% under soil and laboratory degradation respectively, showing that the plastic was biologically degradable. This study provided insight into how preservation methods affect the sensorial quality and microbiological safety of the indigenous mushroom, giving findings which could be used as references in future selection of ways to preserve indigenous mushrooms. On the other hand it gave an insight on a route that could be taken to utilize spoiled mushrooms.

4.1 Introduction

4.1.1 Impact of preservation on microbial and sensorial quality of mushrooms

Mushrooms are fungal resources that have been exploited for food and medicine for a long time both in urban and rural areas (Okhuoya et al., 2010; Osarenkhoe et al., 2014). Edible mushrooms include many fungal species that are either harvested in the wild especially during the rainy season or cultivated in house (Cheung, 2013; Ching et al., 2011). In Zimbabwe, cultivated mushrooms are available all year round whereas wild indigenous mushrooms are available during the rainy season (Reid et al, 2016; Mutema et al., 2019). Both these cultivated and wild

mushrooms generally have high moisture contents and they lack an outer protective layer so this makes them more susceptible to pathogenic microorganisms which include bacteria, yeasts, and molds (Wakchaure, 2011; Jiang et al., 2010, Morebu, 2016).

Molds that cause spoilage of fresh mushrooms through producing mycotoxins which cause discoloration, sliminess and decay include *Aspergillus spp, Penicillium spp and Tichoderma spp* (Pitt & Hocking, 2009; Tsai et al., 2008). Bacteria that cause spoilage of mushrooms include *Pseudomonas spp* which cause sliminess of the tissues of the mushrooms, *Bacillus spp* which usually produces spores which are not affected by heat thereby causing spoilage of preserved mushrooms and *Enterobacter spp* which also cause sliminess, bad odor and loss of colour of the mushrooms, (Beuchat, 1998). Fresh mushrooms are also prone to spoilage by fungi that include *Verticillium spp* which causes rotting of the mushrooms and *Mycogone spp* which cause discoloration of mushrooms, (Fletcher & Gaze, 2008).

Due to quick spoilage, fresh mushrooms generally have a short shelf life which ranges from 1 to 3 days under ambient temperatures (Jiang et al., 2010). From this point one can notice that the preservation of mushrooms is important especially for indigenous mushrooms which get spoiled quickly and also become unavailable after the rainy season and will not last long in their fresh state,(Ruan-Soto et al., 2017; Tamimola et al.,2022; Rai et al., 2008; Sethi et al., 2000). Preservation methods that have been used on mushrooms in various parts of Africa as well as around the world include different dying methods such as freeze drying, microwave drying, sun drying and solar drying as well as other methods such as canning, pickling, steeping in brine solution, freezing, coating and use of chemicals, (Wabali & Simeon, 2013; Argyropoulus et al., 2011; Marcal et al., 2021; Ruan-Soto et al., 2017).

These methods have different effects on the microbiological composition of the mushrooms (Kamal et al., 2010, Royse & Sanchez-Vazquez, 2003; Elbah et al., 2017). Generally a noticed trend from other researches involving preservation of mushrooms showed that these methods inhibit or slow down continuous growth of the microbes on mushroom samples (Shonte et al., 2024; Jaworska et al., 2020; Borges et al., 2023). They also enhance the microbial safety of mushrooms thereby increasing their shelf lives to periods longer than the few days they last in their fresh state, (Royse & Sanchez-Vazquez, 2003).

The sensorial attributes of different mushrooms are also affected by preservation methods differently depending on the type of preservation method used and type of mushroom involved, (Fernández-Gutiérrez et al., 2020; Guan et al., 2017). According to Rapetti et al. (2019) preservation methods such as canning, pickling, drying and freezing cause changes in the aroma, flavour and texture of mushrooms likewise freezing and drying increase firmness of mushrooms whilst pickling and canning introduce new sensorial qualities because of the solutions used for these processes.

In overall, an understanding of how preservation methods affect different types of mushrooms in terms of sensorial quality is of importance so as to have an idea of methods that maintain the best sensorial qualities for each mushroom type. It is also of importance to have knowledge on the types of preservation methods which enhance the microbial safety of the different mushrooms for a longer period as well since different preservation methods have different impacts on the microbial quality of the mushrooms. It was therefore one of the aims of this research to evaluate the impact of preservation on the microbial and sensorial quality of indigenous mushrooms. The preservation methods which were used in this research included pickling in vinegar and spices, steeping in brine solution, oven drying and freezing. The mushrooms which were preserved were *C. miombensis*, *Catharellus spp* and *A. Zambiana*.

4.1.2 Downstream processing of spoiled mushrooms to produce biodegradable plastic

According to an assessment which was done by Mutema et al. (2018) on the profitability of oyster mushroom in Harare urban and peri urban areas, 6.6% of the respondents mentioned that they faced losses due to microbial spoilage of their harvests and 40% of the respondents also mentioned losses due to contamination by insects. In a preliminary market research that was done by this author, it was observed that out of the various branches of modern markets that were surveyed, a total of 35.37% of mushrooms meant for sale in these stores ended up spoiling before being bought and these mushrooms are collected and discarded off as biological refuse by city councils.

On the other hand, cultivation of mushrooms in Zimbabwe is continuously increasing it is anticipated that by 2026, mushroom production in Zimbabwe would have reached 951 metric tonnes per year. Considering the fact that because market losses are being noticed already, it would be ideal to have processes that can take in the usage of these spoiled mushrooms. A

possible way to make use of these spoiled mushrooms could be processing them to produce biodegradable plastic using starch harnessed from them.

Starch from sources like cassava and bananas has also been used to make biodegradable plastic (Mohapatra et al., 2010; Gustafsan et al., 2015). The use of synthetic plastics has been raising concerns since they are non-degradable and this was seen through efforts by the Environmental Management Agency of Zimbabwe to ban the use of synthetic plastics in the country (Gogo, 2018; Ngaza et al., 2018). It is therefore essential to provide possible sources of plastics that are biodegradable. Spoiled mushrooms can be possibly used as sources of supplementary starch to add on the already available biological sources of starch which can be potentially used to produce biodegradable plastic. It was therefore another objective of this research to produce biodegradable plastic from spoiled mushrooms.

The two objectives of this research will try to address the issue of underutilization of indigenous mushrooms due to microbial spoilage as well as to provide alternatives of profitably using cultivated mushrooms which inevitably get spoiled whilst on the shelves.

4.2 MATERIALS AND METHODOLOGY

4.2.1 Sample Collection

Mushroom samples which included *A. zambiana*, *Cantharellus spp*. and *C.miombensis* were bought from the Chinhoyi Market. Identification of the samples of the mushrooms was archived by comparing the photographical, morphological and anatomical characteristics with the descriptions given in the manuals on identifications of Southern African mushrooms, (Sharp, 2011; Ryvarden et al., 1994).

4.2.1 Preservation procedures

i. Preliminary processes

Washing under running water to remove grit was done before the implication of all the preservation processes. All samples were blanched in 2% brine solution at 95^0C for five minutes firstly before being preserved, (Rai et al., 2008).

ii. Oven drying

Oven drying was achieved through a method by Cancler, (2012). The oven will was preheated to 60^0C. The mushrooms were dried for 3 hours and cooled at ambient temperatures. Dried mushrooms were stored in plastic packaging for analysis.

iii. Steeping in brine solution

Steeping was done through following a method by Wabali & Simeon, (2013). Steeping solutions were prepared and samples of mushrooms weighing 100 grams were transferred into jars containing 100mls of 15%, 18%, and 20% sodium chloride solutions. Samples were stored for analysis.

iv. Pickling in vinegar and spices

An adjustment of a method by Rai et al. (2012) was used. The indigenous mushrooms were blanched in water which was boiling at a temperature of 95^0C containing 2% sodium chloride and 0.1% citric acid for five minutes. The mushrooms were drained and added into another saucepan which contained 500mls of distilled water mixed with 250 ml olive oil, 250 ml white vinegar, 5 grams of black peppercorns, 10g chili powder, 20g of turmeric powder and 5g of pickling salt. Glass jars were sterilized then equal amounts of the hot mushroom (100g mixed with 100mls of the pickling ingredients) mixtures were added up to a 20 millimeter headspace of the jars. The mixtures were evenly distributed in the jars. The rims of the jars were cleaned with vinegar soaked towels. The jars of the pickled marinated mushrooms were then processed in boiling water for twenty minutes and stored for analysis.

v. Freezing

Mushrooms were blanched in distilled water which was boiling at a temperature of 95^0C, cooled at a room temperature of 25^0C for 45 minutes. Blanched samples weighing 100g were then packed into packaging plastics and were then transferred into a Capri upright laboratory refrigerator which was set at 22^0C and they were stored for analysis.

4.2.2 Microbiological analysis of the preserved indigenous mushrooms

Microbiological analysis was done according to the total plate count in accordance to (Nwachukwo & Ezeigbo, 2013). Analysis of each sample was done in triplicate. Nutrient agar

was prepared through weighing 38g of the media and dissolving it in distilled water. The media was sterilized in an autoclave at 121^0C for 20 minutes. After sterilization the media was left to cool off and then poured into petri dishes. Mushrooms samples were homogenized in a tris buffer using a motor and pestle and serial dilutions of the samples were carried out up to 10^4 using distilled water. 0.1 ml of each sample was added into a petri dish containing the nutrient agar using a pipette. The plates were covered and incubated for 48 hours at 37^0C. Total microbial count was done and the cfu/ml using the equation

Cfu/ml = (Number of colonies × Dilution factor)/ volume plated

Whereby

- Number of colonies- number of colonies observed and counted on the plate

-Dilution factor-the overall dilution factor referring the productof total dilution factors for every step

-Volume plated- total volume of the diluted sample plated on the petri dish

4.2.3 Sensory evaluation of the preserved mushrooms

Sensory evaluation was on the basis of appearance, aroma, texture and overall acceptability using a 9 point hedonic scale. The sensory evaluations were carried out by ten voluntary panelists per each time of analysis. The voluntary panelists were Chinhoyi University of Technology students. For each assessment five males and five females participated and their age ranges were from 23 to 25 years. All the preserved mushrooms were cooked using the stir frying method for 5 minutes in which 5mls of olive oil was heated in a pan followed with addition of 50g of each sample for cooking, (Wabali & Simeon, 2013). A small sized warm portion of 3 table spoons was presented to each participant for scoring. The assessment was done at ambient temperature and distractions were controlled through giving each participant their own bench to carry out their testing on. The voluntary panelists scored the samples according to how they felt about the colour, aroma, texture and overall acceptability.

Ranking for Colour, Texture, Smell, and Overall Acceptability, (Wabali& Simeon, 2013).
Colour 1-9: 0-2 Very Bad, 2-4 Bad, 5-7 Good, 8-9 Very Good

Aroma: 0-2 Very Bad, 2-4 Bad, 5-7 Good, 8-9 Very Good

Texture: 0-2 Slimy, 2-4 Soft, 5-7 Firm, 8-9 Very Firm

4.3 Processing of the spoiled mushrooms

4.3.1 Extraction of starch

Oyster mushrooms were left to spoil at room temperature (3000g), and the spoiled mushrooms were sliced into smaller pieces of 5cm width using a sterile blade so as to prepare them for easy homogenization. They were homogenized through bending in distilled water at high speed for five minutes. The homogenized mixture from the blender was then filtered through a 1mm sieve and then through a 0.25mm sieve. The filtrate was left for 72 hours at 4˚C and the starch settled in the bottom. The liquid part was discarded cautiously. The sediment starch was washed thrice with sodium hydroxide aqueous solution (0.2% w/w), thrice with deionized water and subsequently twice with anhydrous ethanol by centrifuged at 3000 RPM for 15 minute. The starch obtained from the washing was then spread on aluminum foil paper and put to an oven for drying at 60 ˚C for 3 hours. Dried starch obtained from the previous process was then crushed manually using a mortar and pestle to produce fine starch. The fine starch was then stored in airtight containers, awaiting next processes, (Demiate et al., 2022).

4.3.2 Starch Yield calculation

The yield was calculated as a percentage. The total weight of the obtained dried starch was calculated as a fraction of the total spoiled mushroom weight used, and expressed as a percentage, (Demiate et al., 2022).

4.3.3 Biodegradable Plastic Preparation

Production of the bioplastic from the extracted starch was carried out by following the procedure of Yaradoddi et al. (2016). 20ml of distilled water was mixed with 50g of the spoiled mushroom starch and heated on a heater plate with a magnetic stirrer for 5 minutes. 18ml of 0.1M HCl was added into the mixture followed by 18ml of 0.1M NaOH for neutralization. 20ml of 1% glycerol was also added and the mixture was heated at 90˚C for 30mins using a stirrer for even distribution of heat. After heating, the mixture was left on the bench top to gradually harden to

form a gel and the magnetic stick was removed from the system. It took about 1 hour 5 minutes for the mixture to form an opaque gel. The gel was poured on petri-dish molds of varying thicknesses, the thinnest being 2 mm. The samples were left to dry for 5 days at a room temperature of 25^0C to 28^0.

4.3.4 Characterization of the Biodegradable Plastic

i. Laboratory biodegradability test

The Bushnell and Haas agar (basal mineral salt medium) was used for testing the ability of microorganisms in degrading plastics in accordance to Arvind et al. (2023).The biodegradability tests were carried out using flasks containing 100 ml of the prepared basal mineral salt medium, 600 µl of *E. Coli* and *Pseudomonas spp* inoculum separately and strips of the bioplastic of known initial weight, which had been washed with 70% ethanol thoroughly, then rinsed with distilled water aseptically. For each bacterial strain 4 flasks containing strips weighing 0.1g, 0.2g, 0.3g and 0.4g of the strips were used. The flasks were incubated on a rotary shaker (120 rpm) at a temperature of 25˚C. Weight loss measurements were carried out aseptically after 5, 10, 15, 20, 25 and 30 days after incubation.

ii. Calculation of weight loss of the bioplastics after laboratory degradation tests

Weight loss measurements were carried out in accordance to Arvind et al. (2023). The flasks containing the media and plastic samples were washed thoroughly with ethanol. The strips were left to dry on the bench top for 20 hours and the percentage weight loss was determined using the following formula:

$$\text{Weight loss (\%)} = [(\text{Initial weight} - \text{final weight})/ (\text{initial weight})] \times 100$$

iii. Soil burial degradation test

The biodegradability of the bioplastic samples was also tested outside the laboratory using soil burial degradation test as per the method by Karisajan &Ngouajiao, (2012). Bioplastic samples of weights 0.2g and 0.4g were buried in the soil, so that they would be degraded. The degradation could be seen from the mass reduction of respective specimens buried in the ground. The bioplastic samples were buried in the ground at 8-cm depth. They were observed at varied durations (3, 6, 9, and 12 days). Prior to burial, the initial weight (weight before degradation)

was determined. The weight after degradation (final weight) of the each bioplastic sample was measured. Values were expressed as weight loss as follows;

Weight loss (%) = [(Initial weight−final weight)/ (initial weight)] ×100

4.3 Data analysis

Data for microbiological analysis was expressed as means of 3 replicates ± the standard deviation. Differences between means were tested for using a t test ($p<0.05$). Statistical analysis of the data was also done using the Analysis of Variance procedure of Microsoft excel statistical tool at $p<0.05$. Data for the experiments of the biodegradable plastic was also analyzed using Microsoft Analysis of Variance procedure of Microsoft excel.

4.4 Results

4.4.1 Results of fresh mushroom samples

Table 4. 1showing plate counts of fresh samples in cfu/ml

Mushroom Type	Plate Count log cfu/ml
CM	4.95 ± 0.12^{a}
CS	4.81 ± 0.09^{b}
AM	4.98 ± 0.06^{c}

CM- C. miombensis, CS- Cantharellusspp., AZ-A. zambiana, (n=3), results shown as mean ± standard deviation, mean values with different superscripts down the column are statistically different at $p<0.05$

As shown in table 4.1, the fresh mushroom samples had high microbial counts which ranged from 4.81 to 4.95 log cfu/ml.

Table 4. 2showing plate counts of frozen samples in log cfu/ml

Mushroom	**Number of Months**					
Type	**One**	**Two**	**Three**	**Five**	**Seven**	**Nine**
CM	4.77±0.08a	4.76±0.04a	4.77±0.06a	4.76±0.02a	4.77±0.06a	4.77±0.08a
CS	4.56±0.07a	4.56±0.04a	4.57±0.05a	4.58±0.07a	4.57±0.08a	4.56±0.03a

AM	4.79±0.11a	4.79±0.09a	4.78±0.10a	4.79±0.07a	4.79±0.05a	4.81±0.03a

CM- C miombensis, CS- Cantharellus spp, AZ- A. zambiana, (n=3), results shown as mean ± standard deviation, mean values with same superscripts across the rows are not statistically different $p<0.05$

Table 4.2 shows the cfu/ml counts of frozen samples. It can be noted that no significant changes in the cfu/ml counts of these samples were noted over the nine month period of preservation.

Table 4. 3showing plate counts of dried samples log cfu/ml

Mushroom	**Number of Months**					
Type	**One**	**Two**	**Three**	**Five**	**Seven**	**Nine**
CM	4.50±0.04a	4.49±0.03a	4.50±0.05a	4.48±0.01b	4.47±0.04b	4.47±0.03b
CS	4.42±0.07a	4.42±0.04a	4.43±0.06a	4.42±0.05a	4.43±0.04a	4.41±0.04a
AM	4.62±0.03a	4.62±0.04a	4.62±0.02a	4.61±0.04a	4.62±0.03a	4.61±0.01a

CM- C. miombensis, CS- Cantharellusspp, AZ-A. zambiana, (n=3), results shown as mean ± standard deviation, mean values with same superscripts across the rows are not statistically different $p<0.05$

Table 4.3 shows the cfu/ml counts of dried samples. It can be noted that slight significant changes in the cfu/ml counts of these samples were noted over the nine month period of preservation.

Table 4. 4 showing plate counts of pickled samples in log cfu/ml

Mushroom	**Number of Months**					
Type	**One**	**Two**	**Three**	**Five**	**Seven**	**Nine**
CM	4.79±0.04a	4.71±0.03b	4.71±0.05a	4.71±0.01a	4.69±0.04b	4.69±0.03b
CS	4.67±0.05a	4.67±0.04a	4.66±0.06a	4.65±0.05a	4.51±0.03a	4.49±0.04a
AM	4.83±0.04a	4.81±0.04a	4.81±0.02a	4.80±0.04a	4.80±0.03a	4.78±0.01a

CM- C. miombensis, CS- Cantharellusspp, AZ- A. zambiana, (n=3), results shown as mean ± standard deviation, mean values with different superscripts across the rows are statistically different $p<0.05$

Table 4.4 shows the cfu/ml counts of pickled samples. It can be noted that significant decreases in the cfu/ml counts of these samples were noted over the nine month period of preservation.

Table 4. 5 showing plate counts of steeped samples in log cfu/ml

Mushroom Type	Number of Months					
	One	Two	Three	Five	Seven	Nine
CM 15%	4.71±0.03a	4.71±0.04a	4.70±0.04a	4.70±0.03a	4.70±0.06a	4.70±0.04a
18%	4.68±0.02a	4.68±0.05a	4.67±0.05a	4.68±0.03a	4.69±0.04a	4.69±0.02a
20%	4.57±0.04a	4.57±0.04a	4.65±0.04a	4.56±0.04a	4.56±0.04a	4.55±0.04a
CS 15%	4.67±0.05a	4.66±0.04a	4.67±0.06a	4.74±0.05b	4.82±0.03c	4.82±0.04d
18%	4.66±0.05a	4.63±0.04a	4.65±0.09a	4.74±0.08b	4.76±0.10b	4.76±0.07b
20%	4.64±0.04a	4.51±0.05a	4.68±0.05a	4.50±0.04b	4.49±0.06b	4.49±0.04b
AM15%	4.79±0.06a	4.79±0.11a	4.67±0.03b	4.92±0.05c	4.93±0.02c	4.95±0.02c
18%	4.77±0.09a	4.78±0.11a	4.86±0.03b	4.86±0.05b	4.86±0.13b	4.90±0.03c
20%	4.61±0.02a	4.62±0.04a	4.72±0.03b	4.72±0.05b	4.83±0.02b	4.84±0.07b

CM- C. miombensis, CS- Cantharellus spp, AZ- A. zambiana, (n=3), results shown as mean ± standard deviation, mean values with different superscripts across the rows are statistically different $p<0.05$

Table 4.5 shows the cfu/ml counts of samples preserved in brine solution. It can be noted that significant increases in the cfu/ml counts of the *A.zambiana* samples were noted over the nine month period of preservation whereas no significant changes incfu/ml counts of the most of the other samples were noted.

Discussion

Nutrient agar was used to access the changes in the microbial contents because it is a non-selective type of media which supports a wide variety of microorganisms and in this research selection of the different types of the microbes was not to be done, (Downes & Ito, 2001). This microbial count was mainly done to note if the preservation methods had any impact on any microbial counts so thereby giving more room for research on this area afterwards. Before all preservation procedures were done, the samples were blanched. According to Karim et al. (2023), the high temperatures used in blanching reduce microbial load. A significant decrease($p<0.05$) in the microbial loads of the dried samples was observed after month one of preservation for all the treatments shown in tables 4.2 to 4.5.Therefore the blanching procedure

could have contributed to the significant decreases in the microbial loads ($p<0.05$) which were noticed after month one of preservation. The fresh samples of *C. miombensis, Cantharellusspp* and *A. zambiana* had 4.95±0.12 log cfu/ml, 4.81±0.09 log cfu/ml and 4.98 log cfu/ml respectively whereas the preserved samples had microbial loads which ranged from 4.42 log cfu/ml to 4.95 log cfu/ml, (Tables 4.1, 4.2, 4.3, 4.4 and 4.5). The high microbial contents of fresh indigenous mushrooms are mainly attributed by the fact that they sprout in natural environments which generally have a lot of microflora and microbial composition (Stamets, 2005). Wild environments will also not be sterilized, neither would they have undergone antimicrobial treatments and this allows for more colonization of the mushrooms by the surrounding microbes, (Frac et al., 2018). Fresh mushrooms also generally have high moisture contents which support survival of more microbial pathogens, (Frac et al., 2018).

As shown in Table 4.2, the microbial loads of frozen samples did not significantly change ($p>0.05$) after month one. *C. miombensis, Cantharellus spp* and *A. zambiana* samples had 4.77±0.08 log cfu/ml,4.56×10±0.07 log cfu/ml,and 4.79±0.06log cfu/ml after one monthand4.77±0.08 log cfu/ml, 4.56±0.03 log cfu/ml, and 4.81±0.03 log cfu/mlafter nine months respectively. According to Berry et al. (2009), the method of freezing inactivates pathogens and stops them from growing, resulting in a constant number of the microbial contents of the frozen samples since the available microbes are only inactivated.

The microbial loads of dried samples shown in Table 4.3 did not significantly changeover and after the nine-month preservation period ($P>0.05$) likewise after month one.*C. miombensis, Cantharellus spp* and *A. zambiana* samples had 4.50 ±0.04 log cfu/ml, 4.42±0.07log cfu/ml and 4.62±0.03 log cfu/ml respectively and after nine months they were 4.47±0.03 log cfu/ml, 4.41±0.04 log cfu/ml and 4.61±0.01 log cfu/ml respectively. According to Kaur, (2022), drying

is a process which decreases the water content of a food sample thereby substantially inhibiting and delaying growth as well as activity of microbes. In this research the samples were stored in plastic packaging thereby also decreasing further contamination of the samples.

From Table 4.4it can be noted that at $p<0.05$, the microbial contents of pickled *C. miombensis*, *Cantharellus spp* and *A. zambiana* decreased from 4.79±0.04 log cfu/ml, 4.67±0.05 log cfu/ml and 4.83±0.04 log cfu/ml respectively after the first month of picklingto 4.69±0.03 log cfu/ml, 4.49±0.04 log cfu/ml and 4.78±0.01 log cfu/ml respectively after the ninth month of pickling. A research by Tabioloa et al. (2018) on the qualities of pickled *P.ostreatus* showed that the samples had a microbial content of 8.3×10^4 which is a bit higher than all the values which were obtained in this research. This difference maybe mainly due to differences in the initial concentration of the microbial loads in the mushrooms as well as differences in pickling components. According to Iguchi (2021), when acetic acid or vinegar is included in the pickling medium, microbial growth is suppressed. This may also be the reason that also contributed to the suppressed growth of microbes in this research.

Microbial contents of *C. miombensis* samples steeped in 15%, 18% and 20% brine solution did not significantly change ($p<0.05$) over the nine months as shown in Table 4.5. *C. miombensis* samples preserved in 15%, 18%, and 20% sodium chloride solution had microbial counts of 4.71±0.03 log cfu/ml, 4.68±0.02 log cfu/ml, and 4.57±0.04 log cfu/mlafter one month respectively and 4.70±0.04 log cfu/ml, 4.69±0.02 log cfu/ml, and 4.55±0.04 log cfu/ml after month nine respectively. *Cantharellus spp* samples which were steeped in 15% and 18% brine solution had an increased microbial load, ($p<0.05$) from 4.67±0.05 log cfu/ml and 4.66±0.05 log cfu/ml to 4.82±0.04 log cfu/ml and 4.76 ±0.07 log cfu/ml respectively, (Table 4.5).The microbial

contents of *Cantharellus spp* samples in 20% brine solution decreased ($p<0.05$) from 4.67±0.04 log cfu/ml to 4.55±0.04 log cfu/ml.

At a significance level of $p<0.05$, it can also be noted from table 4e that *A. zambiana* steeped in 15% and 18% brine solution had an increased microbial load after nine months, whereas those which were in 20% did not significantly change ($P<0.05$) likewise, after the first month the microbial counts were 4.79±0.06 log cfu/ml, 4.77±0.09 log cfu/ml, and 4.61±0.02 log cfu/ml and after month nine they were 4.95±0.02 log cfu/ml, 4.90±0.03 log cfu/ml and 4.84±0.07 log cfu/ml respectively. In a research by Wabali & Simeon, (2013) on preservation of mushroom in brine solution, it was observed that mushrooms which were preserved in 15%had higher concentrations of microbes than those which were preserved in 20% and 30%. This trend is similar to the one which was observed in this research likewise samples which were preserved in 20% brine solution had lesser microbial quantities. According to Wabali & Simeon, (2013), sodium chloride inhibits microbial growth and higher brine concentration cause higher inhibition, however there are other types of microbes which are resistant to sodium chloride. *A. zambiana* samples had an increased microbial content at $p<0.05$ and this might have been caused by the reason that *A. zambiana* had a greater content of microbes which are resistant to sodium chloride. An overall observation from the results was that steeping in sodium chloride concentration of 20% enhances preservation of mushrooms to a greater extent.

Results of Sensory Analysis

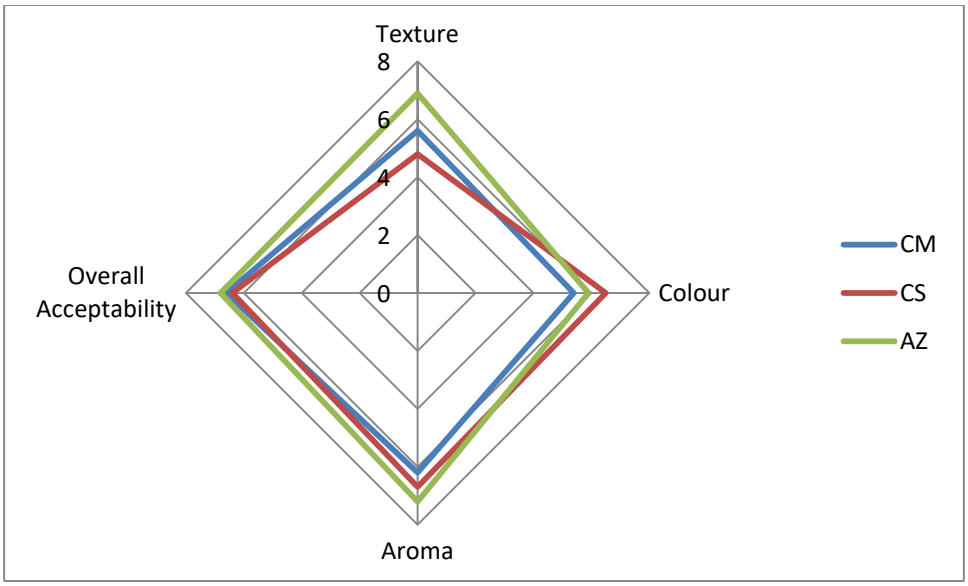

Fig 4. 1 showing mean score values for performances of colour, aroma, texture and overall acceptability of frozen samples (*CM- C. miombensis, CS- Cantharellus spp, AZ- A. zambiana*)

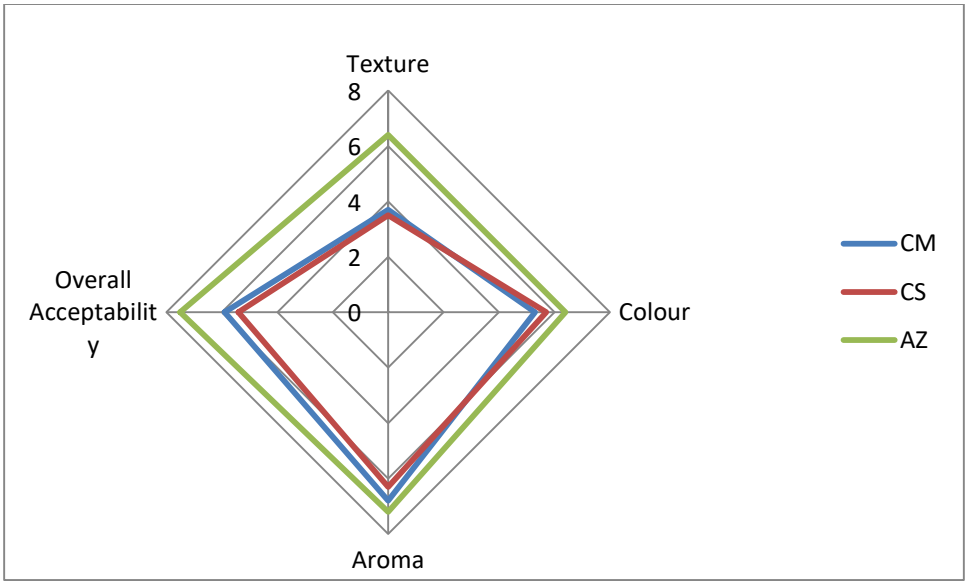

Fig 4. 2 showing mean score values for scores for performances of colour, aroma, texture and overall acceptability ofdried samples (*CM- C. miombensis, CS- Cantharellus spp, AZ- A. zambiana*)

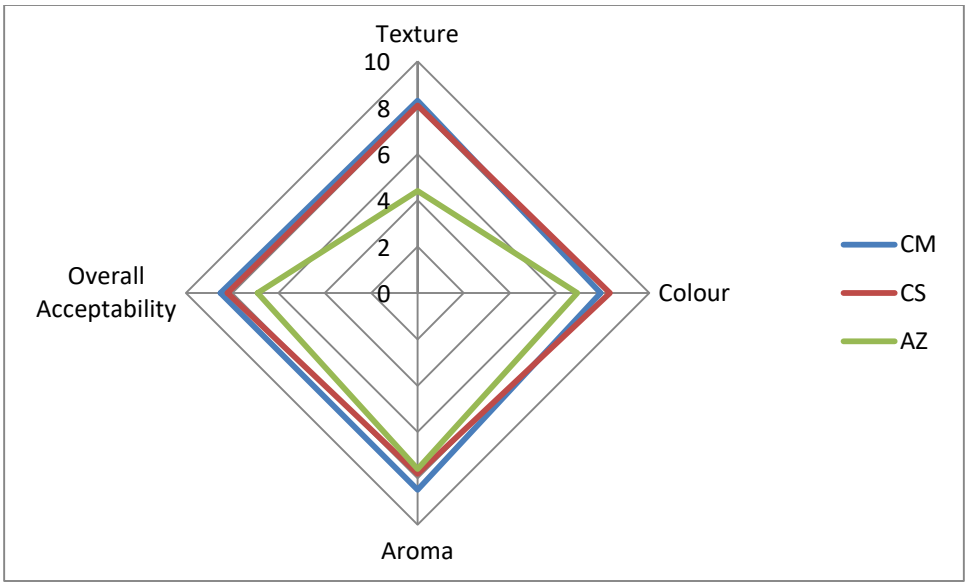

Fig 4. 3 showing mean score values for performances of colour, aroma, texture and overall acceptability of for pickled samples (*CM- C. miombensis, CS- Cantharellus spp, AZ- A. zambiana*)

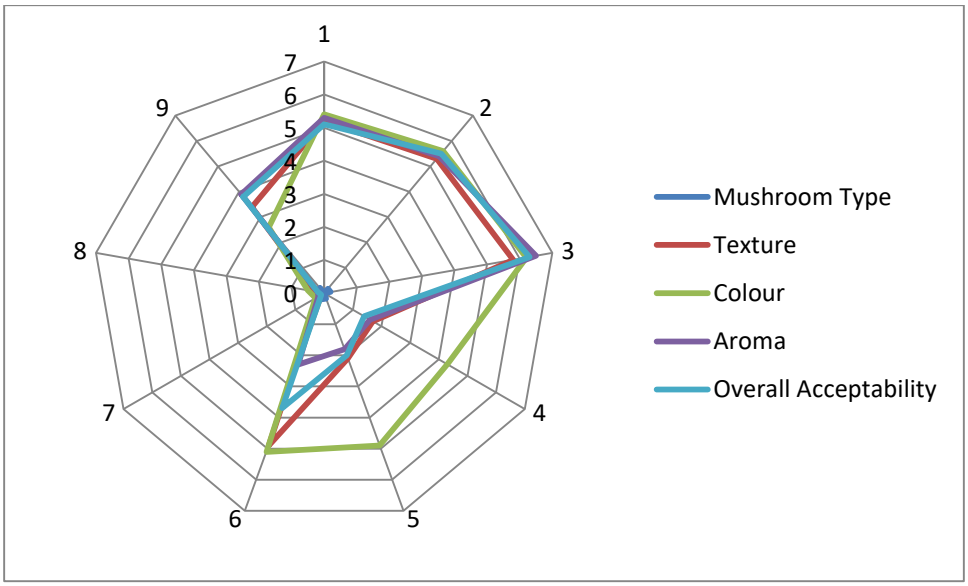

Fig 4. 4 showing mean score values for performances of colour, aroma, texture and overall acceptability of steeped samples (*CM- C. miombensis, CS- Cantharellus spp, AZ- A. zambiana*)

All the results of sensorial evaluation which were collected over the nine months were averaged. As shown in table 4.6, in overall pickled samples had the highest scores of colour, aroma and overall acceptability ranging from 6.9 to 8.3, 7.8 to 8.5 and 6.9 to 8.5 respectively with *C. miombensis*having the overall highest scores. Frozen samples had the second highest scores ranging from 4.8 to 6.9, 5.4 to 6.5, 6.2 to 7.2 and 6.4 to 6.8 for texture, colour, aroma and overall acceptability respectively. A look at the scores of the frozen samples shows that, *A. zambiana*had the highest scores. Dried samples had scores that rangedfrom3.7 to 6.4, 5.3 to 6.9, 6.3 to 7.2 and 5.4 to 7.5 for texture, aroma, colour and overall acceptability respectively with *A. zambiana* samples having the highest scores amongst the dried samples as well. Pickled samples scored the highest because the spices which were used results in better aroma and the vinegar also preserved their colour and texture and these observations resonate with those of Taminola et al., (2022) who cited that spices enhance the aroma of pickled mushrooms.

Samples preserved in brine solution had the lowest scores compared to those which were preserved using the other three methods (table 4.6). It was noted that *C. miombensis* preserved in 20% brine solution are the ones which had more scores compared to the other two samples. Scores of *C. miombensis* across the three concentrations of 15%, 18% and 20% ranged from 5.2 to 5.8, 5.4 to 6.2, 5.3 to 6.5 and 5.1 to 6.3 for texture, colour, aroma and overall acceptability respectively with samples in 20% having the highest scores. *A. zambiana* samples had the lowest

scores ranging from 0.1 to 0.6 across the three percentages. These samples had more slimy texture and an off smell. Sensory quality results from a research by Wabali & Simeon, (2013) also showed that mushroom samples which were preserved in concentrations of 20% and above had higher mean scores of sensory evaluation because they would have maintained their attributes due to the high concentration of sodium chloride. *A zambiana* samples showed spoilage and that is why they had lower scores.

4.5.2 Starch yield

A total of 90 g of fine starch was obtained from the 3000g of mushroom used.

Mushroom starch yield$= \frac{94g}{3000g} \times 100 = 3.14\%$

4.5.3 Biodegradable plastic preparation

The biodegradable plastic which was formed from the starch was transparent and clear in colour, as shown in figure 4i.

The bio plastic

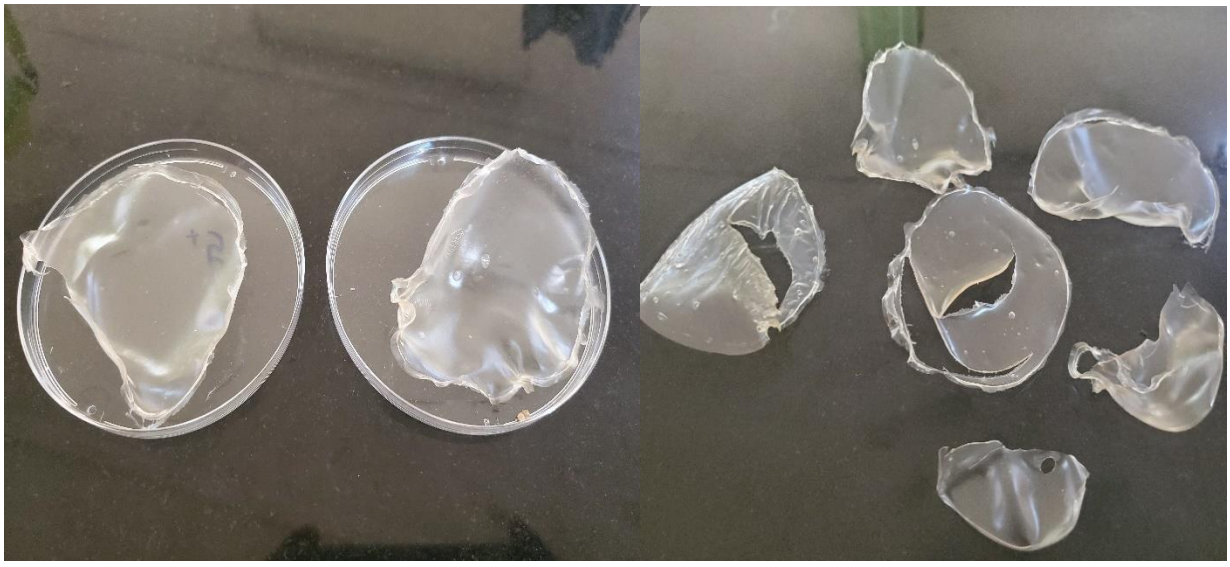

Figure 4. 1shows biodegradable plastic samples produced from mushroom starch after drying

i. **Results of bacterial degradation**

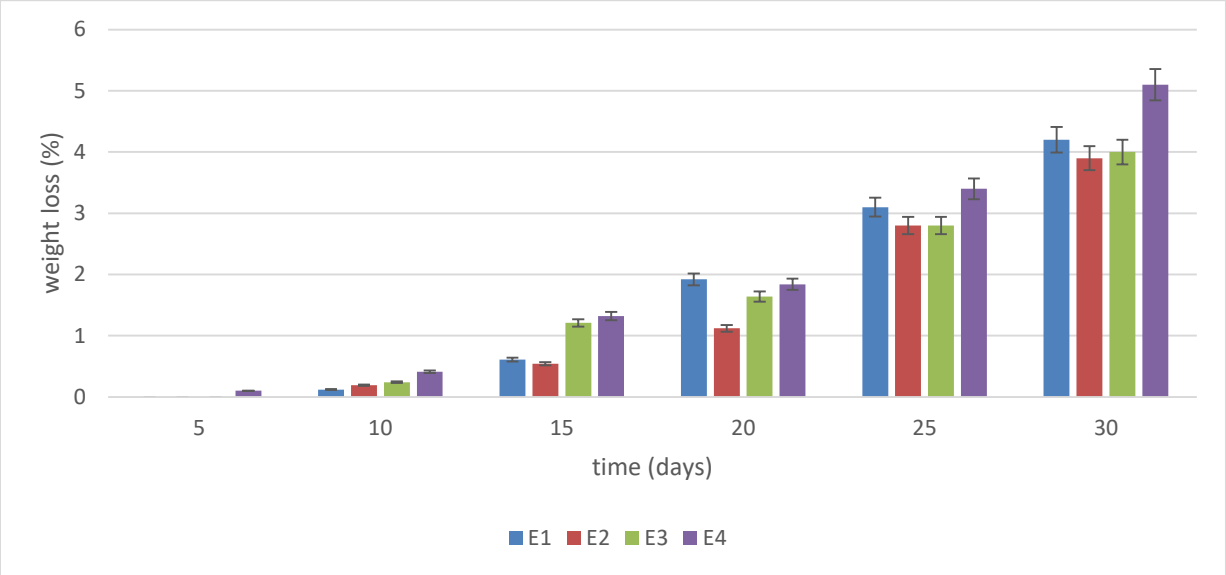

Figure 4. 2shows Plot of the percentage residual weight loss against time for the four *E. coli* treatments numbered E1 to E4.

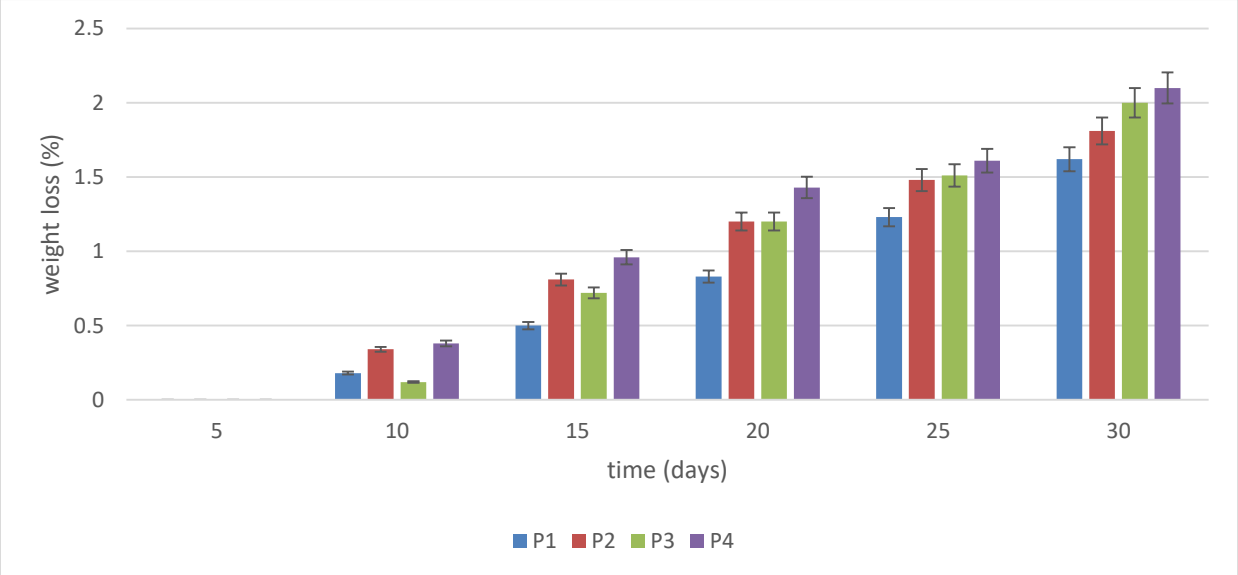

Figure 4. 3showing plot of the percentage residual weight loss against time for the four *Pseudomonas Spp*. treatments

i. **Results of the soil biodegradation tests**

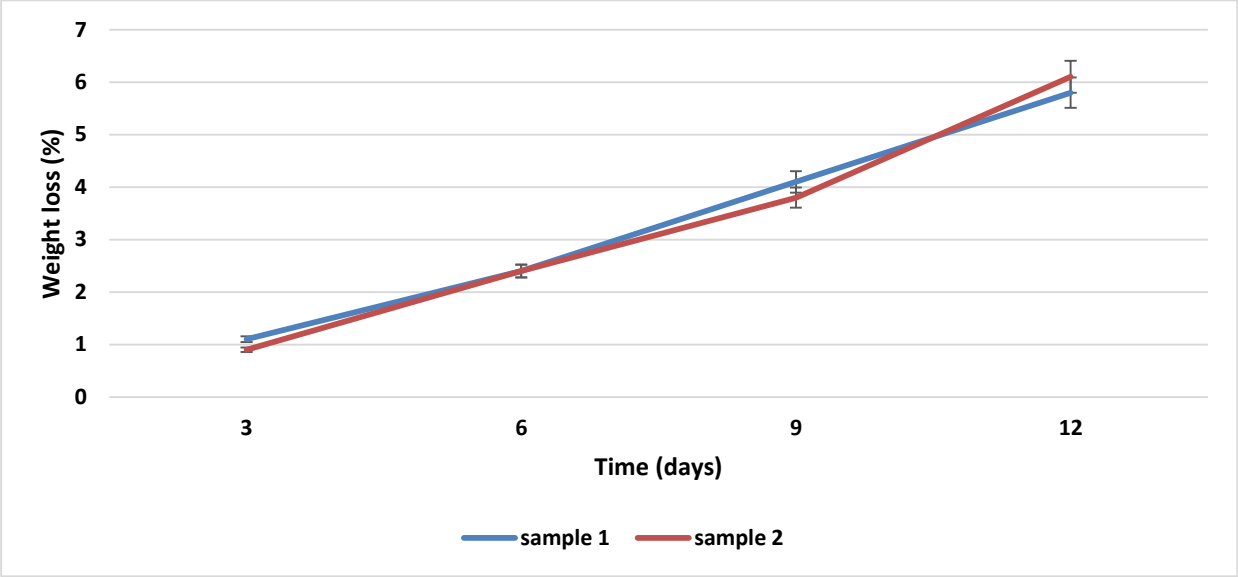

Figure 4. 4shows residual weight losses (%) for two bio-plastic samples buried in soil at 3 day intervals for a 12 day period

As shown in fig 4ii, after 30 days the weight of the bioplatics in the *E.coli* mediums weighing 0.1g, 0.2g, 0.3g and 0.4g had significantly lost weight percentages of 4.2%, 3.9%, 4% and 5.1% respectively, ($p<0.05$). Bioplastics weighing 0.1g, 0.2g, 0.3g and 0.4g in the *Pseudomonas spp* mediums had significantly lost weight percentages of 1.62%, 1.81%, 2% and 2.1% respectively, ($p<0.05$) after 30 days of inoculation. Higher percentage losses ($p<0.05$) were noted in the *E.coli* degraded plastics which therefore means that the bioplastis were more susceptible to degradation by *E.coli*. In both the Ecoli degraded samples and the Pseudomonas degraded samples, higher percentages of weight losses ($p<0.05$) were recorded in the samples weighing 0.4g. This could have been attributed to the fact that the 0.4g samples had more surface areas for the bacteria to degrade. The weight loss results which were recorded in the laboratory degraded bioplastics resonate with those of Arvind et al., (2023) who recorded weight loses in bioplastics of weight 0.1g, 0.2g and 0.3g embedded in *Pseudomonas spp* mediums. More weight losses were recorded in samples weighing 0.3g.

The bioplastics which were buried in the soil weighing 0.2g and 0.4g had significantly reduced weight ($p<0.05$) by about 5.8% and 6.1% respectively after the 12^{th} day (figure 4iv). Weight loss which was noted in soil-buried samples was due to the degradation of bioplastic materials

through digestion by microbes. This degradation was possibly contributed by several bacteria found in the soil such as *Pseudomonas spp*., *Streptococcus spp.*, *Staphylococcus spp.*, *Bacillus spp.*, *and Moraxella spp*, (Andrea et al., 2023)

A common fact which could also have contributed to degradation of the bioplastics in both the laboratory degraded samples and the soil buried samples could have been due to the breaking of polymer chain of mushroom starch containing hydroxyl (OH) carbonyl (CO), and also ester (COOH) into monomer (Nissa et al., 2022). Higher weight losses were recorded in soil buried samples compared to the ones in the laboratory bacterial mediums (figure ii, figure iii and figure iv). These variations might be attributed to the differences in the bacterial species since the soil possibly contained a variety of bioplastic degrading agents, (Andrea et al., 2023).

4.6Conclusion

It was also noted that the different preservation methods had different effects on the microbial composition and sensorial attributes of the mushrooms. Furthermore, from the results it was noted that pickling was the most favorable preservation method, followed by freezing, drying and lastly steeping. Tasting was not done due unavailability of resources to carry out full toxicity tests of the preserved mushrooms therefore further research is required to fully establish the safety of the preserved mushrooms for consumption.

Bioplastic samples obtained from the starch of the spoiled mushrooms were successfully degraded in the laboratory and the soil. Thus spoiled mushroom starch can be used for bioplastic production. These bioplastics can be employed as alternative packaging materials as they have a lot of advantages over the conventional plastics. However, considering the low starch yield, the spoiled mushrooms would need to be used as supplementary source of the starch in cases where supply will be limited. More research is also needed on production process optimization procedures which result in more starch production from the spoiled mushrooms. This study is certainly a great milestone for both economic and environmental friendly products using bioplastics or bio-polymers. This study can be a centralized project that can be scaled up to produce large quantities of bioplastics to supplement the needs of the packaging industry.

Chapter 5

5.1 Overview/ Synthesis

Mushrooms have been continuously proved by various researchers that they are a nutritious source of food, (Marcal et al., 2021). In Zimbabwe both indigenous mushrooms and cultivated mushrooms are found around the country, (Mutema et al., 2019; Reid et al., 2016). Indigenous mushrooms are found during the rainy season only, (Gleaner, 2023). During this time, people scout for these mushrooms for consumption in their homes and some for sale on the roadsides and the informal markets, (Mlambo & Maphosa, 2017). Efforts to cultivate these Indigenous mushrooms are still being made through research however as it stands they are still being underutilized due to their seasonal availability, (Reid et al., 2016). This means there is a need to provide a variety of methods that can be used to preserve these mushrooms and enhance their availability. Cultivated mushrooms are being found all year round on both the informal and the formal markets of Zimbabwe and they are supplied by local farmers, (Mutema et al., 2019). Cultivation of these mushrooms is increasing as more people are continuously joining the mushroom cultivation business, (Mutema et al., 2019).

Both these cultivated mushrooms and indigenous mushrooms are highly perishable and quickly get spoiled, (Agomuo et al., 2022). In Zimbabwe people who harvest indigenous mushrooms usually dry off their excess harvest and a few who have the resources, freeze them off, (Reid et al., 2016). Different methods have been used to preserve mushrooms which include pickling, canning, steeping, use of coatings, use of chemicals, microwave drying, freeze drying and fermentation (Castellenos-Reyes et al., 2010; Zhang et al., 2018). In the case of the cultivated mushrooms which are meant for sell in their fresh state, some get spoiled before they are all sod off to consumers, (Mutema et al., 2019). This brings forth a possible need for downstream processing of these spoiled mushrooms so that the mushroom farmers will not incur complete losses out of the spoiled mushrooms. Looking at the growing need for alternatives to synthetic plastics these spoiled mushrooms can aid as a supplementary source of biodegradable plastic.

The main objective of this research as therefore to preserve indigenous mushrooms as well as to process spoiled mushrooms to produce biodegradable plastic. The first objective was focused on mainly establishing the current state of modern market of availability of indigenous mushrooms so as to justify whether fresh and preserved indigenous mushrooms are being found in the formal markets of Zimbabwe and the main conclusion that was made was that indigenous mushrooms

were not being sold on the formal markets which were surveyed, either fresh or preserved forms. This conclusion therefore justified the need for preservation of the indigenous mushrooms so as to make them available for market take up.

The second objective covered impact of the preservation methods on the proximate nutritional contents of the indigenous mushrooms. The indigenous mushrooms which were preserved included *C. Miombensis*, *Cantharellus spp* and *A. Zambiana*. Methods of preservation which were used included oven drying at 60^0C, freezing at -22^0C, pickling in vinegar and spices and steeping in 15%, 18% and 20% solutions of brine and the preservation period was nine months. Results showed that the preservation methods had different impacts on the mushrooms. A comparison of the nutritional contents of the fresh samples and the preserved samples at a significance level of $p<0.05$ showed that all the methods cause decreases of protein content, lipid content also decreased expect for the pickled samples which had increased lipid content, the moisture content also decreased across all the methods and mushrooms which were preserved over the nine months. A decrease in the crude fibre contents of the frozen and dried mushrooms was noted after the first month only but did not continuously decrease over the nine months. The ash content of the mushrooms also decreased however for the frozen and dried samples, the ash contents did not continue to decrease over the nine months of preservation.

The third objective involved microbiological analysis of the preserved samples. In overall it was noted that after the nine months the method which resulted in lesser amounts of cfu/ml at $p<0,05$ was pickling, followed by freezing, drying and lastly steeping. Mean results of sensorial evaluation of the nine months also showed that pickling was the most favorable preservation method, followed by freezing, drying and lastly steeping. A bioplastic was formed out of starch which had been extracted from the mushrooms, however due to the low starch yield, this source of spoiled mushrooms could be factored in as a supplementary source.

5.3 Conclusions

This research provided a stepping stone towards enhancing utilization and availability of indigenous mushrooms in different preserved forms in modern markets after they become unavailable in the forests off season and an overall conclusion from the research is that preservation methods can be used to enhance the shelf life of indigenous mushrooms as they reduce activities of microbial pathogens. On the other hand as much as they have an impact on

the nutritional contents, most of them still leave the mushrooms with appreciable levels of nutritional contents as was noted in this research. In Zimbabwe, if these preservation methods are to be used, market availability of these indigenous mushrooms could be enhanced. This could lead to more utilization of the indigenous mushrooms. On the other hand, spoiled mushrooms can be taken up as a supplementary source of biodegradable plastic, rather than being discarded off. This point comes from the fact that it was noted that out of 3000g of spoiled mushrooms, about 3.14g of starch was produced which in turn was used to produce about 2.8g of bioplastic. In this way the mushroom cultivators will not incur complete losses and at the same time a gain of the biodegradable plastics will also be made thereby reducing the environmental harm being caused by synthetic plastics.

An overall conclusion at 95% significance level was that both the null hypothesis were accepted after this study that is the preservation methods have an impact on the nutritional contents, sensorial attributes and microbial composition of the indigenous mushrooms and on the other hand biodegradable plastic can be produced from spoiled mushrooms.

5.3 Recommendations

More research needs to be done to establish the safety of the preserved mushrooms for consumption. This will include toxicity tests as well as certification by a food safety regulatory board. More mushrooms also need to be researched on in terms of preservation so as to add a variety of the preserved indigenous that can be made available on the markets. More research also needs to be done to establish other sources of starch for biodegradable plastics beside the spoiled mushrooms. This could also include wild mushrooms which are non-edible.

3.6 Appendices

3.6.1 Questionnaire

CHINHOYI UNIVERSITY OF TECHNOLOGY

SCHOOL OF HEALTH SCIENCES AND TECHNOLOGY

BIOTECHNOLOGY DEPARTMENT

MUSHROOM MARKET SURVEY

My Name is Tashian Silibaziso Ncube. I am an Mphil student currently researching on Postharvest preservation of indigenous mushrooms and downstream processing of spoiled mushrooms to produce biodegradable plastic. May you kindly assist me by participating in this survey through which I would like to gain more detail on the current availability of indigenous mushrooms on market shelves as well as the current state of mushroom spoilage loss being incurred so as to justify the importance of my research. Your identities will be kept confidential and results will be shared with you if you are interested. Your participation is greatly appreciated.

1. Do you sell fresh cultivated mushrooms in your supermarket? Yes …….. No …….
2. If Yes, What types of cultivated mushrooms do you sell?

   …….

3. Where do you usually get your mushroom supplies? .....
4. How long is the shelf life of your mushrooms? .....
5. What is the estimated percentage of mushrooms which get spoiled whilst o the shelves

   ……..

6. How do you get rid of spoiled mushrooms
7. Do you sell any types of fresh indigenous mushrooms? Yes…….. No……..
8. If No, Would you be interested in selling indigenous mushrooms
9. Do you sell any types of preserved mushrooms? Yes..… No……
10. If No, Would you be interested in selling preserved mushrooms?

    Yes…… No……

11. If Yes, Which types of preservation methods would you prefer for the mushrooms? (Please Tick Below)

    Pickled/Canned... Dried… Refrigerated….Frozen...Other (please specify)……

Thank you

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