Analyzing effects of different substrates (Maize Stalks, Cotton Husks and Wheat Straw) on performance of Oyster Mushroom *(Pleurotus Ostreatus) in Zimbabwe*

A dissertation submitted in partial fulfilment of the requirements for the Master of Science Degree in Food Security and Sustainable Agriculture (Production)

Bindura University of Science Education

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DECLARATION

I hereby declare that the research project entitled "**Analyzing effects of different substrates (Maize Stalks, Cotton Husks and Wheat Straw) on performance of Oyster Mushroom *(Pleurotus Ostreatus) in Zimbabwe***" submitted to Bindura University of Science Education, Department of Agricultural Economics, Education and Extension is a record of an original work done by me under the guidance and supervision of **MR MUTSENGI** and this work is submitted in partial fulfilment of the requirements for the award of a Master of Science Degree in Food Security and Sustainable Agriculture. The results embodied in this thesis have not been submitted to any University or Institute for the award of any degree of diploma.

Author: Makunda Blessing Simbarshe
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DEDICATION

I would like to dedicate this research project to my wife Manguwo Bridget and my daughter Makunda Onai Berlin

ABSTRACT

There is a lot of lignocelluloses agricultural waste being burnt in farms posing a lot of threat to biodiversity and the environment. Mushroom farming can prove to be the way to go since it's production can recycle agricultural waste and produce nutritious mushrooms that can improve human diet. Malnutrition is becoming a perennial problem especially in developing countries. The main objective of the project was to analyze the effects of maize stalks, cotton husks and what straw on performance of oyster mushroom (*Pleurotus ostreatus*) since the effect of these substrates is not well documented for Zimbabwean conditions. Randomized complete block design was used to analyze the effects of three substrates on the performance of oyster mushroom. The three treatments were cotton husks, wheat straw and maize stalks. The trial was replicated three times. Kits placement was used as blocking factor. The substrate type have an effect on stipe length ($p<0.05$). Cotton husks gave the longest stipe length (4.55cm). Substrate type also have an effect on cap diameter ($p<0.05$). Oyster mushroom from cotton husk gave the widest cap diameter (9.25cm). Substrate type had no significant effect on total yield ($p>0.05$). The type of substrate had an effect on all nutritional status parameters ($p<0,05$). The type of substrate had an effect on energy ($p<0.05$), with wheat straw providing the highest energy (29.74calorie/100g). The effect of substrate was seen on protein content ($p<0.05$). Wheat straw had the highest protein content of 2.66g/100g. There was significant difference in means for fat produced from different substrates ($p<0.05$). Wheat straw produced oyster with highest fat (1.59g/100g). Effect of substrate was observed on fibre content ($p<0.05$) with wheat straw producing more fibre (3.41g/100g). The effects of substrate type was also observed zinc and iron ($p<0.05$) with wheat straw giving the highest Zn and Fe (9.42mg/100g and 19.49mg/100g respectively). Carbohydrate was affected by substrate type ($p<0.05$) with maize stalks giving the highest content (1.32g/100g). Substrate type affected moisture content ($p<0.05$). Cotton husks had the highest moisture content of 91.91%. As a conclusion, oyster grown from cotton husks performs best in terms of growth and yield but oyster from wheat straw is more nutritious. It is recommended to grow oyster from wheat straw as it has more nutrients. However it can also be recommended to blend main substrates with supplements to realize higher growth performance, improved yield and highly nutritious oyster mushroom.

Keywords:

Pleurotus Ostreatus, stipe length, cap diameter, total yield and nutritional composition

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First and for most I would like to thank God for giving a life to carry out this research in good health. More gratitude is also extended to my supervisor Mr Mutsengi for his valuable advice, patience and time he spend to make this project complete. Thanks to all my classmates who encouraged me to work hard on this piece of work especially Cyprian Mahuni and Faith Dondo.

LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis Of Variance
AOAC	Association of Official Analytical Chemists
C/N	Carbon to Nitrogen
EU	European Union
FAO	Food and Agricultural Organization
GAM	Global Acute Malnutrition
GoZ	Government of Zimbabwe
RCBD	Randomized Complete Block Design
SAZ	Standard Association of Zimbabwe
SPSS	Statistical Package for Social Scientists
UNICEF	United Nations International Children Emergency Fund
ZAGP	Zimbabwe Agricultural Growth Programme
ZimVAC	Zimbabwe Vulnerability Assessment Committee

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

In African cultures, mushrooms were known to be wild but with the evolution of advanced technology a wide range of mushrooms is now being cultivated. Some mushrooms are poisonous and others are edible with numerous medicinal and nutritional benefits. Asemota *et al* (2014) noted that edible mushrooms belong to the Fungi Kingdom, Division Basidiomycota, Class Basidiomycetes and Order Agaricales/Ascomycetes (Mercela, 2013). Additionally, according to scientific classification cited by Randive (2012) oyster mushroom belongs to genus *Pleurotus* and species *Ostreatus*. An early cultivation of Oyster mushroom (*Pleurotus Ostreatus)* began in 1900's (Tisdale, 2004). The cultivation of *Pleurotus Ostreatus* in which natural spawn were used for inoculation of wood logs and stumps started in Germany around 1917 (Martinez-Carrera, 1998). But according to Frempong (2000) mushroom cultivation by human originated in France in the 17th century during the Napoleonic era and other nations adopted the traditional mushroom cultivation biotechnology, thus increasing productivity with time. It was further discovered that a wider range of oyster species can be grown on lignocelluloses and agricultural waste such as cotton seed hulls, wheat straw, soybean straw, maize stalks, maize cobs, saw dust, thatch grass, sugarcane baggase and rice straw (Tisdale, 2004). Since oyster mushrooms are unable to manufacture their own food; they feed by excreting digestive enzymes through the tips of their hyphae (Woller, 2007). These enzymes include peroxidises, laccases, cellulose, hemi cellulases and xylanases which makes the mushroom well adapted to feed on these lignin and cellulose containing substrates (Cohen *el al*, 2002).

Mushrooms are increasingly being recognized as important food products for their significant role in human health, nutrition and disease prevention (Kinge *et al*, 2016). Several species of mushrooms are of great importance because of their medicinal properties, for example, they are active against hypercholestorolemic conditions, hypertension, diabetes, cancer and other infections (Alam *et al*, 2007 cited by Kinge *et al*, 2016). This is attributed to its extract ability to lower cholesterol levels as effectively as dietary supplements. Additionally, *Pleurotus Ostreatus* has potent antinociceptive, antitumors, antioxidants and immunological activities that contribute

significantly to human diet as it reduces cancers and oxidative damages. Gunde ad Cimerman (1999) noted that oyster mushrooms have two of the most prominent medical attributes known as cardiovascular and cholesterol controlling benefits. They naturally produce mevinolin (lovastatin) in portions of the fruiting bodies which is a key enzyme in cholesterol biosynthesis in the liver and reduces cholesterol absorption (Bobek *et al*, 1998). Mushrooms have been defined as the poor man's meat due to its protein content which is considered to be intermediate between that of vegetables and animals (Iqbal *et al*, 2016). This implies that, in terms of nutritional values, mushrooms can be used as better substitutes compared to other vegetable, milk as well as other meat products since proteins, carbohydrates, crude fibre, fat/lipids, vitamins, micronutrients and energy are found in them but at varying degrees dependent on substrate used.

1.2 Statement of the Problem

A lot of lignocellulose agricultural waste is being burnt in farms and this is posing a huge threat to environmental sustainability. FAO (2002) noted that mushrooms can be used as agricultural waste recycler as they have the ability to convert lignocellulosic materials to protein rich healthy food. The problem is it is not known how mushrooms perform under alternative media which are locally available. Therefore it is of relevance to analyze how different substrates can have an improved yield that will provide a higher income to the farmer and also assess the nutritional composition of oyster produced from these substrates.

1.3 Objectives of the study

1.3.1 Main objective

- To analyze effects of different substrates on performance of Oyster Mushroom (*Pleurotus Ostreatus)* in Zimbabwe.

1.3.1.1 Specific objectives

- To assess the effects of different substrates on the growth of Oyster mushroom (stipe length and cap diameter)
- To compare the yields produced from different substrates
- To ascertain the effects of different substrates on nutritional composition of Oyster mushroom produced

1.4 Hypothesis

- Substrate type has an effect on the growth of Oyster mushroom
- Substrate type has an effect on yield of oyster mushroom
- There is no significant difference in nutritional composition of oyster mushroom produced from different substrate types

1.5 Significance of the study /Justification

The majority of people in Zimbabwe are said to be food insecure in terms of access, availability, utilization and stability. Oyster mushroom farming is cost effective and provides nutritional balance in human diet. Agricultural waste can be used to produce a product with adequate amounts of phosphorus, iron, protein, lipids, riboflavin and thiamine (Iqbal *et al*, 2016). Most developing countries are facing a series of challenges including malnutrition, unemployment and effective waste management principles. According to ZimVAC (2017) report, the national prevalence of Global Acute Malnutrition (GAM) was 3.2% and this is below the 5% emergency threshold. This implies a serious need of nutritional intervention to counter the arising problem. Generally, most households in Zimbabwe are relaying on casual labor as the most important source of income (30%) followed by salary (10%) and remittances within and food crop production/sales both at 8% (ZimVAC, 2019). This is a severe challenge in terms of accessing food to improve human healthy. Fresh Oyster mushroom is also rich in carbohydrates, protein, fats, ash and fibre. Scientific research works showed that mushrooms are often considered equal to meat in nutritional value (Thongnaitham, 2012). However, the research seeks to investigate agricultural waste that may result in high production, growth and yield. The research goes further to sort information on substrate for farmers to use with high nutritional combination for a healthy diet. This research is important as a hub of knowledge to help farmers have a climate smart approach to get rid of agricultural waste as well as making revenues. Mushrooms are natural recycler of agricultural wastes because they can convert lignocellulosic materials into protein rich healthy food (FAO, 2002). Additionally, malnutrition is a problem in developing countries and there is great need to produce nutritious food such as mushroom especially from agricultural waste without polluting the environment. This is further supported by Eswaran and Ramabadran (2000) who noted that mushrooms with their flavor, texture, nutritional value and high productivity per unit area have been identified as an excellent food source to alleviate malnutrition in developing countries. Oyster mushroom with higher nutritional status will result

in recommendation of substrate in which it is produced. This helps in lowering the challenges of malnutrition that has been persisting in Zimbabwe since 2014 (ZimVAC, 2017).

1.6 Scope of the study

The experiment was conducted in Harare South in a mushroom house measuring 3m x 4m. The study started on the 1st of November 2019 and end in the first week of March 2020. The focus of the experiment was centred on how maize stalks, cotton husks and wheat straw affects growth rate, yield and nutritional composition of oyster mushroom. The growth parameters involved stipe length to be measured with Vanier callipers and diameter of mushroom head/cap. The yield was measured in terms of mushroom weight using an electronic balance. Nutritional composition was confined to percentage moisture content, protein, fats, carbohydrates, total ash and crude fiber content and two micronutrients (iron and zinc). The experiment was restricted to randomized complete block design (RCBD) where 3 treatments were replicated 3 times and blocked into three. Samples were taken by the researcher for analysis of nutritional composition at University of Zimbabwe department of Food Science laboratory. The data was statistically analyzed using one way ANOVA and descriptive statistics from SPSS version 16.0.

1.7 Limitation of the study

The experiment was faced challenges sourcing for spawn which was then procured at PaHowa (SIBBS) Spawn Laboratories in Epworth, Harare. Nutritional composition analysis is solely depended on mass spectroscopy and forier transform infrared spectroscometer which might not be available at Bindura University or might have restrictive conditions at Standard Association of Zimbabwe (SAZ). However the researcher managed to have access to Food Science Laboratory at the University of Zimbabwe were the samples were tested. The experiment is also expected to be affected by shortage of equipments to measure and control temperature and humidity during the course of mushroom production. To counter these factors, the researcher employed the use of water containers to increase humidity and reduce temperature.

1.8 Outline of Thesis

The research was outlined as follows:

Chapter 1: Introduction of the study.

Chapter 2: Literature review.

Chapter 3: Research Methodology.

Chapter 4: Growth and yield level of oyster from maize stalks, cotton husks and wheat straw

Chapter 5: Nutritional composition of oyster from maize stalks, cotton husks and wheat straw.

Chapter 6: Summary, conclusions and recommendations.

1.9 References

- **Alam, N, Khan, A, Hossain, M.S, Amin, S.M.R. and Khan, L.A. (2007).** Nutritional Analysis of dietary Mushroom *Pleurotus florida* Eger and *Pleurotus sajorcaj* Fr.) Singer. *Bangladesh J. Mushroom*. **1**, 1-7.
- **Cohen, R., Persky, L., and Hadar, Y. (2002).** Biotechnological application and potential of wood-degrading mushrooms of the genus *Pleurotus*. *Appl Microbio Biotechnol* **58**: 582 – 594
- **Eswaran A and Ramabadran R. (2000).** Studies on some physiological, cultural and post harvest aspects of oyster mushroom, *Pleurotus ostreatus*. Tropi Agric Res 12: 360 – 374.
- **Frempong, A. (2000).** A study of the profitability of mushroom cultivation in the Greater Accra regions. A dissertation to the University of Ghana. Accessed on 10/06/2014 from http://ugspace.ug.edu.gh
- **Gunde-Chimerman, N.** (**1999)**. *International J. Medicinal Mushrooms.* 1: 69-80.
- **Iqbal, S. M, Rauf, C. A. and Sheikh, M. I, (2016).** Yield Performance of Oyster Mushroom on Different Substrates, *International Journal of Agriculture & Biology*, Vol. 7, No. 6, 900–903.
- **Martínez-Carrera, D., (1998).** Cultivation of Oyster Mushrooms, *McGraw-Hill Yearbook of Science & Technology, Ed.: M. D. Licker. McGraw-Hill, Inc.,New York.* 242-245.
- **Randive, D.S (2012).** Cultivation and study of growth of oyster mushroom on different agricultural waste substrate and its nutrient analysis. Adv Appl Sci Res;3:1938-1949.
- **Tisdale, T. E, (2004).** Cultivation of the Oyster Mushroom (*Pleurotus sp.*) on Wood Substrate in Hawaii, *university of Hawaii*, 99.
- **Thongnaitham, M, (2012).** Organic Mushroom Cultivation Manual, FreelandFoundation, 591.
- **Woller, R. (2007).** The Pearl Oyster Mushroom, Accessed on 31/08/2014 from http://bioweb.uwlax.edu/bio203/2011/woller_ryan/nutrition.htm

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

This chapter starts off by defining the crop under study, its scientific classification. Description of the overall production of oyster is paramount as well as the literature on the how different substrates influence the parameters under spotlight such as stipe length, cap diameter, yield and the nutritional elements. The chapter explores the opportunities of oyster production at global level as well as the regional acceptance of mushroom growing in Africa which was unfamiliar. The chapter ends by conceptualizing the whole research into a visual framework for easy understanding.

2.1 Defining Oyster Mushroom

Mushrooms belong to a special group called Fungi. According to Cho and Kang (2004) mushroom refers to the fruit produced and it must be large enough to be seen with a naked eye. Ganopedia (2011) has given a more comprehensive definition of mushroom which has described it as fungus consisting of a stem, a cap and gills which produce spores from underside of the cap. Visually the fruit bodies of oyster mushroom have three distinct parts namely spatula cap (pileus), central stalk called stipe and long ridges under pileus called gills. Mushrooms are not only basidiomycetes, they can also be ascomycetes, grow underground, have a non-fleshy texture and could be inedible (Chang, 2007). Oyster mushroom belongs to the class Agaricomycetes, order Agaricales**,** family Pleurotaceae *or* Tricholomataceae, genus *pleurotus* and species *ostreatus.* Scientifically, oyster mushroom is known as *Pleurotus ostreatus* (Kuo, 2011). Oyster mushrooms are the third most cultivated mushrooms in the world and are used primarily as human food. Its production is divided into two main phases: vegetative and reproductive phase. Vegetative phase starts from spawn up to when the hyphae fully colonize the lignocelluloses used whilst reproductive phase starts when the hyphae develops into primordia (mushroom fruit).

2.2 Oyster Mushroom Production overview

The life cycle of oyster is a simplified one which is separated into two main biological phases: first is the vegetative phase consisting of mycelia expansion and maturation. This is followed by the reproductive phase of fruiting body production (Tisdale, 2004). The initial mycelium culture can be obtained through tissue culture of existing mushroom or spores from a selected stock.

This mycelium is then propagated on a sterilized cereal grains producing spawn. The spawn is used to inoculate the mushroom substrate and allowing it into incubation phase also known as spawn run period (Tisdale, 2004). At full colonization the mycelium will be all over the substrate and ready for reproduction phase. If adequate environmental conditions are met the primordia starts to form and develop into a mature mushroom ready for harvesting. This production overview saw followed starting from when the spawn is inoculated on different substrates while the spawn itself was purchased. The cycle of mushroom production has to be understood by local farmers in order to increase total yields and reduce contamination of kits (mushroom bags).

2.3 Substrates on Oyster mushroom

2.3.1 Growth and yield effect

Different types of substrate were looked at using a range of measurable parameters from literature to explore how oyster mushroom itself can perform. According to a research by Salama *et al* (2016), barseem straw gave the highest number of fruit per bag and corn cob gave heaviest weight of fruit body per bag among other substrate such as wheat straw, saw dust, soybean and rice straws. This implies that mushroom produced on wheat straw and soybean straws have a lighter fruiting body weight compared to its counterpart, corn cob. The number of fruits per bag and its weight is very important to take into consideration as they contribute significantly to the total yield produced. These parameters are greatly affected by environmental conditions such as temperature, humidity and the amount of carbon dioxide in the atmosphere. Obodai *et al* (2003) reported that *Pleurotus Ostreatus* pinhead formation can take four to six days after complete colonization of the substrate with the first harvest coming after 10-12 days. Pinhead formation can also spark great interest especially when considering different substrates. If *Pleurotus Ostreatus* pin heads can take 10-12 days, what does it mean for oyster mushroom produced from maize stalks, cotton husks and wheat straws in terms of growth rate and the absolute yield level?. It can generally be assumed if more pin heads are formed in shortest possible number of days, therefore, more fruits are produced and contribute positively to the final yield.

In a recent study by Dubey *et al* (2019), the highest yield with highest stipe length and cap diameter was obtained from rice straw compared to wheat straw, banana leaves and sugarcane baggase. The study justified that rice straw provide a reservoir of cellulose, hemicelluloses and lignin which is used for growth and fructification. This is in contrary to study by Iqbal *et al*

(2016) showing that maximum yield can be produced from wheat straw compared to rice straw, sugarcane baggase, maize straw and sorghum straw. These two studies have created a gap which needs to be explored with more experiments. But it is important to consider the conditions under which the two experiments were conducted in terms of environmental conditions prevailing (humidity and temperature) and the sterilizations methods involved.

In a study by Nurudeen *et al* (2013), the fruiting bodies from different mushrooms produced from corn cob, saw dust and coconut husk substrates were quantified using the length of stipe and diameter of cap parameters. Table 2.1 showed the result summary were harvest was done up to five times with length of stipes averaged for each substrate per flush. The mean length of stipe ranged from 5.8 to 7.2 cm. The mushroom from corn cob exhibited a steady decrease in mean stipe length from second flush to the fifth flush (7.1-6.0 cm) while mushroom from coconut husk showed an increase in mean length of stipe as the flushes increased. The same was observed for saw dust produced mushrooms.

Table 2. 1: *The mean length of stipe (cm)*

Substrate	**flush 1**	**flush 2**	**flush 3**	**flush 4**	**flush 5**
Corn- Cob	6.4	7.1	5.7	6.2	6·0
Sawdust	5.8	6.3	6.1	7.2	6.8
Coconut-Husk	6·0	6.3	6.8	7.1	7.2

Source: *Nurudeen et al (2013)*

The average diameter of the caps was also recorded as shown in Table 2.2. The mushroom from saw dust recorded the widest diameter in flush 4 (7.2 cm) followed by coconut husk and corn cob with values 7.1 and 6.4 cm respectively.

Table 2. 2: *The mean diameter of cap (cm)*

Substrate	flush 1	flush 2	flush 3	flush 4	flush 5
Corn- Cob	6·0	6.4	5.3	5.1	6.2
Sawdust	6.3	6·0	5.7	7.2	6.8
Coconut-Husk	6.1	7·0	6.8	7.1	6.2

Source: *Nurudeen et al (2013)*

The study by Nurudeen *et al* (2013) is important to this experiment as it is taking a similar approach to measure the growth rate and yield. But the substrates involved are different which make it more interesting to investigate.

Kinge *et al* (2016) showed that oyster mushroom cultivated on sawdust possesses better growth. Sharma *et al*, 2013 brought a new twist in literature as they proved that the highest yield can be obtained from rice straw (381.85g) followed by rice straw mixed with wheat straw then rice straw with paper. But the lowest yield can be obtained from saw dust (247.87g). Kumari and Achal (2008) also carried an experiment of *Pleurotus Ostreatus* on different substrates and reported that the highest yield of oyster was on wheat straw followed by wheat straw mixed with maize stalks. Taurachand (2004) showed that sugarcane baggase contains celluloses and sucrose which can easily be degraded by oyster mushroom for growth and improved yield but on the other experiment by Sharma *et al* (2013) it was reported that even though sugarcane baggase is rich in cellulose and sucrose, it produced the lowest yield level in comparison to other substrates. The substrates used by different authors are producing different outcomes of oyster growth and yield which is prompting for more experiment to bring about consistence in results. This research narrows down to investigate how maize stalks, cotton husks and wheat straw substrates on growth and production on oyster mushroom.

2.3.2 Nutritional composition

In a study by Dunkwal and Jood (2009), oyster mushroom produced on wheat straw and brassica straw showed no significant difference in crude protein (25.30 and 26.99%) and total carbohydrates (52.34 and 50.52%). The same was observed in both mushroom in terms of vitamins, amino acids and dietary fibre. Kinge *et al* (2016) came up with some mixed nutritional properties of oyster from different substrates. The substrate has influence on nutritional values as

maximum protein was observed on mushroom from saw dust (29.45%) and minimum protein was from corn cob (25.12%). In contrary, maximum crude fibre (16.69%) was from corn cobs and minimum crude fibre (5.08%) was observed from saw dust (Kinge *et al*, 2016). Woller (2007) highlighted that oyster mushrooms obtain their protein by secreting a potent toxin to kill nematodes or roundworms that may be present on lignocelluloses substrates. The hyphas of the fungi then secrete enzymes that digest these microorganisms and absorb the resulting nutrients produced. A wide range of substrates have different degrees of microorganism infestation that impacts of nutrient composition of oyster mushroom yielded. According to Kang (2004) the substrate on which the mushroom is grown should supply specific nutrient requirements needed and the main sources of these nutrients are cellulose, hemicelluloses and lignin. The main sources of carbohydrates i.e. celluloses and hemicelluloses are incrusted in lignin, which forms a physical seal around celluloses along with nitrogen content of residues affect mycelia growth, oyster mushroom quality and the yield (Philippoussis and Diamantopoulou, 2011).

In a study by Roy *et al* (2015), two species of mushrooms (oyster (*Pleurotus ostreatus*) and Reishi (*Ganoderma lucidum*) were analyzed to evaluate the nutritional composition. More emphasis was on the moisture content of the two species since it is necessary for most of the physiological reactions in plant tissue. In the research it was concluded that higher percentage of moisture content was found in oyster (85%) than in Reishi (47%). This is paramount to understand in this research paper since there is need to also analyze the differences of moisture content in oyster mushrooms produced from a wide range of substrates.

The level of moisture content was also perceived as important in a research by Nurudeen *et al* (2013) were a proximate analysis of fruiting bodies of *Pleurotus sajor-cafu* on three different substrates (sawdust, corn cob and coconut husk) was done. *Pleurotus sajor-cafu* is similar to *Pleurotus ostreatus* since they belong to the same genus. The research used analysis of variance and descriptive statistics supported by statistical package of social sciences (SPSS) version 16.0. The results showed that the fruiting bodies of *Pleurotus sajor-cafu* from saw dust substrate has the highest value of moisture content followed by coconut husk and corn cob with values 88.30, 87.00 and 85.50 respectively. Furthermore the highest protein was found in mushroom from coconut (40.10) followed by saw dust (30.12) and lastly corn cob (29.61). According to

Nurudeen *et al* (2013) high protein value exhibited from coconut husk was attributed to high nutritional content present in coconut husk. In terms of ash content produced, mushroom from saw dust has the highest ash content compared to coconut husk and corn cob with values of 7.42, 6.00 and 5.71 respectively. These results were the same with those produced by Ekpo *et al* (2008).

In a study by Dubey *et al* (2019) it has been shown that the fruit bodies are quite rich in protein ranging from 22.89%-25.97% on dry weight basis. The highest protein, carbohydrate, energy and fiber were obtained from oyster produced from rice straw. According to Wang *et al* (2001) this can not only be attributed to the protein content on fruit bodies but protein content also depend on the used substrate and how it easily release nitrogen that can be extracted for the production of protein. Dubey *et al* (2019) also added that the highest fat (1.03%-1.50%) and fiber (12-14%) was obtained from saw dust and highest total ash was obtained from rice straw mixed with paper. This was widely contrary to findings by Wang *et al* (2001) which showed that the fat and fiber from saw dust was much lower, 2.50%-2.82% and 5.97%-6.42% respectively. In the present experiment by Sarder *et al* (2020) the moisture, protein, fat, fibre, carbohydrates and ash content in oyster mushrooms were ranging; 83-91%, 18-28%, 1.1-3.7%, 9.6-14.6%, 52-61.2% and 5.5-7.3% respectively. The experiments on different parameters of nutritional composition are slightly deferring from different authors. This is further supported by Sangwana and Saini (1995) attributes variations to biological, chemical differences and the C/N ratio of the substrates.

2.4 Opportunities of mushroom cultivation at global level

In India, the Biovillage Programme in connection with mushroom growing was initiated by the M.S. Swaminathan Research Foundaton with aim to improve livelihoods of villagers in several localities. This offered new enterprises to bring new incomes, encourage the need for cooperation among enterprises and training workshops to expand villagers farming systems (ACDI & SIDA cited by Marshall and Nair, 2009). The programme also assisted farmers in marketing their produce and empower once marginalized rural women in the village. Market linkages were established with local markets which further extended to cities.

It has proved to be a tough challenge in organizing farmers in Northeast India due to its political economy but Pranjal Baruah and the NGO Ashoka worked through to break the barrier. Mushroom cultivation systems were developed to empower farmers and develop strong farmer's

networks which later enabled price and quality of mushroom to be standardized (Ashoka, 2008). The organization established a mushroom lab to create a sustainable constant supply of quality spawns at low cost. This was a major stride to the positive direction as most of less developed countries are having fast growing interest in mushroom cultivation but continuously drawn back by spawn availability and its high cost when available. The organization has also stretched arms to involve unconventional groups like prisoners (Ashoka, 2008).

Livelihood opportunities have also reached shiitake (*Lentinus edodes*) mushroom growers in the rural economy of the Republic of Korea (case of Cheongyang-Gun). The area in known for vast availability of oak logs but the majority of growers do not own forests and grow the mushroom under artificial shades (Youn, 2004). The growers buy saw dust of ark logs from timber merchants and use them as substrate in mushroom cultivation. The farmers have established a cooperative known as The Mushroom Growers Club which provides farmers with loan service while the government provides technical support (as cited by Marshall and Nair, 2009). This cooperative proved to be strategic as it traded both in fresh and dried mushrooms: members close to towns specialized in fresh produce while those situated far focused more on dry produce.

In 2007 FAO Regional Office for Asia and the Pacific has been seen pioneering programme in creating opportunities mushroom growing for the disabled in Thailand. The programme was biased towards rural people with disabilities, empowering them to be self-reliant, unfold their capabilities and be re-integrated in their communities. The programme proved to be one of the best as trainees gained self-satisfaction, self-esteem and majority became physically stronger. This improves grower's income giving them financial power to support their families.

2.5 Regional acceptance of mushroom cultivation

Mushroom cultivation is considered a recent venture in a country such as Ethiopia. Back in the time, mushroom consumption was confined to rural inhabitants who gathered wild mushroom in forests, farmlands and waste dumpsites. According to Dawit (2008) small scale mushroom farms started in 1997 where oyster mushroom was cultivated in Ethiopia. This has seen a readily growing demand of the produce at a rate of around 36 tons per year (20% annual growth). Despite the fact that mushroom farms are now in place, they still produce less than the local

market demand (Michael *et al,* 2010). This was attributed to poor farm management, low quality mushrooms, low productivity and improper preservation techniques.

In most parts of Africa people have perceived mushroom as poisonous which had made it so difficult to be accepted. Hai district of Northeastern Tanzania has of late demystified the perception of mushrooms as poisonous through intervention of Horticultural Research Institute Tengeru supported by FARM-Africa's Maendeleo Agricultural Technology Fund resulting in almost 300 farmers adopting oyster mushroom production in their homes (New Agriculturist, 2007). Kilimanjaro highlands in Hai district was once a thriving banana and coffee growing region but now with the falling world market prices for coffee and impact of climate change resulting in low rainfall has left farmers vulnerable to consequences of food insecurity. With the coming in of Hort-Tengeru in 2005, the mushroom growers are now able to buy livestock, pay school fees and household basics, improving diets and others investing in mushroom cultivation expansion (New Agriculturist, 2007).

2.6 Micronutrients

According to UNICEF (2011) an estimated 161 105 children die before reaching the age of five giving an under-five mortality rate of 84/1000. This has been attributed to many factors including malnutrition and prevalence of micronutrients deficiencies across all age groups in Zimbabwe. About 58% of pre-school children are also anaemic together with 47% of pregnant women. They are grave human and economic consequences of the current micronutrient deficiencies in Zimbabwe resulting in about 7 700 children and mothers dying every year. The commonly cited micronutrient deficiencies largely involve iron, zinc, vitamin A and folic acid. This study has narrowed down focus to only zinc and iron that can be found in oyster mushroom. But there is great need to investigate the amounts of these micronutrients that can be found in oyster mushrooms produced from different substrates.

2.6.1 Zinc

Generally, Zinc is a bluish-white metallic element (atomic number 30, atomic weight 65.4), which makes up about 0.02% of the earth's crust and is the twenty third most abundant element. Zinc is a transitional element on the periodic table and possesses specific chemical properties that make it useful and important in biological systems. According to Brown *et al* (2001) Zinc is associated with more than 50 distinct metalloenzymes, which have a diverse range of functions,

including the synthesis of nucleic acids and specific proteins, such as hormones and their receptors. This makes Zinc important in cellular growth, differentiation and metabolism. Evidence of human zinc deficiency began to emerge in the 1960s, when cases of zinc responsive dwarfism and delayed sexual maturation were first reported among Egyptian adolescents. This is further supported by clinical studies of children with acrodermatitis enteropathica an inborn error of zinc metabolism resulting in poor zinc absorption and consequent severe zinc deficiency, have confirmed the critical role of zinc in physical growth and gastrointestinal and immune function (Brown *et al*, 2001). Zinc is highly important during the periods of rapid growth (pre and post natal). Zinc nutritive nature plays a critical function most on pregnancy outcome, susceptibility to infection and neurobehavioral development. On that note, Zinc has been taken for investigation under this study.

2.6.2 Iron

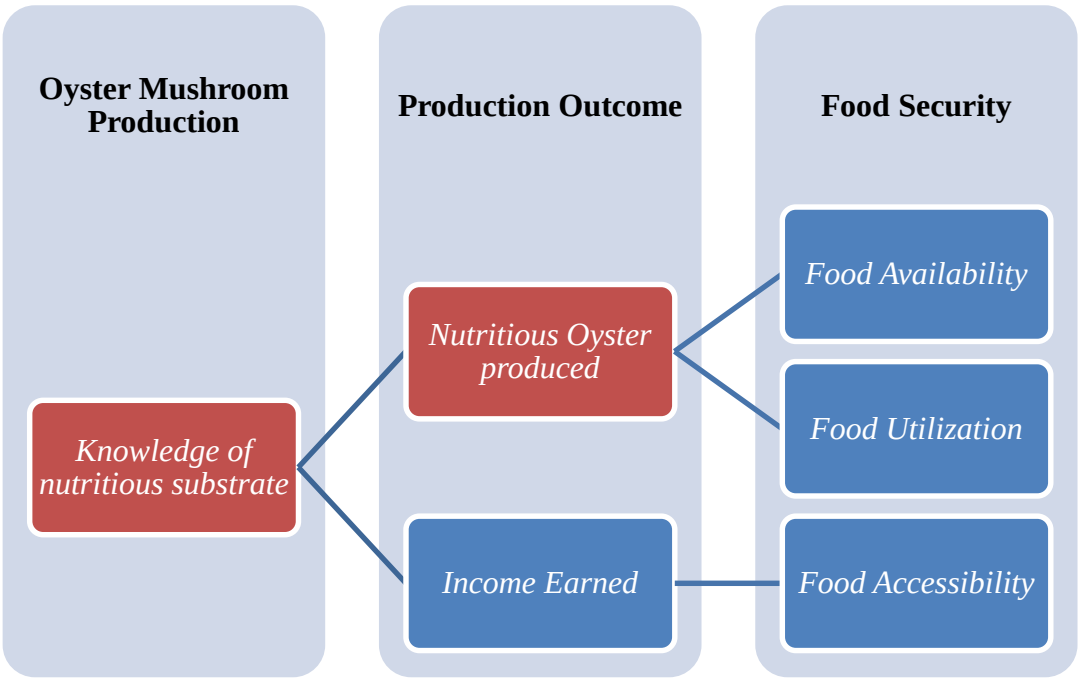
The data collected in Zimbabwe from 1980 to 2006 have shown that a significant proportion of preschool children, school children and adult women (lactating or pregnant) experienced malnutrition with significant proportions of these groups suffering from vitamin A and iron deficiencies (Reilly *et al*, 2012). Iron is important as it forms the central part of haemoglobin which is functional in the transportation of oxygen in the human body. As cited by Malhotra (1998), iron plays an essential role in cellular respiration as a component enzymes involved in biological oxidation such as cytochromes. According to Larkin and Rao (1990) Iron is required for proper myelination of spinal cord and white matter of cerebellar folds in brain and is a cofactor for a number of enzymes involved in neurotransmitter synthesis. Iron is involved in synthesis and packaging of neurotransmitters, their uptake and degradation into other iron-containing proteins which may directly or indirectly alter brain function (Beard, 2001). If the human body is lacking iron; this can be manifested as deficiency disease or anemia. Tan et al (2006) reported that iron deficiency has a role in brain development and in the pathophysiology of restless legs syndrome. This was further supported by Beard (1999) who noted that iron defiency is associated with alterations in many metabolic processes that may impact brain functioning, among whom are neurotransmitter metabolism, protein synthesis and organogenesis. Sadzadeh and Saffarri (2004) also brought a new twist in literature as they discovered that iron accumulation has been related to some neurologic disorders such as Alzheimer disease, Parkinson disease and type-1 neuro-degeneration. Excessive accumulation of iron in the liver,

pancreas, heart, lungs and other tissues cause haemosiderosis and when this is accompanied by bronze pigmentation of the skin, the condition is called haemochromatosis (Malhotra, 1998)

According to Seth (2002) the human brain is sensitive to dietary iron depletion and uses a host of mechanisms to regulate iron flu homostatically. Literature has now proven that even though iron is a micronutrient it has major roles to play in human health. This has motivated the inclusion of iron content investigation in oyster mushrooms grown from different substrates, in this case, maize stalks, cotton husks and wheat straws. It builds on knowledge board if the farmers understand correct substrates around them that can be used to improve iron content in both their diets and consumer nutrition.

Figure 2. 1: Conceptual framework

Figure 2.2: *The conceptual framework for oyster mushroom production*



Oyster mushroom requires substrates with less nitrogen and more carbon, living materials high in cellulose, hemicelluloses and lignin desirable. The knowledge of substrates with high lignocelluloses is important in producing higher yields and product with high nutritional content. This is the core of the experiment. The production of oyster mushroom can be of economic value where farmers can earn revenue which can be used to access other food items. Constant demand of the mushroom has led to increase in the price (Roach, 2006). This makes the farming business

even more lucrative. The production outcomes shown in figure 2.2 all points out to attainment of food security

2.8 Summary

The chapter started by defining the oyster mushroom as belonging to genus *Pleurotus* and specie *Ostreatus.* This was done to specifically highlight the mushroom under study. The chapter went on to describe the production overview of oyster mushroom which was divided into two main phases: the vegetative and reproductive phases. The vegetative consisting of mycelia expansion while reproductive phase defined as the fruiting period. This was followed by detailed description of effects of different substrates on oyster growth performance, yield and nutritional composition. This was streamlined to focus on parameters under investigation including stipe length, cap diameter and nutritional components (moisture content, ash, crude fibre, protein, carbohydrates, zinc and iron). This was further linked to literature as produced by other different authors around the globe. The issue of regional acceptance was not left out since in Africa specifically Zimbabwe, mushrooms were believe to be 'wild borne'. The chapter concluded by consolidating the concepts under study into a broader framework to have a wider view on how they fit to food security in Zimbabwe.

2.9 References

- **Beard JL (1999).** Iron biology in immune function, muscle metabolism and neuronal functioning, J. Nutr. 131: 5685-5695
- **Brown, K.H., Wuehler, S.E. and Peerson, J.M. (2001).** The importance of zinc in human nutrition and estimation of global prevalence of zinc deficiency. Food and Nutrition Bulletin, vol.22, no.2, The United Nations University.
- **Chang, S. T. (2007).** Training manual on mushroom cultivation technology, APCAEM, Accessed on 27/08/2014 from http://www.un-csam.org/publication/TM-Mushroom.pdf
- **Cho, S. B., and Kang, W. S. (2004)**. Oyster mushroom cultivation; what is mushroom. Mushroom Growers Handbook 1, MushWorld limited. Accessed on 27/08/2014 from 77
- http://www.scribd.com/doc/97323446/Mushroom-Growers-Handbook-1-Oyster-Mushroom-Cultivation
- **Dawit, A. (2008).** Mushroom Cultivation in Ethiopia: status and opportunities. Paper presented at Addis Ababa University, 15 May 2008, Science Faculty, Addis Ababa
- **Dubey, D., Dhakal, B., Dhami, K., Sapkota, P., Rana, M., Poudel, N.S. and Aryal L (2019)** Comparative study on effect of different substrates on yield performance of oyster mushroom, GJBAHS 7: 7.
- **Dunkwal V and Jood S, (2009).** Effect of substrates on nutrient composition of oyster mushroom (*pleurotus sajor caju*). Department of Foods and Nutrition, CCS Haryana Agricultural University, Hisar-125 004, India
- **Ekpo , E.N,. Olasupo, O.O. and Eriavbe, M.A (2008).** Effect of different supplement on the growth and yield of *Pleurotus florida*. Obeche Science journal .Vol 27(1): 23-25
- **Ganopedia (2011).** Classification of fungus. Word Press. Accessed on 13/12/2013 from http://ganopedia.com/2011/07/classification-of-fungus-2/
- **Kang, W. S. (2004).** What is oyster mushroom, Mushroom Growers‘ Handbook 1. Accessed on 08/06/2014 from http://www.fungifun.org/mushworld/Oyster-Mushroom-Cultivation/mushroom-growers-handbook-1-mushworld-com-chapter-3.pdf
- **Kumari, D., and Achal, V. (2008).** Effect of different substrates on the production and nonenzymatic antioxidant activity of *Pleurotus ostreatus*. *Life. Sci. J.*, 5: 73-76.

- **Kuo, M. (2011).** Mushroom taxonomy: the big picture. Accessed on 13/12/2013 from the *MushroomExpert.Com* Web site: http://www.mushroomexpert.com/taxonomy.html
- **Larkin EC, Rao CA (1990).** Importance of fetal and neonatal iron; adequacy for normal development of central nervous system, In: Brain behavior and iron in the infant diet (Dobbing J ed), London, UK pp. 43-63.
- **Malhotra VK (1998)**. Biochemistry for Students. Tenth Edition. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India.
- **Michael, H.W., Bultosa, G., & Pant, L.M. (2010).** Nutrient composition and sensory characteristics of selected edible oyster mushrooms grown on two substrates at Haramaya, Ethiopia. M.Sc. ThesisPresented to the School of Graduate Studies of Haramaya University. Haramaya
- **Nurudeen T.A; Ekpo E.N; Olasupo O.O and Haastrup N.O. (2013).** "Yield and Proximate Composition of Oyster Mushroom (Pleurotus sajor- caju) Cultivated on Different Agricultural Wastes" Science Journal of Biochemistry, Volume 2013, Article ID sjbt-189, 5 Pages, 2013. doi: 10.7237/sjbt/189
- **Obodai, M., Amoa-Awua, W. and Odamtten, G. T. (2010).** Physical, chemical and fungal phenology associated with the composting of Wawa‘ sawdust (*Triplochiton scleroxylon*) used in the cultivation of oyster mushrooms in Ghana, *International Food Research Journal* **17**:229- 237
- **Philippoussis, A., and Diamantopoulou, P. (2011).** Agro-food industry wastes and agricultural residues conversion into high value products by mushroom cultivation, National Agricultural Research Foundation. Accessed on 08/06/2014 from http://wsmbmp.org/proceedings/7th%20international%20conference/1/ICMBMP7-Oral-4-2-%20Philippoussis.pdf
- **Reilly, L., Nausch, N., Midzi, N., Mduluza, T. and Mutapi, F. (2012).** Association between Micronutrients (Vitamin A, D. Iron) and Schistosom-Specific Cytokine Responses in Zimbabweans Exposed to *Schistosoma haemoglobium.* Journal of Parasitology Research. University of Zimbabwe: 2
- **Sadrzadeh SM, Saffari Y (2004).** Iron and brain disorders. Am. J. Clin. Pathol, pp. 64-70.

- **Salama A.N.A, Abdou A.A.K, and Helaly A.A, (2016).** Cultivation of *Pleurotus ostreatus* L on different substrates based on JuNCAO technology in EGYPT. *Nature and Science*;14(4):59–66.
- **Sharma, S., Kailash, P., Yadav, R., Chandra, P. and Pokhrel, P. (2013)**. Growth and yield of oyster mushroom (*Pleurotus ostreatus*) on different substrates. *J. New Biolog. Rep*., 2(1): 03-08. doi: 10.1186/s13568-016-0265-1
- **Tan JC, Burns DL, Jones HR (2006).** Severe ataxia, myelopathy and peripheral neuropathy due to acquired copper deficiency in a patientwith history of gastrectomy. J. Paenteral Nutr. 30: 446-450
- **Wang, D., Sakoda, A.K., Suzuki M. (2001).** Biological efficiency and nutritional values of *Pleurotous ostreatus* cultivated on spent beer grain. Bioresour Technolo 78:93-300

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Introduction

This chapter focused on describing the study area, experimental design, the process of substrate preparation and the production process of oyster mushroom. Sampling techniques used in selecting samples to be studied was discussed. The methods and materials used in collecting data for the research as well as the tools used for data analysis were also looked at.

3.2 Description of study site

The research was conducted in Harare South located at coordinates 17.824858 (Latitude) and 31.053028 (longitude). The area is found in natural farming region 2b with an average annual temperature of 18.4°C and average rainfall of 831mm per year. According to Köppen-Geiger climate classification, Harare lies 1479m above sea level and its climate is classified as warm and temperate. The environmental conditions in Harare favours mushroom production as the temperatures are relatively low with annual rainfall promoting high humidity. The mushrooms were grown in a mushroom house measuring 3m x 4m made from farmer bricks and thatched roof. The temperature was maintained around 28 degrees Celsius and humidity above 80% moisture.

3.3 Experimental design

The research used randomized complete block design (RCDB) with three treatments; maize stalks, cotton husks and wheat straw, replicated three times. Level of bag placement was used as a blocking factor.

The mushroom bags (kits) were arranged in the mushroom house. The block was defined by three kits from different treatments hanged vertically one on top of the other. Each treatment was equally represented within each block. This was done to reduce errors involved during management practices such as even distribution of water during irrigation and fair air circulation, temperature and humidity distribution (see **APPENDIX 1**)

3.4 Preparation of kits used

3.4.1 Sources of substrate and spawn

Three different substrates were used in this research; maize stalks, cotton husks and wheat straw. These substrates were selected basing on their easy availability compared to other agricultural substrates. Wheat straw was procured at a road side market in Mt Pleasant as wheat bales. Maize stalks used were obtained from own local peri urban field in Harare South. The cotton husks were procured from Surface, local company producing cooking oil in Chitungwiza. *Pleurotus Ostreatus* spawn was purchased at PaHowa (SIBBS) Spawn Laboratories in Epworth, Harare.

3.4.2 Substrate preparation

The substrates used were wheat straw, maize stalks and cotton husks. Wheat straw was cut into small pieces of 3-4cm in length using a machete. The maize stalks were also cut small pieces of up to 4cm in length. Cotton husks are small in size and were used in their original form. The substrates were soaked in excess water for 24 hours. Water was then drained from the substrates and washed with clean water until clear water can be observed after passing through the substrates. The substrate was then spread on a clean surface ready for pasteurization.

3.4.3 Pasteurization

Pasteurization is the process of heating mushroom substrates in order to kill weeds, disease and pests (Kurtzman, 2010). This process is useful to reduce competitors in the substrate thereby giving the mycelia an upper hand in growth and produce more yields (Mushroom Appreciation, 2008). However, this research employed the method and used a 200liters capacity metal drum for pasteurization. The substrate was loaded into the metal drum and boiled at 100 degrees for 1hour the top covered with a black plastic.

Figure 3. 1: *Pasteurization process*

3.4.4 Spawning

After pasteurization substrate was then removed from the drum and spread on clean surface to cool. The substrate was packed in transparent polythene bags. Substrate packing and spawning was done simultaneously. Once the bags were full (kits), they were tied on top and small holes were punched around the bag to improve aeration as well as allowing effective irrigation. The bags were then transferred to the mushroom house for incubation. The time needed during incubation for the mycelia to colonize the substrate depends on the rate and distribution of spawn, the moisture content and the nature of the substrate used and temperature: as such, a completed spawn took 21 days to fully colonize the substrate. During spawn run the required temperature of 28 degrees celcius and humidity of 65-80% and water content of substrate of 65% was maintained, this was in-line with Thakkar (2010).

3.5 Sampling Procedure

Samples for determining growth rate and nutritional compositions were collected using a systematic random sampling method. Samples were taken from every 5^{th} fruiting body within a treatment. Specific mushroom was then picked at random within a fruiting body. 100g of sample was collected from each treatment for nutritional composition analysis. The size of sample to be collected for growth rate determination was depended on fruiting bodies emerged. After taking samples weight of fresh mushrooms were recorded. Then non-edible spoiled portions were separated and removed. Mushrooms were then cut into small pieces. Small amounts (2-3g) in triplicate (from each treatment) were taken for the determination of moisture. The same amounts were also weighed for the determination of protein, carbohydrate, fat, fiber, total ash and minerals (Zn and Fe).

3.6 Data collection procedure

3.6.1 Growth and Yield level

The average growth rate of Oyster mushroom was measured as stipe length and average diameter of mushroom cap. The stipe length as measured in centimetres using a ruler starting from the point of attachment on junction up to where it attaches the cap. Both stipe length and cap diameter were measured as averages. The yield level was measured as total yield weight produced from different mushrooms in different substrates. The total yield determined by summing up the weight of oyster harvested from first, second and third flushes.

3.6.2 Nutritional composition

Analytical methods Standard procedures of AOAC were used to determine the moisture content, crude fibre, crude fat, total nitrogen (Kjeldahl method) and ash (AOAC, 2002). In the fruit body of edible mushrooms, a large amount of nitrogen is actually contained in non-protein compounds; hence, the conversion factor of total nitrogen into crude protein is 3.45 to 4.38 (Braaksman and Schaap, 1996; Shah et al., 1997). In this study, crude protein was calculated using the conversion factor of (N x 4.38); a correlation factor adopted for mushrooms in food composition tables (Crisan and Sands, 1978). Mineral constituents (zinc and iron) were determined by atomic absorption spectrophotometry (AOAC, 2002). The percentage of crude protein, crude fat, minerals and ash were combined and subtracted from 100 to obtain the total carbohydrate percentage for each sample.

3.6.2.1 Determination of Moisture Content

Moisture content was determined by AOAC (2005) by drying the sample in an oven until a constant weight was obtained. Two grams (2 g) of mushroom in triplicates were accurately weighed into previously dried and crucibles. The crucibles with the samples were then placed in thermostatically controlled oven (Gallenkamp, England) at 105 °C overnight till a constant weight of solid material was obtained. The crucibles were then removed and cooled in a desiccator and then weighed.

Calculation of moisture content

$$(\%)\ \text{moisture} = \left[\frac{W_1 - W_2}{W_1}\right] x100$$

Where, w_1 = Weight (g) of original sample

w_2 = Weight (g) of sample after drying

3.6.2.2 Determination of Crude Protein Content

Crude protein was determined by AOAC (2005). Two grams (2 g) of mushroom in triplicates was placed in a Kjeldahl digestion flask also containing a Selenium based catalyst and 25 ml of concentrated H2SO4 added in a fume chamber. The flask was swirled gently to effect proper mixing and heated in a digestion chamber until digestion was complete after 3 hours. The digest was cooled and transferred into a 100 ml volumetric flask and made up to the mark with distilled water. Ten milliliters (10 ml) of the diluted digest was put in the steam distillation unit, which was previously flushed with distilled water. 18 ml of 40% NaOH was then added to the solution in the steam distiller. Twenty milliliters (25 ml) of 2% boric acid was pipetted into a conical flask and two drops of bromocresol green- methyl red mixed indicator added. This mixture was placed under the condenser outlet of the distillation system, with the tip of the condenser completely immersed in it. The distillation was carried out until all the boric acid solution turned from pink to yellowish green. The solution in the conical flask was titrated against 0.1 M HCl solutions and the end point recorded. The distillation and titration processes were done with triplicate samples of the diluted digest. A blank was also taken through the same procedure using distilled water in place of the sample. The crude protein content was then calculated using a factor of 6.25.

Calculation of crude protein content

(%) Crude Protein = $\left[\frac{(A-B}{W}\right]$ x Nx6.25x100

Where, A = volume (ml) of 0.2N HC L used sample titration.

B = Volume (ml) of 0.2N HCL used in blank taken

N = normality of HCL

W= weight (g) of sample

6.25 = the protein nitrogen conversion factor f

3.6.2.3 Determination of Crude Fat Content

Crude fat was determined based on the soxhlet extraction method of AOAC (2005). Two grams (2 g) of the dried sample was weighed into each of two paper thimbles. The thimbles were sealed and placed in soxhlet extractors. Fifty milliliters (50 ml) of petroleum ether was poured into each of the previously dried and weighed round-bottomed flasks attached to the extractors. Extraction was carried out for 4 h. After this the petroleum ether was recovered from the soxhlet with only small amounts left in the flasks. The flasks were then removed and placed in an oven (with the door partially closed) for the ether to completely evaporate. The flasks were cooled in a desiccator, weighed and the fat content calculated.

Calculation of crude fat content

(%) Crude Fat = $\left[\frac{W3-W2}{W3}\right]$ x100

w_2 = Weight (g) of sample after drying

w_3 = Weight of original sample

3.6.2.4 Determination of Ash Content

Ash was determined by AOAC (2005). Two grams (2 g) of sample was weighed into previously dried and weighed porcelain crucibles and heated for about 20 min over a boiling water bath till they were visibly dry. The crucibles with their contents were then transferred into a muffle furnace (Gallenkamp, England) at 600 °C and incinerated for 5 hours. The crucibles were

removed, placed in a desiccator to cool then weighed and the ash content calculated and expressed as a percentage.

Calculation of ash content

$$\text{Percentage (\%) ash} = \left[\frac{\text{weight of ash}}{\text{weight of original sample}}\right] \text{x}100$$

3.6.2.5 Determination of Crude fibre

Crude fibre will be determined as cited by Iqbal *et al* (2016). The oyster mushroom sample (5g from each substrate) will be heated with 200ml of sulphuric acid solution at 80degrees Celsius for 30mins. The samples will then be washed with hot water to remove free acid. The sample will then be heated again with 200ml sodium hydroxide at 80degrees Celsius. Sample will be washed 3 to 4 times with hot water to remove alkali. A muslin cloth will be used to filter the mushroom samples which will then be allowed to dry. The samples will be taken to a furnace where they will be heated at 550 degrees Celsius for 4hours until ash turns grey, then weighed. The crude fibre will be calculated using the formula:

Calculation of crude fibre

$$\text{Crude fiber \%} = \left[\frac{\text{dry weight after digestion} - \text{weight of ash}}{\text{weight of moisture and fat free sample}}\right] \text{x}100$$

3.6.2.6 Determination of Total Carbohydrate Content

The content of the available carbohydrate was determined by the following equation following Raghuramulu et al., 2003.

$$\text{Carbohydrate (g/100g)} = 100 - [\text{Moisture} + \text{Protein} + \text{Fat} + \text{Ash} + \text{Fibre}]$$

3.6.2.7 Determination of Gross Energy

A bomb calorimeter was used to measure the energy.

3.6.2.8 Determination of micronutrients in mushrooms.

The sample was digested with nitric acid to release Fe and Zn, which were determined by atomic absorption. Instrument used was Shimadzu AA-6701F Atomic Absorption/Flame Emission Spectroctrophotometer, Serial number A3042-3400190SU

3.7 Data analysis procedure

The data to be collected will be analyzed using statistical methods in SPSS version 16.0 software and Microsoft office excel 2010. The analysis of variance will be conducted a means will also be separated and compared by least significant difference (LSD) at 5% significance level for the tests (Bhattacharjya et al, 2015).

3.8 Ethical considerations

According to Plummer (2001) ethics are central to data collection methods in every piece of research. Ethics has to be observed in research (Burgess, 1987). During the experiment the author will respect intellectual property rights and acknowledge works done by other writers. The researcher will give objectivity a priority in order to maintain relevance of the experiment. Honesty and integrity will be respected which allows for correct and accurate data collection. It is imperative to consider these ethics to promote validity of the experiment.

3.8 Summary

The chapter provided details on the item under study, parameters involved and how they were measured. This was incorporated under research area, experimental design, methods of data collection, data analytical tools used, to mention a few. The chapter also looked at procedure carried out in setting up the experiment such as preparation of kits used in the study. All parameters for nutritional analysis were described and methodologies for their determination were explained. The chapter concluded by describing the ethics which were observed during the study.

3.9 References

AOAC (2005). Official Methods of Analysis -17th ed. Association of Official Analytical Chemist, Maryland

AOAC (Association of Official Analytical Chemists). (2000). Official Methods of Analysis. 17th ed., Gaithersbersburg MD. Accessed on 27/08/2014 from http://www.aoac.org/iMIS15_ Prod/AOAC.

Bhattacharjya, D.K, Paul R.K and Ahmed K.U, (2015). Effect of different saw dust substrates on the growth and yield of oyster mushroom (*Pleurotus ostreatus*).Iosr Journal of Agriculture and Veterinary Science (IOSR-JAVS) e-ISSN:2319-2380.

Braaksman, A., Schaap, D.J. (1996). Protein analysis of the common mushrooms Agaricus bisporus. Post. Harv. Biol. Technol. 7: 119-127.

Crisan, E.V., Sands, A. (1978). Nutritional value. In: Chang ST and Hayes WA (Eds). The Biology and Cultivation of Edible Mushrooms. London, Academic Press Inc, pp 137-165

Iqbal, S. M, Rauf, C. A. and Sheikh, M. I, (2016). Yield Performance of Oyster Mushroom on Different Substrates, *International Journal of Agriculture & Biology*, Vol. 7, No. 6, 900–903.

Mushroom Appreciation (2008). How to pasteurize straw for growing mushrooms. Accessed from http://www.mushroom-appreciation.com/pasteurize.html on 26/01/2014

Plummer, K. (2001). Document of Life2: An Invitation to a Critical Humanism, Revised Edition. London: Sage Publication.

Shah, H., Khalil, I.A., Jabeen, S. (1997). Nutritional composition and protein quality of Pleurotus mushroom. Sarhad J. Agric. 13:621-627

Thakkar, N. (2010). Cultivation of oyster mushroom and performance of its protein analysis, Accessed on 28/01/2014 from http://www.researchgate.net

CHAPTER 4

RESULTS (Growth and Yield of Oyster Mushrooms)

Abstract

There was a significant difference in mean for stipe length and cap diameter was p=0,001 and p=0.000 respectively at p<0.05 level. For total yield (p=0.896), the differences in mean were not significant at p<0.05 thus it was analyzed using descriptive statistics on stipe length and cap diameter of oyster mushrooms from maize stalks, cotton husks and wheat straw. The p values for total yield was more than 0.05 meaning there was no significant differences between the means, it occurred only by chance. However where the actual difference lays was determined through post hoc comparison to evaluate pair-wise differences between stipe length, cap diameter and total yield and the treatments using Tukey test. All the mean differences of parameters were very significant at p<0.05 level, however, the p values of total yield was ranging from 0.804 to 0.986 (**APPENDIX 9**). Descriptive statistics and literature review were used. The results on stipe length, cap diameter and total yield is presented in the following subsection. It is recommended to grow oyster mushroom on cotton husks in order to realize higher growth and finally total yield.

Key Terms: Stipe length, cap diameter, total yield and *Pleurotus ostreatus*.

4.1 Introduction

The cultivation of *Pleurotus ostreatus* is one of the profitable agribusiness for small scale farmers. Oyster mushroom belongs to class Basidiomyccets, subclass Hollobasidiomycetidae and order Agricals. They are edible with excellent taste and flavour. Most organic matters containing cellulose, hemicelluloses and lignin can be used as mushroom substrate, for example, rice straw, cotton seed hulls, corncob, heat straw, waste paper, leaves and so on (Sharma *et al* (2013). Oyster mushrooms need less of nitrogen and more of carbon materials for optimal growth. The demand for oyster mushroom has been on a steep rise in recent years with population growth, market expansion and changes in consumer preferences. However, this section focused on different lignocelluloses; maize stalks, cotton husks and wheat straw as substrate that can be used in oyster production and investigate how they affect the growth performance and yield of oyster

mushrooms. This was narrowed to focus on stipe length and cap diameter to measure growth performance and yield weigh for the yield.

4.2 Material and Methods

P. *ostreatus* spawn was purchased at PaHohwa (SIBBS) Spawn Laboratories in Epworth. The maize stalks were gathered from local peri urban fields in Harare South, cotton husks were purchased at Surface in Chingwiza and wheat straw was also purchased at a roadside market in Mt Pleasant Harare. The maize stalks and wheat straws were chopped into 2-4cm small pieces before pasteurized and spawned into kits. The kits were store under incubation for 21 days to allow for mycelia development and full colonization of the substrate. After 21 day the kits were hang on bamboos in the mushroom house following a randomized complete block arrangement.

4.3 Description of study area

The study was conducted in Harare South in a mushroom house built from farm brick and a thatched roof with a bamboo ceiling to hang the prepared kits. The mushroom house measured 3m x 4m. Harare South is located at coordinates 17.824858 (Latitude) and 31.053028 (longitude). The area is found in natural farming region 2b with an average annual temperature of 18.4°C and average rainfall of 831mm per year. Inside the mushroom house, temperature was maintained around 28 degrees Celsius and humidity above 80% moisture. The humidity was maintained by putting sand on the floor and water regularly. The light was only allowed inside the house to initiate fruiting.

4.4 Research Design

The study was set up as a randomized complete block design (RCBD). There were three treatments; maize stalks, cotton husks and wheat straw which were replicated three times. The level of kit placement was used as a blocking factor.

4.5 Sampling procedure

The systematic random sampling procedure was employed in selecting samples to be measure in terms of stipe length and cap diameter. Labels for each kit per treatment was placed in a hat and picked at random. The fruiting bodies were randomly selected from the kit, this was the starting point, and every 5^{th} fruiting body was measured for the stipe length and cap diameter. The averages were recorded. This was done just before harvesting. The measurements were taken three times (meaning 3 flushes) and the average recorded.

4.6 Data collection procedure

4.6.1 Stipe Length

The stipe length was measured from the base of the stipe to where it attaches the pileus (cap) using a transparent ruler. The stipe length was taken from at least two fruiting bodies per bag of each treatment. All the recordings were averaged.

4.6.2 Cap Diameter

The cap diameter was measured using a string and a ruler to improve on accuracy. The sample to be measured was selected using systematic random sampling procedures and at least two fruiting bodies per of each treatment was measured. The measurements were then averaged.

4.6.3 Yield level

The yield was collected in all three treatments; three times from three flushes considered. This was weighed using an electronic digital scale. Prior to weighing all the non edible materials were removed.

4.7 Data analysis procedure

The data was analyzed using descriptive statistics from SPSS version 16.0. Additionally the statistical evaluations were done using ANOVA and the comparison of mean realized by Tukey LSD at $p<0.05$

4.8 Challenges encountered during data collect

The fruiting bodies have mushrooms that may have wider variation in terms of stipe length and cap diameter. The COVID 19 movement restrictions made it difficult for researcher to have more physical consultations to supervisors and other stakeholders. Contamination of kits caused a lot of challenges as first and second attempt failed.

4.9 Results and Discussion

4.9.1 Effect of substrate type on stipe length

Table 4.1 presents the average stipe length from all the treatments; maize stalks, cotton husks and wheat straw.

Table 4.1: *The average stipe length (cm)*

Substrate	**Stipe Length**
Cotton husks	4.55 ± 0.14^{a}
Wheat straw	3.89 ± 0.18^{b}
Maize stalks	3.43 ± 0.22^{b}

Means with different letters are significantly different according to Tukey's HSD test at the 0.05 level ($p < 0.05$).Stipe length data is expressed as mean±standard deviation of mean (SDM);

The type of substrate used in oyster mushroom production had an effect on stipe length ($p<0.05$). Oyster from cotton husks had the longest mean stipe length (4.55cm) compared to maize stalks with the shortest length (3.43cm) and wheat straw (3.89cm). According to a study by Salama *et al* (2013) the highest value of stipe length was found from corn cop substrate which recording the number of 3.18cm while wheat straw recorded the shortest stipe length of 2.60cm. This has contradicted with the result findings. The variations in these parameters maybe attributed to texture and substrate formulations as well as nutrients in these substrates possibly affecting the composition of the final mushroom growth and qualities such as water holding capacity and degree of aeration (Kurtman, 2010).

4.9.2 Effect of substrate type on cap diameter

Table 4.2: *The average cap diameter (cm)*

Substrate Type	**Cap diameter**
Cotton husks	9.25 ± 0.09^{a}
Maize stalks	7.33 ± 0.25^{b}
Wheat straw	4.33 ± 0.22^{c}

Means with different letters are significantly different according to Tukey's HSD test at the 0.05 level ($p < 0.05$).Cap diameter data is expressed as mean±standard deviation of mean (SDM)

The average cap diameter of oyster mushroom produced from maize stalks, cotton husks and wheat straw substrates was recorded in Table 4.2. The substrate type had an effect on cap diameter in oyster mushroom production at $p<0.05$. The p values on cap diameter ($p=0.000$) proved that there is strong significant difference in mean testing at 5% significance level (**APPENDIX 9**). Oyster with the widest cap diameter was produced using cotton husks as substrate (9.25cm) followed by that from maize stalks (7.33cm) and lastly from wheat straw (4.31cm). The substrate type had an effect on average cap diameter ($p<0.05$). The mean difference in cap diameter of oyster mushroom from maize stalks and cotton husks was 1.92 ($p=0.000$), maize stalks and wheat straw 3.00 ($p=0.000$) and cotton husks and wheat straw 4.92 ($p=0.000$). The results showed that substrate type proved greater significant differences in mean from oyster produced on cotton husks and wheat straw. Slight difference in mean was observed on oyster from maize stalks and cotton husks. There is a positive linear relationship between cap diameter and the final total yield. According to Nurudeen (2018) oyster mushrooms from maize residues can have a mean cap diameter of 5.1-7.2cm which is not significantly different from the experiment findings.

4.9.3 Effect of substrate type on total yield

You have to separate the means, 950, 860 and 830 using the LSD as you did for the other parameters, if you can, you can separate the means by flush or you can separate by total yield

Table 4.3: *The total yield (grams)*

Substrate Type	**Total Yield**
Cotton husks	950
Wheat straw	860
Maize stalks	830

Means with different letters are significantly different according to Tukey's HSD test at the 0.05 level ($p < 0.05$).Total yield data is expressed as mean±standard deviation of mean

Harvesting was done in three flushes and recorded in table 4.3 below. The p value for total yield (ranging between 0.804 and 0.986) is greater than 0.05, then there was no significant difference in yields of the three substrates ($p>0.05$). According to Wang (2010), wheat straw substrate was found to be very susceptible to drying and this was further supported by Royes (2002) adding that this affects sporophore formation. Putting this together with wheat straw poor water holding capacity, it all contributes to loss of fruiting body weight. He *et al* (1995) added that cotton husks high total yield may be attributed to increased water holding capacity and reduced the mortality of young fruiting bodies due to water shortages. Yang *et al* (2013) noted that cereals such as rice straw, wheat straw and maize residues dries up quickly due to their physical nature and high porosity resulting in reduced total yield of mushrooms. However, in a study by Ipbal *et al* (2016) different substrates were evaluated on growth performance of oyster and it was concluded in all three flushes wheat straw produced a total yield of 1360g followed by rice straw with 1230g the sorghum and maize straw with total yield of 1165g and 960g respectively. The contradiction of these results and own findings may be attributed to different environmental conditions in which the experiment was exposed.

4.10 Conclusion

Oyster mushrooms produced from cotton husks have a higher average growth performance in terms of oyster mushroom from maize stalks and wheat straw. There is a positive linear

relationship between stipe length and total yield and also between average cap diameter and total yield.

4.11 Recommendations

It can be deduced that the average stipe length and average cap diameter has a linear positive relationship with the total yield. This has been shown by oyster from cotton has having longer average stipe length (4.55cm), wider average cap diameter (9.25cm) and absolutely greater total yield (950g). It is therefore important to consider mixing the substrate i.e. wheat straw produces nutritious oyster and cotton holds more water and reduces mortality rate of fruiting bodies. Environmental conditions as well as supplementation of substrates with various additives including nitrogen sources have been reported to improve growth, yield and quality of mushrooms (Royes, 2002)

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4.12 References

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He, H.Q., Bao, D.P., Wen, M.Z., Lu, X.M., (1995). Test on cultivation Pleurotus streatus with different formula of straw and cotton seed hulls. J. Anhui Agrotechnical Teach. Coll. 9, 36–44.

Kurtzman, R.H. (2010). Ventilation for mushroom cultivation: the importance of the needs of mushrooms and of the gas laws. Micol.Apl. Int, 22 (2): 63-78

Royes, D.J., (2002). Influence of spawn rate and commercial delayed release nutrient levels on Pleurotus cornucopiae (oyster mushroom) yield, size and time of production. Appl. Microbiol. Biotechnol. 58, 527–531.

Yang, W., Guo, F.L. and Wan, Z. (2013). Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. Institute of Cash Crops, Hubei Academy of Agricultural Sciences, Wuhan 430064, China

Wang, B.F., (2010). Use of wheat straw residue from a paper mill for Pleurotus ostreatus cultivation. Acta Edulis Fungi 17, 30–31.

CHAPTER 5

RESULTS (Nutritional Analysis of oyster from different substrates)

Abstract

This section of the study focused on the nutritional composition of oyster produced from different substrate type. Oyster mushroom was grown in a 3m x 4m mushroom house made from farm bricks and thatch. Temperature in the mushroom house was maintained around 28 degrees Celsius, humidity (80%) and light was only allowed to initial fruiting. Samples were randomly taken, contaminants removed and sample weighed. Results showed that there were significant differences in mean of all nutritional parameters; energy, protein, carbohydrates, ash, fat, crude fibre, moisture content, zinc and iron ($p<0.05$). Oyster from wheat straw had a significant higher nutritional content in energy (29.75 calorie/100g), protein (2.66g/100g), fibre (3.41g/100g), ash (1.31g/100g) fat (1.56g/100g), zinc (9.46mg/100g) and iron (18.39mg/100g). Oyster mushroom produced on cotton husks had the highest moisture content (91.91%) while that from wheat straw only had the lowest (89.08%) ($p<0.05$). Oyster mushroom from maize stalks was significantly higher in carbohydrates (1.32g/100g) compared to that from wheat straw (1.26g/100g) and cotton husks (1.03g/100g) ($p<0.05$). It was concluded that oyster produced on wheat straw was more nutritious than that from maize stalks and cotton husks. Farmers are therefore recommended to grow oyster mushroom on wheat straw.

- **5 keywords**: Nutritional composition, maize stalks, cotton husks, wheat straw and oyster mushroom

5.1 Introduction

This chapter focused on the nutritional composition of oyster mushroom produced from maize stalks, cotton husks and wheat straw. The main parameters looked at were protein content, carbohydrates, moisture content, ash, energy, fat, crude fibre and micronutrients (zinc and iron). The results were related to literature already in existence. The study area was discussed together with research design used and sampling procedures. The challenges faced during the course of research were also aired as well as the recommendations.

5.2 Material and Methods

The materials used from the beginning of the research include; spawn, maize stalks, cotton husks, wheat straw, polythene bags, knapsack sprayer, mushroom house, electronic scale and chlorine as disinfectant. This was presented in detail in **Table 5.1** below;

Table 5. 1: *Materials used per each production level*

Level of production	**Materials used**
1. Substrate preparation	Maize stalks, cotton husks, wheat straw
2. Soaking	Metal drum, water, filtering cloth, machete
3. Pasteurization	Metal drum, fire wood, hot water, stone to avoid floating
4. Spawning	Spawn, polythene bags, strings
5. Cropping and harvest	Sharp knife, dish, scale

5.3 Description of study area

The whole study was conducted as a two phase study, were there was production of oyster in Harare South and analysis of oyster samples at the University of Zimbabwe. Both areas are found in natural farming region 2b with an average annual temperature of 18.4°C and average rainfall of 831mm per year. The area is 1479m above sea level. A 3m x 4m mushroom house was used which made from local farm bricks and a thatched roof. The analysis of sample for nutritional composition was conducted at the University of Zimbabwe, institute of food, nutrition and family science laboratory. This is in region 2b with environmental conditions similar to those were production was carried out. Inside the mushroom house, temperature was maintained around 28 degrees Celsius and humidity above 80% moisture. The humidity was maintained by putting sand on the floor and water regularly. The light was only allowed inside the house to initiate fruiting.

5.4 Research Design

The experiment used the randomized complete block design (RCBD). It had three treatments: maize stalks, cotton husks and wheat straw. It was replicated three times and the level of bag placement was used as a blocking factor

5.5 Sampling procedure

The sample were collected from each treatment were the first fruiting body was randomly selected and this was followed by a systematic selecting of fruiting bodies were every fifth one was taken. 100g of sample from each treatment was weighed and labeled then taken to the laboratory for nutritional composition analysis. Then non-edible spoiled portions were separated and removed. Mushrooms were then cut into small pieces. A small amount (2-3g) in triplicate (from oyster from maize stalks, cotton husks and wheat straw) were taken for the determination of moisture. The same amounts were also weighed for the determination of protein, carbohydrate, fat, fibre, total ash and minerals.

5.6 Data collection procedure

For the determination of nutritional composition, a wide range of laboratory apparatus was used for specific nutritional parameter to be determined. This is shown in the following sections;

5.6.1 Determination of Moisture Content

Materials used include: spatula, crucible, electronic oven and electronic scale. The moisture content was determined following AOAU (2005) procedures. The sample was dried in an oven until a constant weight was determined. Then 2g of mushroom was taken from each treatment i.e. maize stalks, cotton husks and wheat straw in triplicates. The triplicates were defined as flushes meaning three flushes of sample were taken from each treatment. Flushes were spaced by 10 days, from pin formation to harvesting of oyster mushrooms. The samples were accurately weighed into previously dried crucibles which were then placed in thermostatically controlled oven at 105 degrees Celsius overnight until a constant weight was obtained.

5.6.2 Determination of Crude Protein Content

The materials used include: electronic scale, Kjeldahl digestion flask, selenium, sulphuric acid, 100ml volumetric flask, steam dilation unit, sodium hydroxide, boric acid, pipette, biuret, conical flask, bromocresol green, crucible, hydrochloric acid and distilled water. Crude protein was determined by AOAC (2005). The Kjeldahl method was used to determine the crude protein content calculated using a factor of 6.25. This is a worldwide used method for standard analysis of protein in food

5.6.3 Determination of Crude Fat Content

The materials used include: sample, electronic scale, paper thimbles, soxhlet extractor, petroleum ether, round bottomed flask and even. Crude fat was determined based on the soxhlet extraction method of AOAC (2005). Two of paper thimbles were filled with 2g sample which were then sealed and put in soxhlet extractor. 50ml of petroleum ether was added. Weighed round bottomed flask was attached to extractors. Petroleum ether was recovered from soxhlet with small amounts left in the flask.Extraction was done in 4hours and flasks removed and and placed in an oven for ether to evaporates.

5.6.4 Determination of Total Ash Content

The materials used include: sample, porcelain crucibles, electronic scale, desiccators and furnace Ash was determined by AOAC (2005). Two grams (2 g) of sample was weighed into previously dried and weighed porcelain crucibles and heated for about 20 min over a boiling water bath till they were visibly dry. The crucibles with their contents were then transferred into a muffle furnace at 600 °C and incinerated for 5 hours. The crucibles were removed, placed in a desiccator to cool then weighed and the ash content calculated and expressed as a percentage.

5.6.5 Determination of Carbohydrates

The carbohydrates from oyster mushroom samples was determined through calculations where the summation of moisture content, total ash, crude fiber, protein and fat was subtracted from hundred Raghuramulu *et al*., 2003 .

5.6.5 Determination of Energy

The energy content was determined by a bomb calorimeter and energy emitted was measured form the triplicates of sample.

5.6.6 Determination of Micronutrients of mushrooms

The sample was digested with nitric acid to release Fe and Zn, which were determined by atomic absorption. Instrument used was Shimadzu AA-6701F Atomic Absorption/Flame Emission Spectroctrophotometer, Serial number A3042-3400190SU

5.7 Data analysis procedure

In all the experiments the samples from oyster mushroom in three treatments were analyzed in triplicates. The results were expressed as mean values ± standard deviation (SD). The analysis started by checking the descriptive table from SPSS output for the number of samples (n). The

test for homogeneity was conducted using Levene Statistic test to check for significance difference of variables. The samples were then analyzed using one-way ANOVA followed by Tukey's test for significant difference under Post Hoc test with $\alpha = 0.05$. The whole analysis was conducted in SPSS version 16.0.

5.8 Challenges encountered during data collect

The production of oyster mushroom was affected by contamination of kits resulting in two attempts failing completely. This was due to poor quality of spawn procured. The third attempt was successful as a reputable supplier of spawn was engaged. The experiment was also greatly affected by COVID 19 restriction which made it difficult to move around especially for the laboratory activities.

5.9 Results and Discussions

5.9.1 Nutritional analysis of oyster mushroom from different substrates

The results for nutritional parameters were presented and discussed in the following subsections.

5.9.1.1 Energy content

Table 5. 2: *Energy content*

Parameter	**Wheat straw**	**Maize stalk**	**Cotton husks**
Energy (calories/100g)	29.75 ± 0.17^{a}	27.60 ± 0.22^{b}	21.09 ± 0.18^{c}

Means with different letters are significantly different according to Tukey's HSD test at the 0.05 level ($p < 0.05$).Energy data is expressed as mean±standard deviation of mean (SDM)

Oyster produced from wheat straw showed energy level of 29.75 calories/100g which was significantly higher than that from maize stalks (27.60 calories/100g) and cotton husks (21.09 calories/100g) ($p<0.05$). The total energy contribution of the sample fruit bodies of oyster mushroom ranged between 22.31calorie/100g-26.82calorie/100g in fruit bodies for oyster mushroom (Salama *et al*, 2016). In a study by Sharma *et al* (2013) the amount of energy varied between substrates, rice straw recording 24.83calorie/100g, wheat straw recording 25.32calorie/100g and sugarcane bagasse 2.67calorie/100g. These energy values were slightly

lower compared to experiment findings. This can be attributed to chemical composition of substrates which varies.

5.9.1.2 Protein content

Table 5. 3 *Protein content*

Parameter	**Wheat straw**	**Maize stalk**	**Cotton husks**
Protein (g/100g)	2.66 ± 0.01^{a}	2.52 ± 0.03^{b}	2.09 ± 0.01^{c}

Means with different letters are significantly different according to Tukey's HSD test at the 0.05 level ($p < 0.05$).Protein content data is expressed as mean±standard deviation of mean (SDM)

The protein content is a very important nutritional parameter and its deficiency is the most serious human problem including malnutrition especially in less developed countries such as Zimbabwe. Findings from this study indicate that substrate type had an effect on protein content. Oyster produced from wheat straw had a significantly higher protein content of 2.66g/100g compared to that from maize stalks (2.52g/100g) and cotton husks (2.09g/100g) ($p<0.05$). The protein content of oyster grown on maize stalks was significantly higher than that from cotton husks ($p<0.05$). The differences in protein content may be attributed to differences in Carbon to Nitrogen (C/N) ratio where by wheat straw having lesser C/N ratio compared to maize straw and cotton husks. This was further supported by Yehia (2012) noting that C/N ratio is inversely proportional to protein content. This was further supported by Rai (1995) who reported that chitin nitrogen is responsible for high protein values derived with usual 6.25 factor. However, the protein content may depend on other factors such as pileus size, cultivation time and strains (Yehia, 2012).

5.9.1.3 Carbohydrates

Table 5. 4: *Carbohydrates*

Parameter	Maize stalks	Wheat straw	Cotton husks
Carbohydrates (g/100g)	1.32 ± 0.02^{a}	1.26 ± 0.02^{b}	1.03 ± 0.02^{c}

Means with different letters are significantly different according to Tukey's HSD test at the 0.05 level ($p < 0.05$).Carbohydrates data is expressed as mean±standard deviation of mean (SDM)

Carbohydrate is an important constituent of mushroom and represents the majority of mature bodies accounting for 0 to 64 on a dry matter basis (Nasiruddin *et al*, 2018). Carbohydrate is an important source of energy for metabolic processes in human body. Results from this study showed that substrate type had an effect on the amount of carbohydrate produced in oyster mushrooms. Oyster from maize stalks produced a significantly higher carbohydrate content (1.32g/100g) compared to that from cotton husks (1.03g/100g) and wheat straw (1.26g/100g) ($p<0.05$). Oyster mushroom produced from wheat straw had a significantly higher carbohydrate content compared to that from cotton husks ($p<0.05$). Higher carbohydrate content in oyster mushroom from maize stalks compared to both wheat straw and cotton husks may be attributed to higher C/N ratio in the substrate (Yehia, 2012).

5.9.1.4 Fat

Table 5. 5: *Fat*

Parameter	Wheat straw	Maize stalks	Cotton husks
Fat (g/100g)	1.56 ± 0.03^{a}	1.35 ± 0.02^{b}	0.96 ± 0.02^{c}

Means with different letters are significantly different according to Tukey's HSD test at the 0.05 level ($p < 0.05$).Fat content data is expressed as mean±standard deviation of mean (SDM)

The primary role of fats in the human body is to provide energy for the muscles and metabolism, regulate temperature, aid proper digestion and absorption of nutrients (Khydagi *et al*, 2012). The results indicated that there is a significant difference in fat content of oyster mushrooms

produced from different substrate types. Oyster mushroom from wheat straw had a significantly higher fat content (1.56g/100g) compared to that from maize stalks and cotton husks. The fat content of oyster mushroom grown on maize stalks (1.35g/10g) was significantly higher than that from cotton husks (0.96g/100g). The results were contrary to Wang *et al* (2001) who pointed out that fat ranges from 2.5-2.8g/100g in oyster mushrooms. The variations in findings may be attributed to differences in research methodologies employed. However, the findings were closer to those obtained by Kinge *et al* (2016) who highlighted that the value of fats in oyster mushrooms ranges from 1.97-4.62g per 100g of matter. Furthermore and similarly to this study, Kinge *et al* (2016), confirmed that fat content can be affected by type of substrate used. The fat content from this study concurred significantly with previous results from Chang et al (1981) who showed that fat content from different substrates can range from 1.1-8.0g/100g. In a similar experiment by Yehia (2012) the highest fat content was obtained from wheat straw substrate with records of 0.42g/100g while the lowest fat content was found with maize residue substrate which recorded 0.19g/100g.

5.9.1.5 Crude fibre

Table 5. 6: *Crude fibre*

Parameter	**Wheat straw**	**Maize stalk**	**Cotton husks**
Crude fibre (g/100g)	3.41 ± 0.01^{a}	3.35 ± 0.01^{b}	2.81 ± 0.02^{c}

Means with different letters are significantly different according to Tukey's HSD test at the 0.05 level ($p < 0.05$).Crude fibre data is expressed as mean±standard deviation of mean (SDM)

The results obtained from this study showed that there is a significant difference crude fibre content of oyster mushroom produced from different substrates. The experiment findings have shown that oyster mushroom from wheat straw had a significantly higher crude fibre (3.41g/100g) content than that from cotton husks (2.81g/100g). Crude fibre from maize stalks was slightly close to that of wheat straw (3.35g/100g), possibly, this can be attributed to the same family they belong. According to a study by Khydagi *et al* (2012) wheat straw substrate produced 5.42g/100g of crude fibre compared to soya bean with 5.02g/100g and the lowest crude fibre content was produced in corn cob with only 3.43g/100g. The results obtained by Khadagi et al contradicted with this experimental findings on oyster from wheat straw (5.42g/100g and

3.41g/100g respectively) and for maize residues the results pointed in one direction (3.43g/100g and 3.35g/100g) with a close to insignificant difference. The rich raw substrate in fibre content may give large quantities to be consumed by the growing mushroom fruiting bodies (Yehia, 2012). Patil (2012) highlighted that the variation in fibre might be due to the quality and quantity of fibre available in substrates

5.9.1.6 Total ash

Table 5. 7: *Total ash*

Parameter	**Wheat straw**	**Maize stalk**	**Cotton husks**
Total Ash (g/100g)	1.31 ± 0.02^{a}	1.23 ± 0.01^{b}	0.70 ± 0.01^{c}

Means with different letters are significantly different according to Tukey's HSD test at the 0.05 level ($p < 0.05$).Total Ash data is expressed as mean±standard deviation of mean (SDM)

The results showed that substrate had an effect on the total ash ($p<0.05$). The research found out that oyster from wheat straw substrate had a significantly higher ash content (1.31g/100g) compared to that from maize stalks (1.23g/100g) and cotton husks (0.7g/100g). According to a study by Nasiruddin et al (2018) oyster ash content recorded ranged between 6.41 to 5.66g/100g. The findings contradicted significantly and this may be due to supplementation of rice bran and cotton seed hulls to maize residues and wheat straw. The supplementation improves on substrate nutritional component and water holding capacity.

5.9.1.7 Moisture content

Table 5. 8: *Moisture content*

Parameter	**Cotton husks**	**Maize stalk**	**Wheat straw**
Moisture content (%)	91.91 ± 0.03^{a}	90.10 ± 0.00^{b}	89.08 ± 0.03^{c}

Means with different letters are significantly different according to Tukey's HSD test at the 0.05 level ($p < 0.05$).Moisture content data is expressed as mean±standard deviation of mean (SDM)

The results showed that substrate type had an effect on the moisture content of oyster mushroom produced ($p<0.05$). *Pleurotus Ostreatus* with the highest percentage moisture content was

produced from cotton husks (about 91.91%) followed by that from maize stalks with 90.10% and lastly wheat straw with only 89.08%. This may be attributed to the fact that cotton husks have the ability to retain water for a long time after irrigation implying a consistent supply of it to fruiting bodies. This was supported by a study by Zahid *et al* (2010) showing that moisture content in mushrooms was found to be in a range of 85.95-90.07g %. Moisture content also varied with cropping and watering conditions and type of substrate used (Gupta *et al.* 2004) during cultivation. Moisture content can also be influenced by mushroom age, growing environments, mushroom strains and post harvest environments. The lowest moisture content observed in oyster from wheat straw maybe due poor nature of the substrate in water holding capacity compared to others.

5.9.1.8 Micronutrients in Oyster Mushroom

Table 5. 9: *Micronutrients*

Parameter	**Wheat straw**	**Cotton husks**	**Maize stalk**
Zinc (mg/100g)	9.46 ± 0.00^{a}	6.99 ± 0.03^{b}	5.01 ± 0.06^{c}
Iron (mg/100g)	19.39 ± 0.01^{a}	16.10 ± 0.01^{b}	13.09 ± 0.01^{c}

Means with different letters are significantly different according to Tukey's HSD test at the 0.05 level ($p < 0.05$).Zinc and Iron data is expressed as mean±standard deviation of mean (SDM)

The research findings proved that the substrate type had a significant effect on zinc and iron concentration ($p<0.05$). Zinc is a necessary metal of a large form of completely different enzymes within which it's concerned in chemical change, structural and regulative role. Zinc level reported in literature was 0.33-0.89mg/100g and 0.29-1.58mg/100g (Nasiruddin et al, 2018). As illustrated in table 5.2, oyster mushroom from wheat straw had a significantly higher amount of zinc (9.46g/100g) compared to that of cotton husks (6.99g/100g) and maize stalks (5.02g/100g). Iron is essential for the biosynthesis of haemoglobin in red blood cells and the cytochromes that has a function in cellular respiration to produce energy. The amount of Fe found in the samples (from maize stalks, cotton husks and wheat straw) were much higher than what has been reported by Afiukwa et al (2013)(1.15mg/100g). Oyster mushroom from wheat straw had a significantly higher amount of iron (19.39mg/100g) than that from maize stalks and cotton husks.

5.10 Conclusion

The study was conducted by growing *Pleurotus Ostreatus* on three substrates: maize stalks, cotton husks and wheat straw. Oyster mushroom from wheat straw proved to be highly nutritious compared to those produced from cotton husks and maize stalks. Therefore is can be concluded that farmers can be encouraged to grow oyster mushrooms on wheat straw to improve their nutritional food security.

5.11 Recommendations

There is a wide variation in nutritional status of oyster mushroom produced from maize stalks, cotton husks and wheat straw. Oyster mushroom from wheat straw proved to be high in energy (29.74calorie/100g), protein (2.66), fat (1.59g/100g), fibre (3.41g/100g), zinc (9.42mg/100g) and iron (19.49mg/100g). Oyster from maize stalks was high in carbohydrates (1.32g/100g) while that from cotton husks had the highest moisture content (91.91%). Generally, the main substrate alone may not provide enough nitrogen required for maximum growth and additive such as rice or wheat bran may be necessary as sources of nitrogen (Choi, 2004). For cotton waste, supplements such as wheat bran (5-10%) maybe added to the substrate since cotton as a very high water holding capacity and wheat is better in nutrient releasing.

5.13 References

Chang, S.T., Lau, O.W. and Cho, K.Y. (1981). The cultivation and nutritional value of *Pleurotus sajor-caju*. *Eur. J. Appl. Microbiol. Biotechnol.* **12 (1)**, 58-62.

Choi, K.W. (2004). Shelf cultivation of oyster mushrooms.

Gupta, M., *et al*. (2004). *J. Food Sci. Technol*. **41:**584-586.

Narayanasamy, P., Suganthavel, P., Sabari, P., Divya, D., Vanchinathan, J., and Kumar, M. (2013). *Cultivation Of Mushroom (Pleurotus Florida) By Using Two Different Agricultural Wastes In Laboratory Condition*. The Internet Journal of Microbiology. 2008 Volume 7 Number 2.

Patil, S. (2012). Cultivation of *Pleurotus sajorcajuon* different agro wastes. Science research reporter, 2 (3):225-228.

Patil, S.S,. Ahmed S.A,. Telang S.M. and Baij M.M.V. (2010). The nutritional value of *Pleurotus ostreatus* (Jacq.: Fr.) Kumm cultivated on different lignocellulosic agro-wastes. InnoRoma Food Biotech, 7: 66-76.

Rai, R.D. (1995). In: Advances in Horticlture- Mushroom, (Chadha, K.L. and Sharma, S.R. eds.) pp 537-551.

Sharma, S., Kailash, P., Yadav, R., Chandra, P. and Pokhrel, P. (2013). Growth and yield of oyster mushroom (*Pleurotus ostreatus*) on different substrates. *J. New Biolog. Rep*., 2(1): 03-08. doi: 10.1186/s13568-016-0265-1

Yehia, S.R. (2012). Nutritional value and biomass yield of the edible mushroom *Pleurotus ostreatus* cultivated on different wastes in Egypt. Innovative Romanian Food Biotechnology, 91: 85–91.

CHAPTER 6

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

This chapter focused on discussion of the research summary and conclusion. The results of the research was discussed and how it will help in advocacy and lobbying of polices that will help farmers to adopt new techniques and knowledge generated to improve productivity. Recommendations were made in line with sustainable policy framework. Areas that need further research were described.

6.2 Research summary

Research was based on analysing the effects of different substrate on oyster performance. Three treatments were under spotlight; maize stalks, cotton husks and wheat straw. The substrates were chosen basing on their availability locally. The spawn was purchased from PaHohwa SIBBS laboritories in Epworth maize stalks gathered in local fields, cotton husks bought from Surface in Chitungwiza and wheat straw from a roadside market in Harare. Maize stalks and wheat straw were cut into 2-4cm small pieces before pasteurization using hot water. The substrate was packed into polythene bag simultaneously with spawn during spawning. The bags were taken for incubation which took about 21 days before full colonization was declared. The randomized complete block design (RCBD) was used and bag placement was defined as blocking factor. The three treatments maize stalks, cotton husks and wheat straw was replicated three times. The growth parameters were filtered to focus only on stipe length and cap diameter as well as the total yield. The nutritional composition parameters were; energy, protein, carbohydrates, ash, fibre, moisture content, fat, zinc and iron. The data was collected in triplicates of each treatment. Nutritional composition analysis was conducted at the University of Zimbabwe (UZ) Institute of Food, Nutrition and Family Science. Data for stipe length, cap diameter and yield was collected in three flushes at the mushroom house in Harare South. The oyster mushroom produced from cotton husks has the longest stipe length (4.55cm), widest cap diameter of 9.25cm and the final yield of 950g in three flushes. Oyster mushroom produced from wheat straw was high in energy, protein, ash, fibre, fat, zinc and iron whereas oyster mushroom from cotton husks had high moisture content and that from maize stalks was high in carbohydrates.

6.3 Conclusions

As a conclusion, different substrates have diverse effect on growth, total yield and nutritional composition of oyster mushroom. Cotton husks have the ability to hold water reducing mortality of fruiting bodies. Wheat straw has got physical nature and chemical composition that allows it to produce oyster mushrooms rich in energy, protein, fibre, ash and micronutrients such as zinc and iron. However, wheat straws have poor water holding capacity resulting in drying up of fruiting bodies and end up producing less total yield compared to oyster from cotton husks. It is important to supplement main substrates with wheat bran, rice bran or cotton seed hull in a ratio ranging from 10-15% to improve total yield.

6.4 Policy implication and recommendations

The Government of Zimbabwe (GoZ) is currently racing towards a goal of creating an environment that enhances the sustainable flow of investment into agricultural sector towards enhancing productivity and production, ensure food and nutrition security and promote national economic growth and development through National Agricultural Policy Framework (NAPF). This is evidenced by GoZ engaging European Union (EU) forming Zimbabwe Agricultural Growth Programme (ZAPG) which is meant to help farmers improve productivity in a wide range of agricultural enterprises. Now with the knowledge generated from this paper it is recommended that farmers engage Mushroom Farming using supplemented substrates that produce higher and nutritious Oyster mushroom of good quality that fetches high market price.

6.5 Areas for further research

The research was narrowed to analyzing the effects of maize stalks, cotton husks and wheat straw on growth performance, yield and the nutritional composition of mushroom produced from these substrates. This was more into the output side of the study. There is now a gap that needs to be investigated further from the input side. This is more focused on the nutritional status of the substrates and the chemistry in which there nutrients are released.

6.6 APPENDICES

APPENDIX 1

Experimental Layout in mushroom house

BLOCK 1	**BLOCK 2**	**BLOCK 3**
W1	M2	W3
M1	C2	C3
C1	W2	M3

APPENDIX 2

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confider Lower Bound
Energy	Oyster from Maize Stalks	3	27.5967	.22008	.12706	27.05
	Oyster from Cotton Husks	3	21.0933	.18148	.10477	20.64
	Oyster from Wheat Straw	3	29.7467	.16503	.09528	29.33
	Total	9	26.1456	3.90534	1.30178	23.14
Protein	Oyster from Maize Stalks	3	2.5233	.02517	.01453	2.46
	Oyster from Cotton Husks	3	2.0867	.01155	.00667	2.05
	Oyster from Wheat Straw	3	2.6633	.01155	.00667	2.63
	Total	9	2.4244	.26092	.08697	2.22
Carbohydrates	Oyster from Maize Stalks	3	1.3233	.02082	.01202	1.27
	Oyster from Cotton Husks	3	1.0267	.01528	.00882	.98
	Oyster from Wheat Straw	3	1.2633	.01528	.00882	1.22
	Total	9	1.2044	.13667	.04556	1.09
Fat	Oyster from Maize Stalks	3	1.3567	.02082	.01202	1.30
	Oyster from Cotton Husks	3	.9600	.02000	.01155	.91
	Oyster from Wheat Straw	3	1.5600	.02646	.01528	1.49
	Total	9	1.2922	.26499	.08833	1.08
Crudefibre	Oyster from Maize Stalks	3	3.3533	.01155	.00667	3.32
	Oyster from Cotton Husks	3	2.8133	.01528	.00882	2.77
	Oyster from Wheat Straw	3	3.4067	.00577	.00333	3.39
	Total	9	3.1911	.28445	.09482	2.97
Totalash	Oyster from Maize Stalks	3	1.2267	.01155	.00667	1.19
	Oyster from Cotton Husks	3	.7000	.01000	.00577	.67
	Oyster from Wheat Straw	3	1.3100	.01732	.01000	1.26
	Total	9	1.0789	.28668	.09556	.85
Percentagemoisture	Oyster from Maize Stalks	3	90.1000	.00000	.00000	90.10
	Oyster from Cotton Husks	3	91.9100	.02646	.01528	91.84
	Oyster from Wheat Straw	3	89.0767	.02517	.01453	89.01
	Total	9	90.3622	1.24267	.41422	89.40
Zinc	Oyster from Maize Stalks	3	5.0133	.05686	.03283	4.87
	Oyster from Cotton Husks	3	6.9900	.02646	.01528	6.92
	Oyster from Wheat Straw	3	9.4600	.00000	.00000	9.46
	Total	9	7.1544	1.92966	.64322	5.67
Iron	Oyster from Maize Stalks	3	13.0933	.01155	.00667	13.06
	Oyster from Cotton Husks	3	16.1033	.00577	.00333	16.08
	Oyster from Wheat Straw	3	19.3900	.01000	.00577	19.36
	Total	9	16.1956	2.72743	.90914	14.09

APPENDIX 3

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Energy	.101	2	6	.906
Protein	1.255	2	6	.351
Carbohydrates	.356	2	6	.715
Fat	.341	2	6	.724
Crudefibre	1.556	2	6	.286
Totalash	1.273	2	6	.346
percentagemoisture	4.171	2	6	.073
Zinc	6.090	2	6	.036
Iron	.857	2	6	.471

APPENDIX 4

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Energy	Between Groups	121.796	2	60.898	1.682E3	.000
	Within Groups	.217	6	.036		
	Total	122.013	8			
Protein	Between Groups	.543	2	.271	904.704	.000
	Within Groups	.002	6	.000		
	Total	.545	8			
Carbohydrates	Between Groups	.148	2	.074	246.037	.000
	Within Groups	.002	6	.000		
	Total	.149	8			
Fat	Between Groups	.559	2	.279	546.543	.000
	Within Groups	.003	6	.001		
	Total	.562	8			
Crudefibre	Between Groups	.646	2	.323	2.424E3	.000
	Within Groups	.001	6	.000		
	Total	.647	8			
Totalash	Between Groups	.656	2	.328	1.846E3	.000
	Within Groups	.001	6	.000		
	Total	.657	8			
percentagemoisture	Between Groups	12.351	2	6.176	1.389E4	.000
	Within Groups	.003	6	.000		
	Total	12.354	8			
Zinc	Between Groups	29.781	2	14.890	1.136E4	.000
	Within Groups	.008	6	.001		
	Total	29.789	8			
Iron	Between Groups	59.510	2	29.755	3.347E5	.000
	Within Groups	.001	6	.000		
	Total	59.511	8			

APPENDIX 5

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.
Energy	Oyster from Maize Stalks	Oyster from Cotton Husks	6.50333*	.15535	.00
		Oyster from Wheat Straw	-2.15000*	.15535	.00
	Oyster from Cotton Husks	Oyster from Maize Stalks	-6.50333*	.15535	.00
		Oyster from Wheat Straw	-8.65333*	.15535	.00
	Oyster from Wheat Straw	Oyster from Maize Stalks	2.15000*	.15535	.00
		Oyster from Cotton Husks	8.65333*	.15535	.00
Protein	Oyster from Maize Stalks	Oyster from Cotton Husks	.43667*	.01414	.00
		Oyster from Wheat Straw	-.14000*	.01414	.00
	Oyster from Cotton Husks	Oyster from Maize Stalks	-.43667*	.01414	.00
		Oyster from Wheat Straw	-.57667*	.01414	.00
	Oyster from Wheat Straw	Oyster from Maize Stalks	.14000*	.01414	.00
		Oyster from Cotton Husks	.57667*	.01414	.00
Carbohydrates	Oyster from Maize Stalks	Oyster from Cotton Husks	.29667*	.01414	.00
		Oyster from Wheat Straw	.06000*	.01414	.01
	Oyster from Cotton Husks	Oyster from Maize Stalks	-.29667*	.01414	.00
		Oyster from Wheat Straw	-.23667*	.01414	.00
	Oyster from Wheat Straw	Oyster from Maize Stalks	-.06000*	.01414	.01
		Oyster from Cotton Husks	.23667*	.01414	.00
Fat	Oyster from Maize Stalks	Oyster from Cotton Husks	.39667*	.01846	.00
		Oyster from Wheat Straw	-.20333*	.01846	.00
	Oyster from Cotton Husks	Oyster from Maize Stalks	-.39667*	.01846	.00
		Oyster from Wheat Straw	-.60000*	.01846	.00
	Oyster from Wheat Straw	Oyster from Maize Stalks	.20333*	.01846	.00
		Oyster from Cotton Husks	.60000*	.01846	.00
Crudefibre	Oyster from Maize Stalks	Oyster from Cotton Husks	.54000*	.00943	.00
		Oyster from Wheat Straw	-.05333*	.00943	.00

	Oyster from Cotton Husks	Oyster from Maize Stalks	-.54000*	.00943	.00
		Oyster from Wheat Straw	-.59333*	.00943	.00
	Oyster from Wheat Straw	Oyster from Maize Stalks	.05333*	.00943	.00
		Oyster from Cotton Husks	.59333*	.00943	.00
Totalash	Oyster from Maize Stalks	Oyster from Cotton Husks	.52667*	.01089	.00
		Oyster from Wheat Straw	-.08333*	.01089	.00
	Oyster from Cotton Husks	Oyster from Maize Stalks	-.52667*	.01089	.00
		Oyster from Wheat Straw	-.61000*	.01089	.00
	Oyster from Wheat Straw	Oyster from Maize Stalks	.08333*	.01089	.00
		Oyster from Cotton Husks	.61000*	.01089	.00
percentagemoisture	Oyster from Maize Stalks	Oyster from Cotton Husks	-1.81000*	.01721	.00
		Oyster from Wheat Straw	1.02333*	.01721	.00
	Oyster from Cotton Husks	Oyster from Maize Stalks	1.81000*	.01721	.00
		Oyster from Wheat Straw	2.83333*	.01721	.00
	Oyster from Wheat Straw	Oyster from Maize Stalks	-1.02333*	.01721	.00
		Oyster from Cotton Husks	-2.83333*	.01721	.00
Zinc	Oyster from Maize Stalks	Oyster from Cotton Husks	-1.97667*	.02956	.00
		Oyster from Wheat Straw	-4.44667*	.02956	.00
	Oyster from Cotton Husks	Oyster from Maize Stalks	1.97667*	.02956	.00
		Oyster from Wheat Straw	-2.47000*	.02956	.00
	Oyster from Wheat Straw	Oyster from Maize Stalks	4.44667*	.02956	.00
		Oyster from Cotton Husks	2.47000*	.02956	.00
Iron	Oyster from Maize Stalks	Oyster from Cotton Husks	-3.01000*	.00770	.00
		Oyster from Wheat Straw	-6.29667*	.00770	.00
	Oyster from Cotton Husks	Oyster from Maize Stalks	3.01000*	.00770	.00
		Oyster from Wheat Straw	-3.28667*	.00770	.00
	Oyster from Wheat Straw	Oyster from Maize Stalks	6.29667*	.00770	.00
		Oyster from Cotton Husks	3.28667*	.00770	.00

*. The mean difference is significant at the 0.05 level.

APPENDIX 6

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Inter	
						Lower Bound	U
Stipe Length	Oyster from Maize Stalks	3	3.4300	.22113	.12767	2.8807	
	Oyster from Cotton Husks	3	4.5533	.13796	.07965	4.2106	
	Oyster from Wheat Straw	3	3.8900	.18358	.10599	3.4340	
	Total	9	3.9578	.51439	.17146	3.5624	
Cap Diameter	Oyster from Maize Stalks	3	7.3333	.24826	.14333	6.7166	
	Oyster from Cotton Husks	3	9.2500	.09165	.05292	9.0223	
	Oyster from Wheat Straw	3	4.3300	.22068	.12741	3.7818	
	Total	9	6.9711	2.15457	.71819	5.3150	
Total Yield	Oyster from Maize Stalks	3	2.7667E2	76.63115	44.24302	86.3043	
	Oyster from Cotton Husks	3	3.1667E2	85.04901	49.10307	105.3932	
	Oyster from Wheat Straw	3	2.8667E2	66.16142	38.19831	122.3126	
	Total	9	2.9333E2	68.52554	22.84185	240.6599	

APPENDIX 7

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Stipe Length	.617	2	6	.570
Cap Diameter	1.301	2	6	.339
Total Yield	.057	2	6	.945

APPENDIX 8

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Stipe Length	Between Groups	1.913	2	.957	28.241	.001
	Within Groups	.203	6	.034		
	Total	2.117	8			
Cap Diameter	Between Groups	36.900	2	18.450	466.171	.000
	Within Groups	.237	6	.040		
	Total	37.137	8			
Total Yield	Between Groups	2600.000	2	1300.000	.223	.806
	Within Groups	34966.000	6	5827.667		
	Total	37566.000	8			

APPENDIX 9

Multiple Comparisons

Dependent Variable		(I) sample	(J) sample	Mean Difference (I-J)	Std. Error
Stipe Length	Tukey HSD	Oyster from Maize Stalks	Oyster from Cotton Husks	-1.12333*	.15028
			Oyster from Wheat Straw	-.46000	.15028
		Oyster from Cotton Husks	Oyster from Maize Stalks	1.12333*	.15028
			Oyster from Wheat Straw	.66333*	.15028
		Oyster from Wheat Straw	Oyster from Maize Stalks	.46000	.15028
			Oyster from Cotton Husks	-.66333*	.15028
	LSD	Oyster from Maize Stalks	Oyster from Cotton Husks	-1.12333*	.15028
			Oyster from Wheat Straw	-.46000*	.15028
		Oyster from Cotton Husks	Oyster from Maize Stalks	1.12333*	.15028
			Oyster from Wheat Straw	.66333*	.15028
		Oyster from Wheat Straw	Oyster from Maize Stalks	.46000*	.15028
			Oyster from Cotton Husks	-.66333*	.15028
Cap Diameter	Tukey HSD	Oyster from Maize Stalks	Oyster from Cotton Husks	-1.91667*	.16244
			Oyster from Wheat Straw	3.00333*	.16244
		Oyster from Cotton Husks	Oyster from Maize Stalks	1.91667*	.16244
			Oyster from Wheat Straw	4.92000*	.16244
		Oyster from Wheat Straw	Oyster from Maize Stalks	-3.00333*	.16244
			Oyster from Cotton Husks	-4.92000*	.16244
	LSD	Oyster from Maize Stalks	Oyster from Cotton Husks	-1.91667*	.16244
			Oyster from Wheat Straw	3.00333*	.16244
		Oyster from Cotton Husks	Oyster from Maize Stalks	1.91667*	.16244
			Oyster from Wheat Straw	4.92000*	.16244
		Oyster from Wheat Straw	Oyster from Maize Stalks	-3.00333*	.16244

			Oyster from Cotton Husks	-4.92000*	.16244
Total Yield	Tukey HSD	Oyster from Maize Stalks	Oyster from Cotton Husks	-40.00000	62.33066
			Oyster from Wheat Straw	-10.00000	62.33066
		Oyster from Cotton Husks	Oyster from Maize Stalks	40.00000	62.33066
			Oyster from Wheat Straw	30.00000	62.33066
		Oyster from Wheat Straw	Oyster from Maize Stalks	10.00000	62.33066
			Oyster from Cotton Husks	-30.00000	62.33066
	LSD	Oyster from Maize Stalks	Oyster from Cotton Husks	-40.00000	62.33066
			Oyster from Wheat Straw	-10.00000	62.33066
		Oyster from Cotton Husks	Oyster from Maize Stalks	40.00000	62.33066
			Oyster from Wheat Straw	30.00000	62.33066
		Oyster from Wheat Straw	Oyster from Maize Stalks	10.00000	62.33066
			Oyster from Cotton Husks	-30.00000	62.33066

*. The mean difference is significant at the 0.05 level.

APPENDIX 10

Correlations

		Sample	Stipe Length	Cap Diameter	Total Yield
sample	Pearson Correlation	1	.387	-.604	.063
	Sig. (2-tailed)		.303	.085	.872
	N	9	9	9	9
Stipe Length	Pearson Correlation	.387	1	.470	.226
	Sig. (2-tailed)	.303		.202	.558
	N	9	9	9	9
Cap Diameter	Pearson Correlation	-.604	.470	1	.155
	Sig. (2-tailed)	.085	.202		.691
	N	9	9	9	9
Total Yield	Pearson Correlation	.063	.226	.155	1
	Sig. (2-tailed)	.872	.558	.691	
	N	9	9	9	9