NUTRITIONAL COMPOSITION OF WILD EDIBLE MUSHROOMS GROWING IN KENYA AND THEIR UTILIZATION IN FOOD PRODUCT DEVELOPMENT

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(Food Science and Postharvest Technology)

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

2013

Nutritional Composition of Wild Edible Mushrooms Growing in Kenya and their Utilization in Food Product Development

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A thesis submitted in partial fulfilment for the degree of Master of Science in Food Science and Postharvest Technology in the Jomo Kenyatta University of Agriculture and Technology

2013

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

This work is dedicated to my family**,** for their unwavering support and strong belief that I would make it despite my advance in age. Let this work inspire others who may feel that old age is an impediment to scaling academic heights. It can be done.

ACKNOWLEDGEMENT

First, I wish to acknowledge and thank the Almighty God most sincerely for giving me the energy and motivation to successfully complete both the course work and the thesis within the prescribed timelines.

I really cannot thank Jomo Kenyatta University of Agriculture and Technology enough for the immense support it has afforded me both in my pursuit of the Masters degree and my career progression at the university. I feel humbled and indeed indebted to the university for granting me study leave and for funding the entire MSc. Program. Without the university's financial assistance, undertaking the necessary research work would not have been possible. Thank you very much.

My sincere gratitude goes to my supervisors, Prof. G. M. Kenji and Dr. J. M. Onguso for their assistance and guidance throughout the research work. I further wish to acknowledge and thank both the teaching and technical staff in the Food Science and Technology Department, technical staff in the Horticulture Department and all my colleagues in the 2011 Masters class for their invaluable assistance and encouragement during my study.

I would also like to acknowledge and graciously thank Mr. Peter Mukirae and Mr. Geofrey Mashauri of Malindi; Ms. Mary Mwema and Ms Mary K'onyango from the Ministry of Agriculture Kakamega and Siaya Counties respectively; Mr Ndiema of Mt. Elgon and Mr Njoka of Chinga, Nyeri County for assisting me to obtain wild edible mushrooms which were critical for my research. My heartfelt appreciation goes to Dr. Jefwa J. and all the research officers in the Herbarium Department at Museums of Kenya for assisting me in scientific identification of the mushrooms. I am truly grateful.

Last but not least, I wish to acknowledge and thank all my family members for their encouragement and prayers during my study. Cheering me on all along the way, they gave me the strength and the impetus I needed to forge forward to the end of the program. Thank you very much. May God bless you all.

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LIST OF ABBREVIATIONS AND ACRONYMS

AAS	Atomic absorption spectrophotometry
AOAC	Association of Official Analytical Chemists
CAT	Catalase
CE	Catechin equivalence
CHO	Carbohydrates
DWB	Dry weight basis
DPPH	2, 2-Diphenyl-1-picryl hydrazyl radical
EC_{50}	Extract concentration providing 50% inhibition
EUC	Equivalent ‘umami’ concentration
FA	Fatty acid
FFA	Free fatty acids
GAE	Gallic Acid Equivalent
HPLC	High Performance Liquid Chromatography
LSD	Least Significant Difference
NWFP	Non Wood Forest Products
RSA	Radical scavenging activity
SOD	Superoxide dismutase
TF	Total flavonoids
TP	Total polyphenol
QE	Quercetin equivalent
UV-Vis	Ultra violet- visible spectrophotomètre
WSV	Water soluble vitamins

ABSTRACT

Mushrooms, a group of macro-fungi, have been used as food for centuries all over the world because of their characteristic texture and flavor. They are documented as being good source of nutrients and bioactive compounds beneficial to the human body. In spite of this, consumption of wild mushrooms in Kenya is low. This research aimed at determination of nutritional chemical composition and phytochemical compounds of wild edible mushroom found in selected areas in Kenya and utilization of the mushrooms in product development.

Two commercially grown and ten wild edible mushrooms species were collected from coast, central and western parts of Kenya. They were analyzed for nutrients according to standard procedures. All the analysis was done in triplicate. Proximate analysis showed high levels of nutrients: lipids, 1.6-10.3 %; insoluble fiber 4.5-16.3 %; ash 6.6-21.8%; protein 16.6-35.9 % and soluble carbohydrates 37.5-63.3 %. Mineral analysis showed presence of calcium, sodium, copper, manganese, zinc, potassium, phosphorus, magnesium and iron. Potassium was found to be present in the highest proportion in most of the species (202 -4140 mg/100 g, dwb) and iron most abundant trace element (4.2-42.1 mg/100 g, dwb). Water soluble vitamins $B_{2,}$ Folate and ascorbic acid were detected in all the mushrooms at 0.1-6.4; 0-0.8 and 0.1-1.2, mg/100 g (dwb) respectively.

Phytochemical compounds screening indicated presence of saponins, polyphenols and terpenoids. Total polyphenols values ranged 210.5-1614.1 mg/100g, dwb and flavonoids 214.2-1695.4 mg, Quercetin Equivalent (QE)/100 g dwb. The results showed a positive relationship between both total polyphenols (TP) and total

flavonoids (TF) with the radical scavenging activity (RSA). Mushrooms with high TP/TF had high RSA values. The correlation coefficient between polyphenols and RSA was 0.8. High RSA is a desirable property.

Mushroom flour was used in development of noodles, soup and biscuits. Subsequently consumer preference evaluation was done. Noodles with 25 % oyster and soup with 2.5 % *oruka* mushroom flour received highest consumer preference rating.

The research showed that all mushrooms are rich in nutrients and phytochemical compounds which are necessary for a healthy body. It also showed that all types of edible mushrooms can be used in formulation of value-added products.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Mushroom has been defined as ‘a macro-fungus with a distinctive fruiting body, which can be hypogenous or epigeous, large enough to be seen with the naked eye and to be picked by hand (Chang and Miles, 1989). The term is also used to describe both the fleshy fruiting bodies of some *Ascomycota* and the woody or leathery fruiting bodies of some *Basidiomycota* (Pathak *et al*, 1997). They occur in various ecological conditions, from desserts to forests. Of around 3000 known edible species only 30 are domesticated and 10 are commercially cultivated (Shu-Ting, 2011).

Mushrooms are divided into four groups; edible, medicinal, poisonous and others. The others are those that may have combined characteristics e.g. edible and medicinal (*Shiitake*) or medicinal and poisonous. Shiitake is highly valued edible mushroom and it is incorporated in several medicines. Mushrooms with both medicinal and poisonous properties are avoided Shu-Ting, 2011).

According to nutritional strategy, mushrooms can be divided into three groups; mycorrhizal (symbiotic) species where there is close relationship with the host usually a tree, saprotrophic (saprophytes) species that live on dead organic matter and parasitic species that live on other species in a non-symbiotic relationship (Kalac, 2012). They have ability to convert inedible ligno-cellulosic organic waste into palatable food that is known for characteristic soft texture and mild flavor (Pathak *et al*, 1997).

The world market for the mushroom industry in year 2005 was valued over $45 billion. Commercial varieties include Button, Oyster and Shiitake mushrooms. The white button mushroom was domesticated in France in 17th century and rapidly spread after First World War when reliable spawn was available (Chang and Miles, 1989).

There is a large number of wild mushroom species in North and South America. Native Americans and immigrants from Asia or Europe used wild mushrooms as food, medicine and dyes (Mueller, 2007). Some cultures however, do not use mushrooms as a significant source of food, and in some cases have not used it at all in their diets (Wong, 2003).

Asian cultures are generally mycophilic. China has longest tradition in collecting mushrooms, not just for consumption as food, but also as an important role in traditional medicine. However, unlike most other mycophilic societies, species of mushrooms that are used in China for food and medicine are generally cultivated. Japan is similar to China in having cultivation play a large role in supplying mushrooms for the diet and medicinal needs of its people (Wong, 2003).

European cultures are generally fond of mushrooms but the species favored by each country varies. The Italians prefer the porcini (*Boletus edulis*) and white truffle (*Tuber alba*) while the Germans and Swiss prefer the chanterelle (*Cantharellus cibarius*) (Wong, 2003).

Africa appears to be generally mycophilic. There are some regions, such as Nigeria, where mushrooms are a part of everyday life as food, charms and remedies in traditional medicine. Malawi is also a region of mushroom gourmands, whose women have knowledge of the edible and poisonous species. Over 60 edible species

are recognized in this area, predominantly belonging to the genera *Amanita*, *Cantharellus* and *Termitomyces* (Wong, 2003). The responsibility of foraging and identifying the mushrooms fall upon the women.

In Tanzania, wild mushrooms are used as food. The tradition of collecting edible mushrooms is done almost exclusively by women and children who go on a mushroom foray (Harkonen *et al*. 1995).

Over 60 edible mushroom species have been identified in Tanzania (Buyck *et al*. 2000; Harkonen *et al*. 1995). Mushrooms collected in southern Tanzania is mainly for own consumption. Fresh, but also dried mushrooms are sold at market places and along roadsides.

In rural Zambia and Democratic Republic of Congo, mushrooms are widely consumed during "hunger" months from late November through early April. The species favored for eating are restricted to *Termitomyces* but species of *Lactarius*, *Russula*, *Cantharellus* and *Amanita* are also eaten. Research conducted in Zambia found that communities attain greater sustained livelihoods from wild mushroom sales than from the felling and sale of hard wood timber from host tree species. The conclusion was that collection and consumption of wild mushrooms will not only protect the forest ecosystem but will also be a source of food and vital nutrients to the people (Wong, 2003).

In Kenya, indigenous mushrooms are still consumed from the wild, but mainly by the communities living around the forests. The collection and consumption is mainly done in Kakamega and Kisumu Counties; Mt Elgon and Arabuko Sokoke forests. Wild mushrooms constitute one of the Non Wood Forest Product (NWFP) where some mushrooms and hard wood trees or termites exist in a symbiotic relationship

(Buyck et al. 2000). Wild Living Resources has partnered with four Community Based Organizations (CBO's) located in remnant forest farm areas surrounding Arabuko Sokoke forest. An East African wild Chanterelle mushroom model has been established on the Wild Living Resources Conservancy (Rob *et al*, 2010). This model showcases the Chanterelle production potential of mature host tree species, and provides examples of how Chanterelle can be ranched and intensively farmed. Wild Living Resources provides training for outgrowers from its partner CBOs on sustainable wild harvesting, quality control, Chanterelle mushroom ranching and farming. As the pivotal incentive for valuing host tree species, outgrower livelihoods are assured through Wild Living's assistance in the marketing of fresh and dried Chanterelle in the local as well as international European markets (Rob *et al*, 2010).

Cultivation of mushroom is an emerging industry in Kenya. Button (*Agaricus bisporus*), Oyster (*Pleurotus spp*) and Shiitake (*Lentinula edodes*) are the edible cultivated mushrooms, with the former two dominating the market. Shiitake is grown at very low scale, mainly for hotel industry that caters for foreign and high end clients. Reishi (*Ganoderma lucidun*), a medicinal mushroom is grown on a small scale. Mushrooms are mainly cultivated by small-scale farmers in Kakamega, Kisumu, Nyeri, Kiambu, Malindi and Mombasa Counties (Wambua, 2004). However, mushroom being an emerging food source, limited research has been undertaken to provide clear background information.

1.2 Problem Statement

A wide variety of wild mushrooms grow in Kenya. They are harvested soon after the rains and soon dry out in about one or two week after the rains. Their nutritional composition is not known.

Mushrooms are alleged to have medicinal properties. The medicinal benefits could be due to presence of phytochemical compounds found in mushrooms. However information on type and levels of these compounds in wild edible mushrooms found in Kenya is not available.

Edible wild mushroom processing is largely unexploited while it is possible to develop value-added mushroom products. Research on this is necessary.

1.3 Justification

Our forests have a rich source of edible wild mushrooms. Communities living near forests have learnt to distinguish toxic and non toxic varieties. This knowledge should be tapped and exploited for the benefit of other Kenyans.

Mushrooms are excellent bio-degraders (Oei, 2003). They decompose organic waste which releases the nutrients in celluloses, hemicelluloses and lignin. They therefore help in cleaning the environment and recycling the nutrients. Value addition will provide sustainable agro-based enterprises that will target local, national and international markets. The climate change experienced globally has resulted in prolonged droughts, floods and frostbite, since 2009. This rendered about 3 million Kenyans to face starvation (Mwaniki P, 2012). There is need to seek permanent solution to the stated problems. This study aimed at providing information about the

chemical composition and types/ levels of phytochemicals in wild edible mushrooms found in selected areas in Kenya. Further, the study was meant to come up with formulations that could be used in development of value added products using the mushrooms. This would not only curb wastage of fresh mushrooms but also enable many Kenyans to utilize the mushrooms in the developed products.

1.4 Objectives

1.4.1 Main Objective

To determine the nutritional and phytochemical composition of wild edible mushrooms and two cultivated varieties and to develop value-added products using the mushrooms.

1.4.2 Specific Objectives

The specific objectives were as follows:

1. To determine proximate (moisture, protein, lipids, soluble carbohydrates, ash, and insoluble fibre) and mineral composition and water soluble vitamins of selected mushroom varieties found in Kenya.
2. To screen for the presence of selected phytochemical compounds (saponins, polyphenols, alkaloids, tannins, terpenoids and anthraquinons) and to determine the levels of polyphenols, flavonoids and the radical scavenging activity of the mushrooms
3. To formulate value-added products using mushroom flour, to do sensory evaluation of the developed products and to analyze for any improvement in protein, Iron and zinc content in the product.

1.5 Hypothesis

The null hypothesis tested was:

Edible wild mushrooms in Kenya do not contain nutrients and phytochemical compounds that are useful to the body and that the mushrooms cannot be utilized in product development.

CHAPTER TWO:

2.0 LITERATURE REVIEW

2.1 Mushroom folklore and history

Fossil records show that mushrooms existed about 130 million years ago; long before mankind evolved and it is probable that he has utilized mushrooms for food ever since (Shu-Ting, 2011). Mycophagy is the act of consuming mushrooms as food. Evidence of mycophagy can be dated back to several 100 years BC in China. The Chinese used mushrooms for medicinal properties as well as for food. Man has harvested wild edible mushroom for centuries, dating back to Paleolithic times (Chang and Miles, 1989).

Ancient Egyptian Hieroglyphics reveal that mushrooms were believed to bring immortality and that only the pharaohs, who were thought of as godlike, could receive this privilege (Stamet, 1993). Association of mushrooms emergence with lightning, thunderstorms and rain was common in mythology hence they were also associated with supernatural powers. (Quimio *et al*, 2005).

Romans and Greeks also consumed mushrooms close to 4600 years ago (Stamet, 1993). It is reported that they liked the taste so much that the Roman Ceasers used to have a food taster to taste the mushrooms right before the Caesar consumed it to ensure that the mushrooms were not poisonous. Truffles, underground *Ascomycota* fungi, were unknown in Europe until the 14th century. Being unfamiliar to Europeans, truffles were attributed with powerful aphrodisiac abilities (Del and Stephen, 2006).

In the late 19th century, mushroom cultivation reached the United States. In 1903, pure culture was developed in Pennsylvania and since then commercial mushroom production spread the world over (Del and Stephen, 2006).

Current global mushroom production stands at 5.1 million tons with China's output at 3.92 million tons (Hong, 2012). China is currently the world's largest commercial producer of mushrooms at 76.86 % of total volume followed by Europe and the United States of America (Hong, 2012).

2.2 Mushroom structure and biology

2.2.1 Mushroom biology

Mushroom biology is a branch of mycology that is concerned with scientific study of mushrooms. It deals with all aspects of mushrooms that include taxonomy, development, nutrition, production, edibility, mushroom products and toxicity (Chang and Miles, 1992). Mushrooms are the most visible members of the economically and ecologically important Fungi kingdom. They are the reproductive structures produced by members of the division of fungi known as the Basidiomycota and the ascomycota in the order Agaricales. (Chang and Miles, 1989). Other forms of fungi include puff-balls, cup-fungi, bracket-fungi, club-fungi, rusts and smarts, yeasts, and molds (Shu-Ting, 2011).

The life cycle of a mushroom starts when spores germinate to form haploid mycelia (fig. 1). The undifferentiated hyphae from two haploid mycelia of opposite mating type undergo plasmogamy, creating a dikaryotic nucleus and thus the zygote mycelium that grows faster than the haploid mycelia. Ultimately the dikaryotic mycelia crowds out the haploid parent mycelia. Environmental cues such as rain and

temperature change induce the dikaryotic mycelium to form compact masses that form primodia which develop into mushrooms. Karyogamy occurs in the terminal dikaryotic cells that line the surfaces of the gills. Each cell swells to form a diploid basidium, which rapidly undergoes meiosis and yields four haploid nuclei. The basidium then grows four appendages, and one haploid nucleus enters each appendage and develops into haploid basidiospore (spore). In the life cycle of a sexually reproducing mushroom a haploid phase alternates with a diploid phase. However all the mushrooms do not necessarily follow the two phase production process (Shu-Ting, 2011)

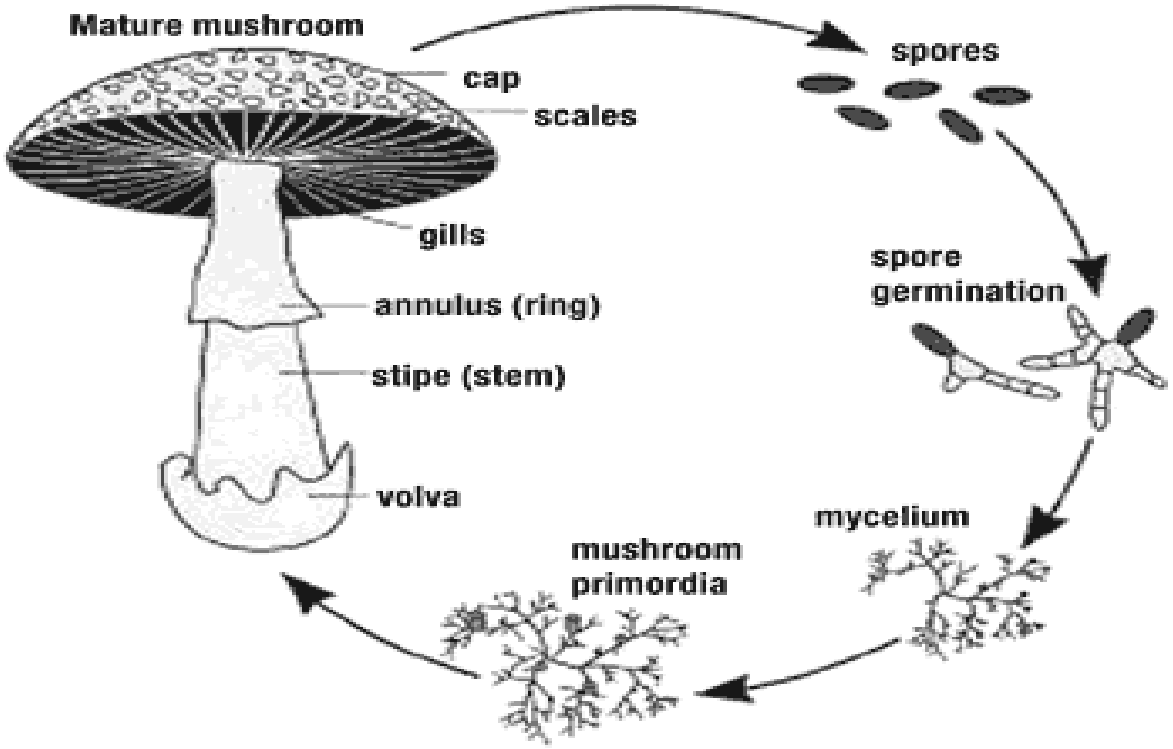


Figure 2.1: Life cycle of a mushroom (Adapted from: (www.mushroomgrow.com)

2.2.2 The Structure of Mushrooms.

A mushroom or a fruit body has three main parts above the substratum, the cap, the gills and the stipe. The cap forms the upper protective layer of the mushroom while the stipe or the stem lifts the spore-producing region above the substratum to enable release of the spores. The gills radiate out on the underside of the cap. The gills are lined with reproductive cells called basidia from which spores are produced (Shu-Ting, 2011).

The spores are usually actively ejected from the gill or tube surface to float down and out of the cap. They are so light that even the slightest breeze is enough to transport them far and wide.

Like all fungi, mushrooms consist of fine microscopic threads, the hyphae which grow and branch within the substratum to form mycelia. Vegetative mycelia are septate and since each cell contains all the organelles for independent growth, fragments of mycelia can regenerate to form new colonies (Binding, 1978). The mycelia produce enzymes that digest complex organic material like celluloses to simple substances that are absorbed by hyphae to be utilized for growth and storage. The energy stored is later used in making the fruiting bodies, the visible mushrooms (Pathak *et al*, 1997).

When climatic conditions are appropriate, spores are produced to be disseminated usually by the wind in order to spread the species. The structure of mushroom is thus designed to enable production and release of spores (Binding, 1978). A mushroom is either an umbrella to keep rain away or a parasol to keep out the heat of the sun (Shu-Ting, 2011).

The applied mushroom biology is a discipline whose aim is to tackle the three basic problems, shortage of food, diminishing quality of human health and pollution of the environment, which human beings will always face, due to the continued increase of the world population. Applied mushroom biology can not only convert these huge lignocellulosic biomass wastes into human food, but can also produce notable nutriceutical/neutraceutical products, feed, fertilizers, and protect and regenerate the environment (Shu-Ting, 2011; Chang and Miles, 1992).

2.3 Medicinal mushrooms

Medicinal mushrooms are mushrooms or extracts that are used or studied as possible treatments for diseases. Mushrooms that are medicinal include reishi (*Ganoderma lucidum*), shiitake (*Lentinula edodes*) and maitake (*Grifola fiondosa*) among others. Shiitake and maitake have both edible and medicinal properties. Reishi is cultivated in several countries with China taking the lead in terms of production, followed by Korea, Taiwan, Japan, USA, Malaysia, Vietnam, and Indonesia (Shu-Ting, 2011). Some mushroom compounds, like polysaccharides, glycoprotein and proteoglycans are reported to modulate immune system responses and inhibit tumor growth (Martin, 2010). Some medicinal mushroom isolates that have been identified also show cardiovascular, antiviral, antibacterial, anti-inflammatory, and anti-diabetic properties (Cheung *et al*, 2003).Medicinal mushrooms take different shapes and colors. A few examples of medicinal varieties are presented in plate 1.

Brown reishi (*Ganoderma lucidum*)

Crab Brittlegill (*Russula xerampelina*)

Turkey tail (*Trametes versicolor*)

Maitake (*Grifola fiondosa*)

Plates 1: Photographs and examples of medicinal mushrooms

(Source: americansmushrooms.com 2006)

Currently, several extracts have widespread use in Japan, Korea and China as adjuncts to radiation treatments and chemotherapy in cancer treatment. In China, more than 700 medical products with mushroom as the main ingredient are commercially available. At least 106 medicines contain *Ganoderma,* 43 *Cordyceps* and 7 *Shiitake* mushrooms components/ extracts (Oei, 2003, Chang and Miles, 1989). Ganoderma wastes have also been converted to chitosan and used as dressing on human wounds (Kurtzman, 2005).

Historically, mushrooms have had medicinal uses, especially in traditional Chinese medicine. Mushrooms have been a subject of modern medical research since the

1960s, where most modern medical studies concern the use of mushroom extracts, rather than whole mushrooms. However, a few specific mushroom extracts have been extensively tested for efficacy (Chen and Phung, 2006).

The available results for other mushroom extracts are based on *in vitro* data, effects on isolated cells in a lab dish, animal models like mice, or underpowered clinical human trials. Studies show that glucan-containing mushroom extracts primarily change the function of the innate and adaptive immune systems, functioning as bio-response modulators, rather than by directly killing bacteria, viruses, or cancer cells as cytocidal agents (Chen and Phung, 2006). In some countries, mushroom extracts like polysaccharide-K, schizophyllan, polysaccharide peptide, and lentinan are government-registered adjuvant cancer therapies (Zhu *et al*, 2011; Chen and Phung, 2006).

2.4 Mushrooms as food

In the affluent countries, mushrooms are considered as an expensive type of vegetable that are eaten for their culinary properties, and for providing a flavor and/or garnish for other foods (Shu-Ting, 2011). In many regions of Europe and Asia, mushrooms and truffles are gathered every year in quantities by specialized professional collectors or amateurs, and immediately conserved (Wong, 2003). In most developing countries wild mushrooms are collected and used fresh, occasionally conservation by sun drying is done. .

Mushrooms are gradually becoming popular since they are reported to be rich in proteins, dietary fiber, minerals and vitamins while low in lipids. Fresh mushrooms have high moisture content, 90-95%, making them highly perishable (Okoro and

Achumba, 2011). They need to be consumed within a day unless refrigerated. Mushrooms have been used in making soups, pickles, and as additives in many food preparations. They are considered as a vegetarian delicacy or diabetics' delight all over the world (Pathak *et al*, 1997).

Examples of edible mushrooms are presented in Plate 2.

A

B

C

D

Plates 2: Photographs of edible mushroom:
A Coprinus *comatu;* B Tuber *melanosporum* (black truffle); C Sarcosscypha *mesocyantha*); D Lentinula *edodes* (Shiitake). (Source: americansmushrooms.com 2006)

2.5 Nutritional aspects of edible mushrooms

2.5.1 Proteins

Many mushroom varieties contain high level of proteins. On dry weight basis, protein content is reported to range 19 to 40 % (Binding, 1978). Proteins have been reported to be composed of albumins, globulins, glutelins, glutelin-like substances, prolamins and prolamin-like substances (Kalac, 2012). However presence of non-protein nitrogen in chitin lowers available protein to 75-90 % (Chang and Miles, 1998). Of equal importance to the amount of protein is the quality of the protein. Mushroom proteins are reported to contain all the essential amino acids, some non-essential amino acids and amides (Chang and Miles1989). A total of 17 amino acids, including all the essential amino acids, were qualitatively identified. Quantitative estimation of essential amino acids showed that, except for methionine and phenylalanine, other essential amino acids are present in fairly high concentration (Bano *et al*, 1963). The reported content of free amino acids range between 0.15-7.20 % in different mushroom species (Barros *et al*, 2007; Beluhan and Ranogoyec, 2011). Free amino acids have been found to be arginine, alanine, glutamine and glutamic acid in 15 species of *Boretus.* These amino acids, together with other components, determine the taste of mushrooms (Kalac, 2012). The protein quality is however lower than that from animal sources like fish, or eggs (Pathak *et al*, 1997).

2.5.2 Lipids

Lipids have been reported to occur in the range 1-10 % on dry weight basis (dwb) (Kalac, 2012). Polar lipids account for more than 50 % in most species. More than 25 different fatty acids (FAs) were found in the mushroom lipids but only three predominate, these are the unsaturated linoleic and oleic which account for about 83

% of the total FAs. Palmitic acid was the main saturated FA (Karin *et al,* 2006). Linoleic acid makes up to 76 % of unsaturated fatty acids and 90 % of polar lipids (Huang *et al,* 1985). It is the presence of linoleic, acid, one of the Omega 6 fatty acid that contributes to mushrooms being a healthy food (Karine *et al*, 2006; Shao *et al*, 2010). The content of odd and branched-chain and hydroxyl fatty acids is negligible. Elaidic acid, a trance isomer of oleic acid was reported present but at less than 0.3 % of tatal fatty acids. Elaidic acid is undesirable in the diet; it contributes to atherosclerosis (Pedneault *et al*, 2008).

2.5.3 Soluble Carbohydrates

Soluble Carbohydrates present in mushrooms account for 30-65 % on dry weight basis. This is made of monosaccharides and their derivatives, oligosaccharides and polymeric glycogen. Trehalose and sugar alcohol, mannitol, are the main representative of oligosaccharides and polyols respectively (Hammond, 1979; Kalac, 2012). Mannitol is the sugar responsible for cell expansion hence very important in mushroom growth (Kalac, 2012). Polymeric compounds include glycogen which serves as the storage form of energy. It accounts for 5-10 % on dry weight basis (Kalac, 2012). The caloric impact of mushroom consumption is low; 100 grams of mushrooms have less than 100 kcal/100 g, dwb (Pathak *et al*, 1997).

2.5.4 Vitamins

Mushrooms are reported to be good source of vitamin B complex; thiamin, riboflavin, nicotinic acid, pantothenic acid and cyanocobalamine. (B_{12}). Concentration of the vitamins in button mushroom (*Agaricus bisporus)* has been

reported to vary from farm to farm, with higher concentrations of vitamin B_{12} detected in outer peel than in cap, stalk, or flesh, suggesting that the vitamin B_{12} is probably bacteria-derived (Koyyalamundi *et al*[a], 2009).

Ascorbic acid is present in low amounts in mushrooms (Roberts *et al*, 2010). Ascorbic acid is an antioxidant; it is involved in elimination of free radicals like hydroxyl, superoxyl and peroxyl radicals, responsible for oxidative stress. Ascorbic acid together with other antioxidants, protect biomembranes against damage by eliminating peroxyl radicals in the aqueous phase before the latter can initiate lipid peroxidation (Davey, 2000). Ascorbic acid is thought to exert a protective role against various oxidative stress-related diseases such as heart disease, stroke, cancer and several neurodegenerative diseases (Helliwel, 1996).

Fat soluble vitamins are present in negligible amounts (Roberts *et al*, 2010). However, mushrooms that have been exposed to ultraviolet (UV) light contain large amounts of vitamin D_2. When exposed to UV light, mushrooms convert ergosterol, a chemical found in large concentrations in many mushrooms, to vitamin D_2 (Koyyalamudi *et al*[b], 2009). This is similar to the reaction in humans, where Vitamin D_3 is synthesized after exposure to UV light (Lee and Lee, 2009; Koyyalamudi *et al*, 2009[b]; Roberts *et al*, 2010).

2.5.5 Minerals

Mushrooms are reported to have high mineral content. One of the functions of fungi in mycorrhizal relationship with plants is the uptake of minerals; their high mineral content is therefore expected (Falandysz, 2008). Mushrooms are rich in minerals; potassium, phosphorus, calcium, magnesium and iron and selenium (Adejumo and Awosanya, 2005; Falandysz, 2008). Potassium and phosphorus are reported to have

very high bioaccumulation; with potassium accumulation of up to 20-40 fold that in the substrate (Kalac, 2012). Different species have varying amounts of individual minerals. Selenium, a mineral found in many mushrooms, works as an antioxidant to protect body cells from damage that might lead to heart disease, some cancers and other diseases associated with aging process. These diseases are brought by accumulation of free radicals in the body (Adejumo and Awosanya, 2005; Kalac, 2012).

2.5.6 Insoluble fiber

Mushrooms have both insoluble and soluble fiber. The insoluble fiber constitutes 22-30 % and it is mainly the chitin, a co-polymer of N-acetyl glucosamine linked to D-glucosamine; with the former predominating (Synytsya *et al*, 2008). It is responsible for the rigidity and support that allows the mushroom to stand upright and be flexible enough to sway without snapping. Chitin is reported to account for 80-90 % dry matter in the cell wall (Kalac, 2012).

Dietary fiber is one of the keys in lowering cholesterol levels and risk of heart diseases, type 2 diabetes, colon cancer and general bowel health (Komura, 2010). Chitin has been documented to act as cholesterol reducer, and as a prebiotic for the desirable gut flora (Kurtzman, 2005). Getting enough fiber has also been linked to a lower body mass index, an indicator of obesity (Chang and Miles, 1989). The total (soluble and insoluble) fiber in mushrooms range between 4-20 % dwb (Tamer and Copur, 2010).

Numerous applications of chitin and its derivative chitosan are found in medicine, agriculture and industry e.g. in coating for fresh-cut fruits and vegetables (Jeong *et al*, 2010). Osteoarthritis has become a common problem all over the world. This is

the loss of joint cartilage, so that instead of the cartilage at the end of the bones rubbing as the joints move, the joint bones rub directly on each other resulting in painful joints. Glucosamine, the product of chemical digestion of chitin, has been accepted as "over the counter cure" for osteoarthritis (Kurtzman, 2005).

2.6 Other health benefits

Mushroom nutriceuticals describe a new class of compounds extractable from either the mycelium or fruit body of mushrooms and embodies both their nutritional and medicinal features. They are consumed in the form of capsules or tablets as a dietary supplement (not a food), which has potential therapeutic applications (Chang and Miles, 1989). These compounds may enhance immunity of the human body or cause regression of the disease (Jeong et al, 2010; Ramkumar et al, 2010).

Mushroom nutraceuticals are enriched food materials which are used for maintenance of healthy diet. These are part of a meal (Chang and Miles, 1989; Shiuan, 2004). Infusion of mushrooms has been used to prevent beriberi. In addition, the decoction has been used for the treatment of abscesses and wounds (Yu *et al*, 2009).

Mushrooms are reported to have phytochemicals like ergothioneine and other compounds which are strong antioxidants. Ergothioneine and other phytochemical compounds seem to mop the free radicals generated during metabolism. Free radicals are produced in the normal metabolism of aerobic cells, mostly in the form of reactive oxygen species (ROS) like superoxide anion (O_2^-) (Isabel, 2008; Zhu *et al*, 2011). Once produced, most of the free radicals are neutralized by cellular antioxidant defense enzymes e.g. Superoxide dismutase (SOD), catalase (CAT) and

non-enzymatic molecules like ascorbic acid and carotenoids or selenium (Barros, 2007; Falandyz, 2004). Maintenance of equilibrium between free radicals production and antioxidant defenses is an essential condition for normal organism functioning (Fang *et al*, 2002). Nevertheless, the equilibrium between ROS production and antioxidant defenses might be displaced either by the overproduction of ROS or by the loss of the cell Polyphenolic compounds are classified into (i) phenolic acids (e.g caffeic, gallic acids), (ii) flavonoid polyphenolics and (iii) non-flavonoid polyphenolics (e.g stilbenes). They all display a large diversity antioxidant defenses. This disequilibrium is known as oxidative stress (Fang et al, 2002). Presence of free radicals in the body interfere with cell integrity hence normal functioning and division are altered (Kwok, 2005). Non-controlled production of free radicals has been related to more than one hundred diseases including several kinds of cancers, diabetes, cirrhoses, cardiovascular diseases and neurological disorders. The overproduction of ROS has also been related to the aging process (Adams et al,2008; Fang et al, 2002).

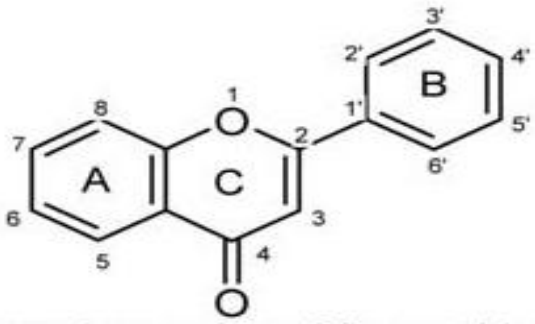
Mushrooms have shown the ability to accumulate a variety of secondary phytochemical metabolites that include polphenolic compounds, terpenes, tannins, saponins and terpenoids among others.

They are reported to have high Radical scavenging activity (Fang, 2002;

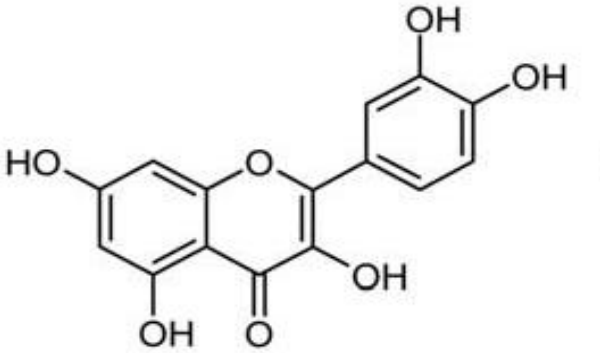
Doughari, 2012). The *in vitro* and *in vivo* studies suggest that they have a variety of beneficial biological properties like anti-inflammatory, antitumor and antimicrobial activities. The antioxidant properties have been attributed to the presence of phenolic acids and flavonoids (Barros, 2007).

Flavonoid polyphenolics, characterized by C6–C3–C6 skeleton, are classified into flavonols, flavones, flavanols, flavanones, anthocyanins and isoflavonoids (Hollman, 2000; Nijveldt, 2001). Over four thousand flavonoids are known to exist and some of them are pigments in higher plants. The radical scavenging activity of phenolic compounds has been correlated to their chemical structures (Nijveldt, 2001). In general, free radical scavenging and antioxidant activity of phenolics (e.g. flavonoids, phenolic acids) mainly depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenol molecules.

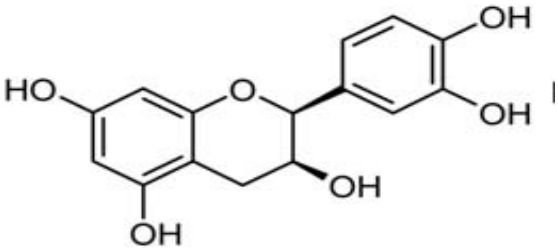
Quercetin, a flavonol, is reported to be the most abundant flavonol (Fang, 2002). To be a good radical scavenger a flavonoid requires ortho dihydroxy groups on B ring and presence of 2-3 double bonds in conjunction with 4-oxo function in C ring. Quercetin activity is due to hydroxyl groups arrangement that allow delocalization of A and B rings. This is not the case with catechin, a flavanol, and it is reported to have lower scavenging activity. Similarly, a glycosylated form of quercetin, rutin, has even lower activity; the activity order is quercetin > (+)-catechin > rutin (Nijveldt et al, (2001); Hollman, 2000). Saponins comprise a large family of structurally related compounds containing a steroid or triterpernoid aglycone attached to one or more oligosaccharide moieties by glycosidic linkage (Hostettmann& Marston, 2005).



General structure of flavonoids:
2-phenyl-1,4-benzopyrone



Quercetin



(+)-Catechin

They are compounds reported to be present in mushrooms. They exert various biological benefits such as hypolipidemic and anti-cancer anti-inflammatory and anti-diabetic activity (Pathak *et al*, 1997; Banno and Rajarathnam, 1986).

Terpenoids are secondary metabolites with molecular structures of carbon backbone made up of isoprene (2-methylbuta- 1, 3-diene) units. Isoprene contains five carbon atoms and as a result, the number of carbon atoms in any terpenoid is a multiple of

five. To date, over 3600 terpenoids have been identified. The terpenoids group show significant pharmacological activities, such as anti-viral, anti-bacterial, anti-malarial, anti-inflammatory, inhibition of cholesterol synthesis and anti-cancer activities (Beattie, 2011).

The soluble fiber, 4-9 %, is mainly beta-glucans (β-glucans) and chitosans which are components of cell wall. β-glucans are polysaccharides of β D-glucose monomers linked by β 1-3 and 1-6 glycosidic bonds. The β-glucans found in numerous mushroom species have shown marked immunity-stimulating effects (Bobek, 1995; Fang, 2002). They contribute to resistance against allergies and may also participate in physiological processes related to the metabolism of fats and sugars in the human body. The β –glucans contained in oyster, shiitake and split gill mushrooms are considered to be the most effective (Cheung et al, 2003).

Chitosan is the deacetylated form of chitin (Lee and Lee, 2009). Chitosan has found use in food industry because of its emulsifying, antimicrobial, antioxidant and gelling properties. It also acts as a functional fiber (Tamer and Copur, 2010).

2.7 Flavor and taste components

Mushroom flavor is appreciated by many consumers. The odorous compounds identified belong to derivatives of octane and octenes, terpenes, aldehydes, sulphur and heterocyclic compounds. The derivatives include the alcohols, their esters with volatile fatty acids and ketones (Combet, 2006). Non-volatile taste compounds have been reported to include 5'nucleotides of adenosine, guanosine, inosine, uridine and xanthosine (Tsai et al, 2008). The equivalent 'umami' concentration (EUC)

expressed as grams monosodium glutamate equivalent (MSGE) have been calculated for the five nucleotides.

2.8 Mushroom processing

Mushrooms have an average moisture content of 90 %. They therefore have a short shelf life. At 4^0 C to 5^0 C fresh mushroom can be stored for four days. Long time storage requires conservation methods (Byung, 2004). These methods include the following.

2.8.1 Drying.

This can be sun drying, cabinet or freeze drying. Sun drying is the most convenient and cheap. Either way the product is packaged in moisture proof package after drying. In dry state mushroom may be stored for six months or longer depending on storage conditions (Byung, 2004)..

2.8.2 Canning

Canning is a technique by which the mushrooms can be stored for long period of up to one year. Most of the international trade in mushrooms is done in this form. The canning process can be divided into various unit operations namely cleaning, blanching, filling and sealing, sterilization, cooling, labelling and packaging. This method is capital intensive and it requires large volumes of fresh mushrooms for the operation to be viable (Rai and Arumuganathan, 2008).

2.8.3 Conservation in oil or vinegar.

In this method mushrooms are washed, blanched in water or broth containing salt and spices, put in acid-resistant containers like glass, the broth is added to the rim and tightly closed. Olive oil or vinegar may be added to boost preservation effect. The method is cheaper than a canning operation.

2.8.4. Use of mushroom extract as processing ingredients.

Research on effect of ergothioneine (ESH) extract on color of minced big-eye tuna and yellow-tail fish meats indicated that the bright red color was stabilized against discoloration that results from oxidation process. Ergothioneine is a potent antioxidant prepared from different edible mushroom species (Huynh *et al*, 2010).

Scelta Mushrooms is recognized internationally as a leader in developing and supplying mushroom extracts to the food industry, food service and retail. Its expertise covers a wide range of processed mushroom products like frozen preserves and extracts. These find their way into pizzas, soups, and sauces (Scelta, 2011).

Mushroom parts and residues from mushroom processing which normally go to waste, such as mushroom stipes and cooking broth have been used to produce concentrates in liquid and spray-dried powder form. The concentrate serves as a mushroom base for soups and sauces. Furthermore, it is used as a flavor enhancer for a variety of dishes without imparting a pronounced mushroom flavor. It is a 100 % natural product with the potential to replace monosodium glutamate (MSG) and reduce the use of salt (Scelta, 2011). The concentrate contains 95 % of the healthy vitamins and minerals.

2.9 Status of mushroom growing and consumption in Kenya

In Kenya, mushroom cultivation is a recent introduction (1970s), but the production is slowly and steadily picking up. The exotic mushrooms currently cultivated in Kenya are Button *(Agaricus bisporous)* Oyster *(Pleurotus spp)* and Shiitake *(Lentinula edodes).*Shiitake is both edible and medicinal. Shiitake is grown at very low scale, mainly for hotel industry that caters for foreign and high end clients. Reishi (*Ganoderma lucidun*) is grown on a small scale for their medicinal values (Wambua, 2004).

Kenya's annual production is estimated at 500 tones with a farm gate value of KES. 225 million and a retail value of KSh. 340 million (Farm Concerne, 2005). The bulk of this production comes from large scale farms which constitutes 90-95 % of the total production. The major commercial mushroom producers in Kenya are Agridutt Kenya Ltd, 35 %; Rift Valley Mushrooms, 30 %; Olive Farm, 20 % and Devan 10 % (Farm Concern, 2005). Small scale production is concentrated mainly in Kisumu, Kakamega, Mombasa and Malindi Counties.

The button mushroom which constitutes the bulk of the production is grown mostly by large scale farmers who export 95 % of volume produced. It is capital intensive and requires sophisticated technology. Consequently this mushroom is expensive and out of reach for most Kenyans million (Farm Concerne, 2005)..

Small scale farmers produce oyster whose capital requirement is lower than that of button (Wambua, 2004). Value addition is done at very low level in the country. Only about 10 percent of the producers process mushroom into flour and incorporate in cakes (Odendo *et al*, 2009). Constraints to production as revealed in a 2007

consultative stakeholders' workshop include high input costs, lack of quality spawn, diseases and pests, lack of proper skills in production and postharvest handling, and a lean government extension service (Farm Concern, 2005) If these constraints are addressed probably more people will enter the industry which would translate to increased supply and lower prices. More people will be encouraged to eat mushrooms while the excess supply will enter the value-added chain.

2.10. Economic implications of mushrooms growing in Kenya

Collection and consumption of wild mushrooms will not only protect the forest ecosystem but will also be a source of food and vital nutrients to the local people.

Mushroom growing improves environmental management. Mushrooms degrade high ligno-cellulosic agricultural waste, thereby recycling the vital nutrients. In the process additional high quality food is obtained in form of mushrooms (Pathak *et al*, 1997). Further to this the spent compost is used as animal feed or as organic fertilizer. Mushroom culture, growing and consumption, offers vast rural employment potential. Mushroom cultivation is low cost, labor intensive which is plenty in rural areas (Quimio, 1990). Land availability for crop or animal production is a limiting factor most of the time. Mushroom cultures require little space- in basements, garages, between houses/trees or other areas around the house. No fertile land or sunlight is required for mushroom growing; there is no competition for space between crops and mushroom growing (Wambua, 2004).

Mushroom growing is women-friendly operation. Women provide most of the labor in farming systems; there is need to diversify the income opportunities available to them and to lighten their workload. The additional income and food will improve

family welfare. Mushrooms have a big export potential. With developed countries turning to mushrooms for neutriceuticals and nutraceuticals demand for fresh and value added mushroom products can only increase (Farm Concern, 2005).

Additionally regular consumption of mushrooms, cited as rich in high quality protein, water soluble vitamins and minerals, will reduce incidence of deficiency diseases which are killing millions of children less than 5 years in developing countries, Kenya included (Siekmann, 2003). There is need to encourage small scale production to create jobs especially for the youth and eradicate malnutrition, poverty and hunger which are rampant in rural areas.

It would be safe to state that mushroom culture if introduced and adopted will revolutionize economic status of the currently majority poor. Their lives will be greatly improved through consumption of balanced meals and establishment of small business enterprises (SMEs) in mushroom production and value-added chain. The value chain has potential to generate millions of jobs.

2.11 Potential hazards associated with mushroom consumption

There are mushroom species that produce secondary metabolites which can be toxic or mind-altering. Mushroom poisoning (mycetism) refers to harmful effects from ingestion of toxic substances present in a mushroom. These symptoms can vary from slight gastrointestinal discomfort to death. The toxins present are secondary metabolites produced in specific biochemical pathways in the fungal cells (Chang and Miles, 1989). Mushroom poisoning is usually the result of ingestion of wild mushrooms after misidentification of a toxic mushroom as an edible species, ‘the look-alikes’. The most common reason for this misidentification is close

resemblance in terms of color and general morphology of the toxic mushrooms species with edible species (Chang and Miles, 1989). The toxic metabolites can be divided into eight groups based on compounds responsible for toxicity (Wong, 2003; Cope, 2007).

Class I: Cyclopeptides, e.g., Amanitoxins. The toxicity symptoms start 6-24 hours after mushroom consumption. These include severe abdominal pains, vomiting and diarrhea that persist for 6-9 hours. Death may occur in 7 days due to gastrointestinal bleeding and kidney failure.

Class II: Gyromitrin, e.g. Monomethylhydrazine (MMH). Symptoms do not begin until 6-8 hours after consumption. They include vomiting, nausea, stomach cramps, loss of muscular control and fever. The victim may make full recovery.

Class III: Orellanine and orelline. Symptoms do not begin until 12 hours to 3 days after consumption. These include intense burning thirst, dry mouth followed by gastrointestinal disturbances, headache, pain in the limbs, and loss of consciousness. Death may occur due to kidney failure

Class IV: Muscarine. Symptoms begin about 30 minutes – 2hours after ingestion. They may include excessive sweating, salivation and tears. Other symptoms include nausea vomiting and diarrhea, blurred vision and urge to urinate. Full recovery may occur.

Class V: Ibotenic acid and Muscimol. Symptoms begin about 1-2 hours after ingestion. These include dizziness, and muscle spasm followed by deep sleep during which euphoria and hallucination may occur.

Class VI: Coprine. Symptoms include nausea, vomiting and cramps that occur within 30-60 minutes. Toxin may last up to 72 hours.

Class VII: Psilocybin and Psilocin. Symptoms may occur within 10-30 minutes after consumption of mushroom. These include inebriation or hallucination, lack of sleep and uncontrollable laughter. Complete recovery possible 5-10 hours after consumption

Class VIII: Gastrointestinal irritants. The group is composed of a number of unrelated compounds. Symptoms may occur within 30 minutes to 2 hours after consumption of mushroom. These include: vomiting, nausea, diarrhea, stomach cramps and sweating. Usually complete recovery in 3-4 days.

The ability of mushrooms to absorb heavy metals, including those that are radioactive, may cause heavy metal toxicity (Garcia *et al*, 2009; Stihi *et al*, 2009). Collection of mushrooms along or near industrial waste should be avoided.

Mushrooms have been found to contain enzyme thiaminase. This enzyme destroys one of the water soluble vitamins, thiamine. However the enzyme is highly heat labile and a mild heat treatment inactivates it. It is subsequently digested as a protein (Kutzman, 2005).

Some examples of poisonous and hallucinogenic mushrooms are presented in plate 3

A

B

C

D

Plates 3: Photographs of poisonous and hallucinogenic mushroom

(Source: Beug, 2000).

A. *Gymnopilus spectabilis.* (hallucinogenic). Toxin, psilocybin or psilocin.

B. *Omphalotus olearius* (Jack O'Lantern)- *glows* in the dark. Toxin, Terpinoid compounds.

C.*Amanita phalloides* (death cap). Toxin, amanitins in lethal levels.

D. *Amanita muscaria.*(hallucinogenic and poisonous) Toxins, muscimol and ibotenic acid

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental design

The project was cross-sectional study, designed to collect wild edible mushrooms in areas where local communities were known to collect and use mushrooms as food. Members of the communities were to be randomly picked and verbally interviewed on types of wild edible mushrooms consumed, places where they were easily available and how to distinguish between edible and toxic varieties. Choice of collection sites was to be made with the assistance of Agricultural Extension Officers and local communities who collect/consume the mushrooms.

3.2 Collection and identification of wild edible mushrooms

Mushrooms collection was done with the assistance of Agricultural officers and members of local community in each case. Mushrooms were collected from Aberdare, Mt. Elgon and Arabuko Sokoke forests and Kisumu, Kakamega and Siaya Counties (Fig 2-6). Permission to enter different forests was sought from Kenya Wildlife Service (KWS) in Nairobi and this was granted. Entering and mushroom collection in different forests was done when accompanied by armed KWS officers for security from wild animals or poachers.

Fresh mushrooms were transported in cool boxes to the Food Science and Technology department, Jomo Kenyatta University of Agriculture and Technology (JKUAT) within 12 hours after collection. Samples of each of the wild mushrooms

were sent to Kenya National Museums, Herbarium department for taxonomic identification by taxonomists

Mushroom collection sites (MCS).

Mushrooms were collected in different parts of the country as shown in figures 2-6.

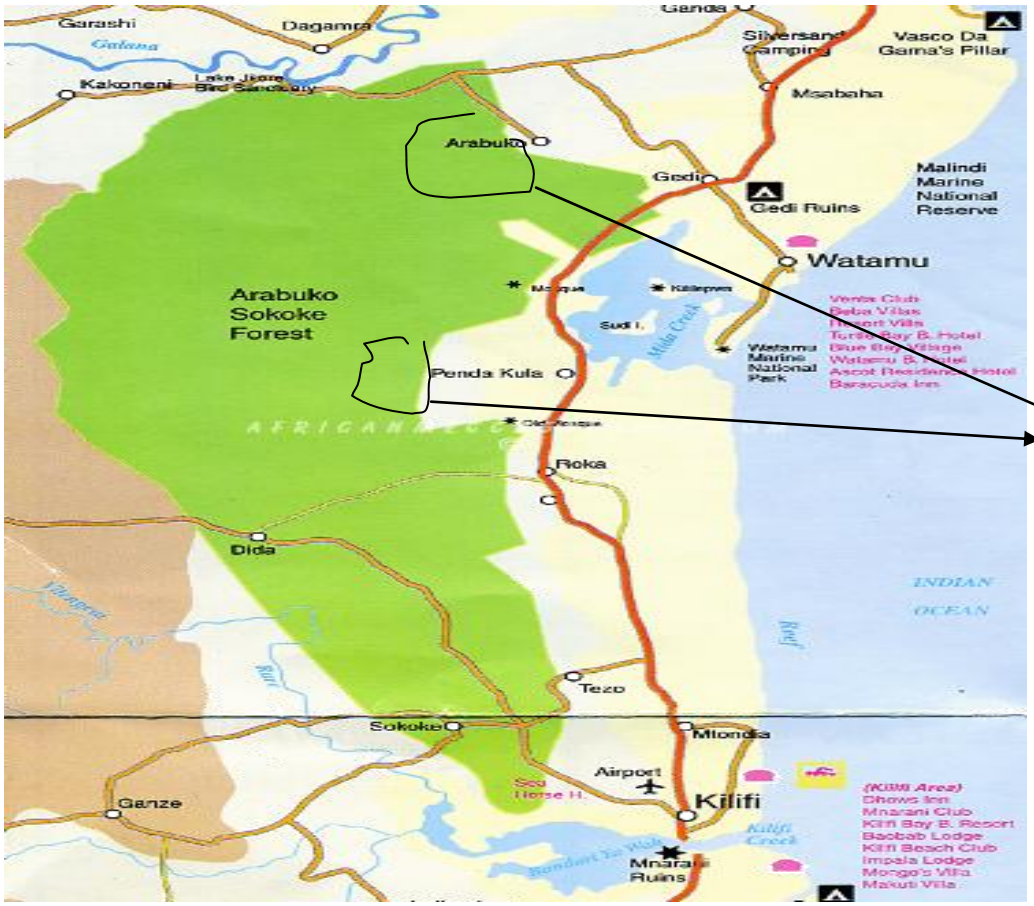


Figure 3.2: Arabuko Sokoke Forest map (Source: http://www.africanmeccasafaris.com 2012

In Arabuko Sokoke forest (figure 2), mushrooms were collected from Penda kula, Kararacha, Arabuko and Mabwani areas.

Figure 3.3: Siaya and Kakamega County map (Source: http://www.africanmeccasafaris.com 2012)

In Siaya and Kakamega Counties (figure 3), collection was done in Alego Kaluo and around Kakamega town respectively. In the region wild mushroom consumption is high by the Luo, Luhya and other communities who inhabit the area.

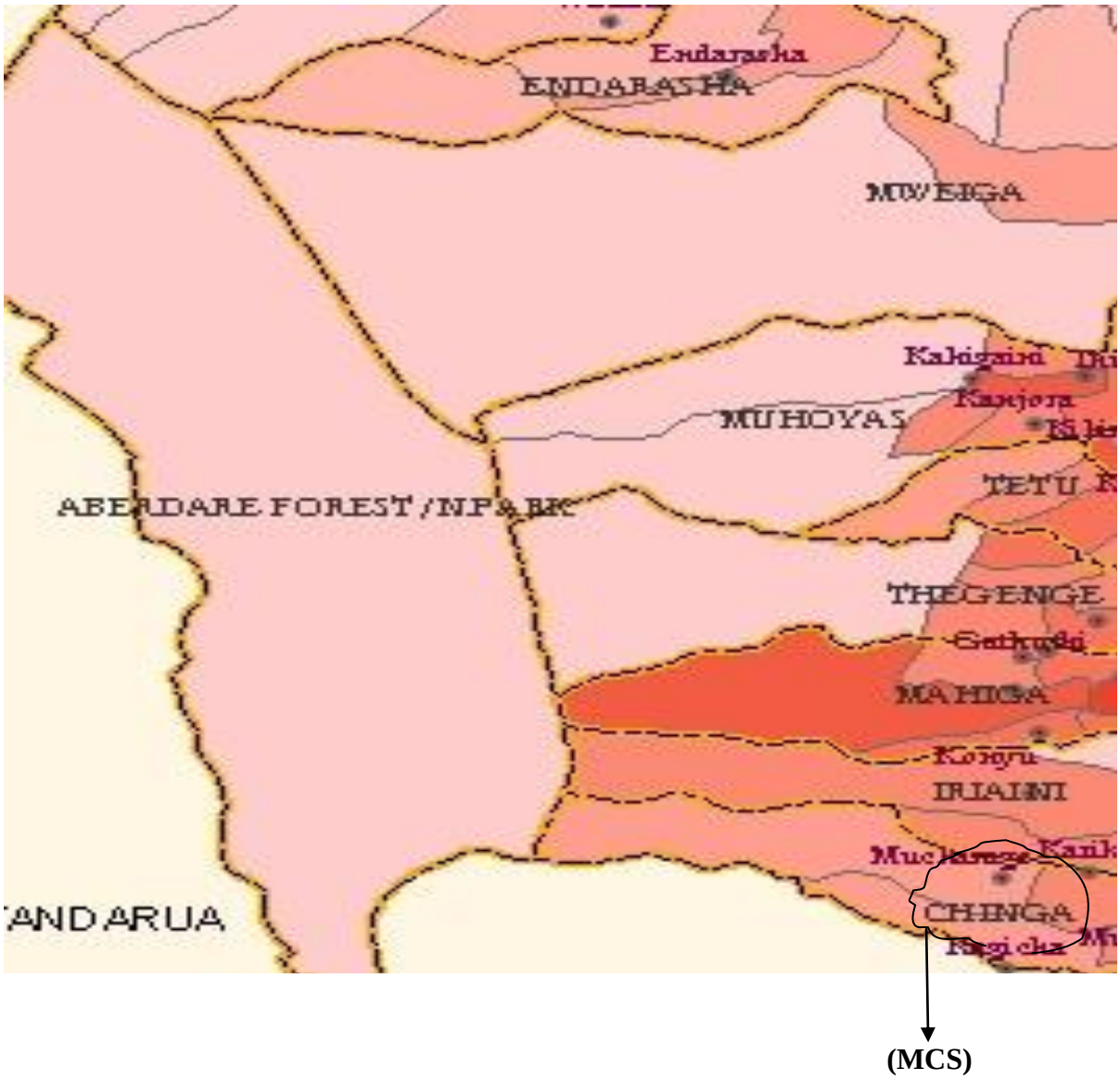
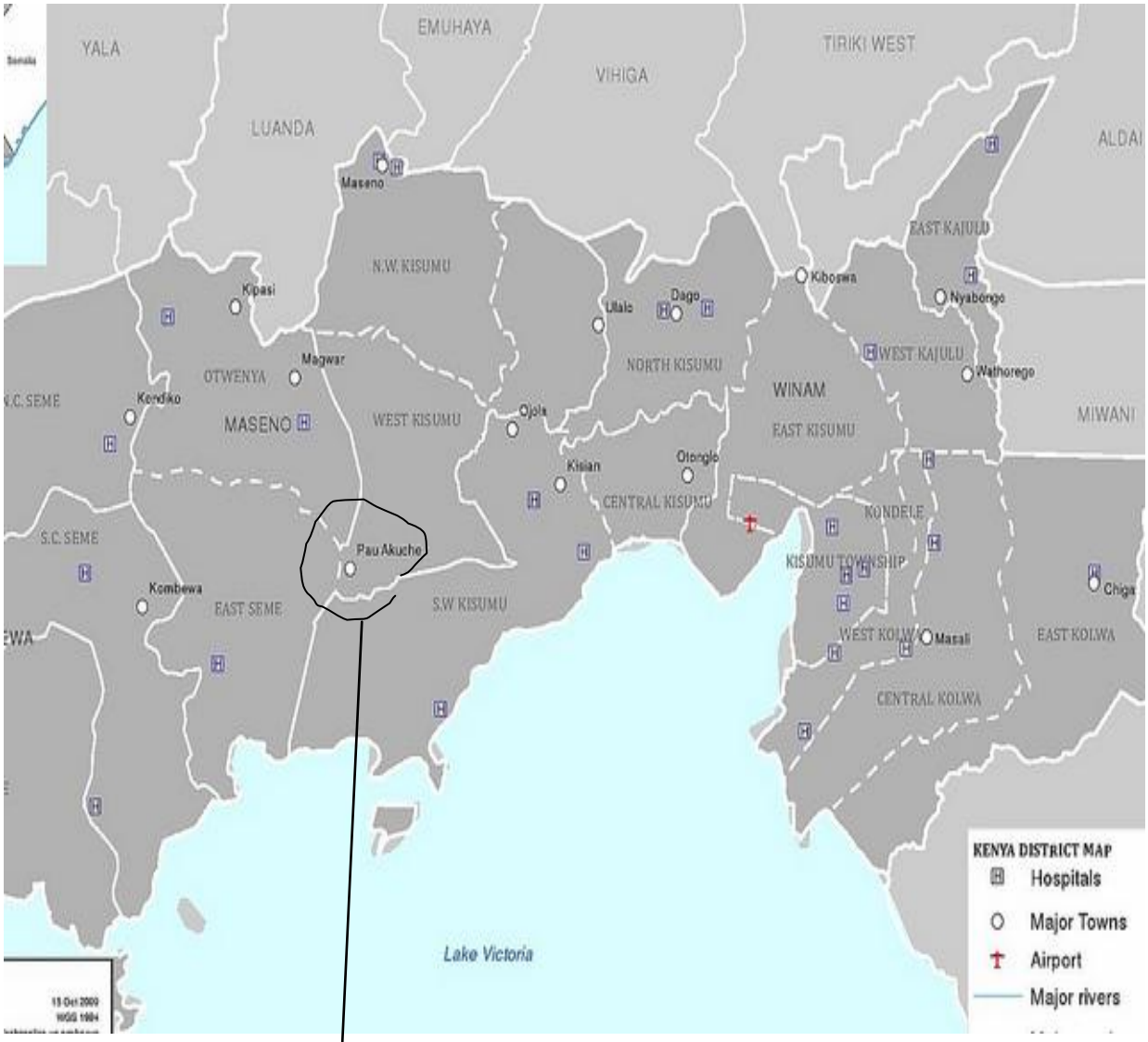


Figure 3.4: Aberdares Forest map (Source: http://www.africanmeccasafaris.com 2012)

In Aberdare forest (figure 4) collection was done in Chinga area (fig. 4). This is an area where collection and consumption is done by few locals. Most consumers are the immigrants from other parts of the country on employment or businesses e.g Luos, Kisiis and Luhyas. The indigenous people, the kikuyus, concentrate on food crops and tea farming. A minority however collect and use the mushrooms as food.



(MCS)

Figure 3.5: Kisumu West County map (Source:http://www.africanmeccasafaris.com 2012).

In Kisumu West County (Fig.5), mushrooms were collected from Pau Akuche.

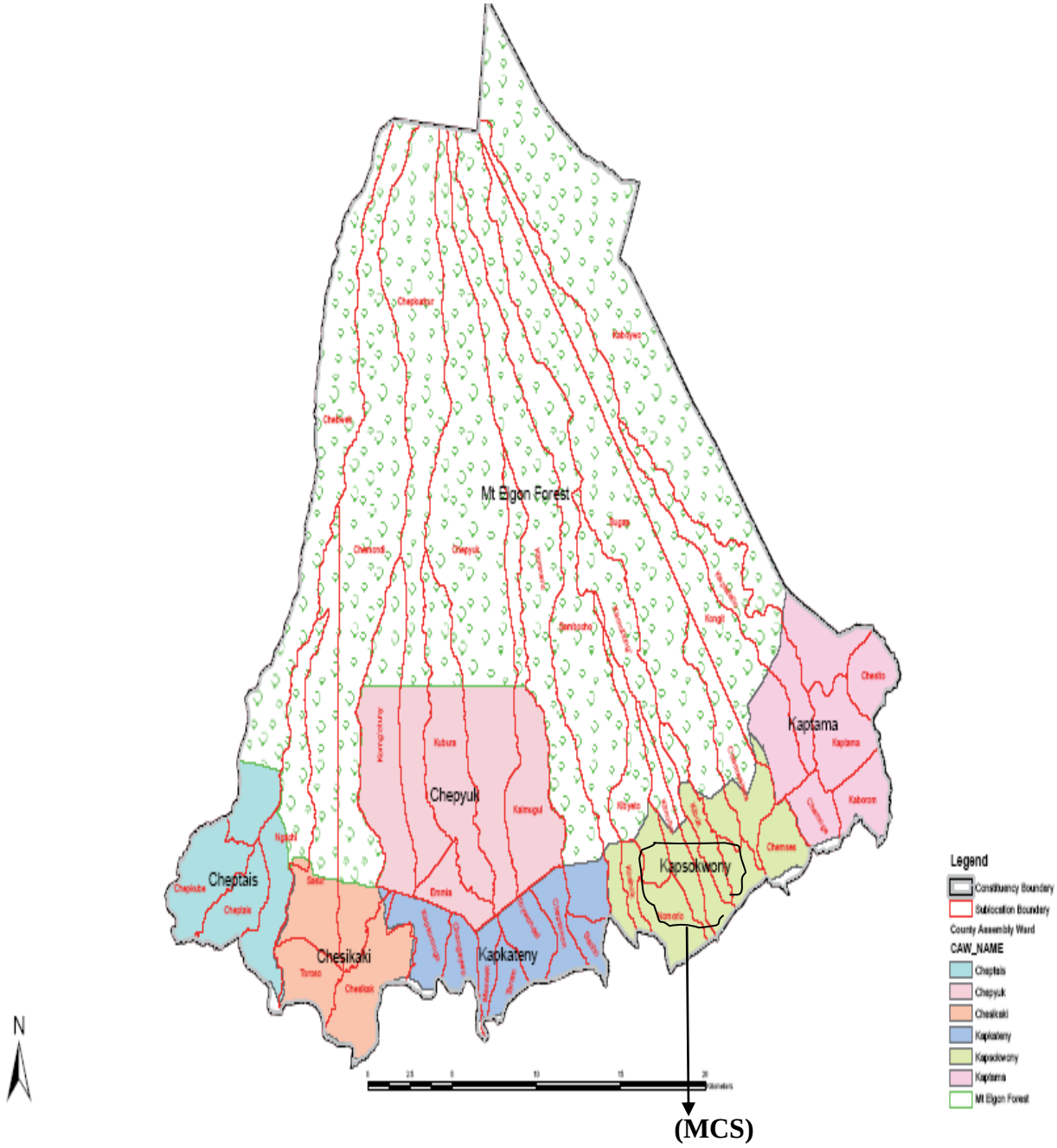


Figure 3.6: Mt. Elgon Forest (district) map

(Source:http://www.africanmeccasafaris.com 2012)

In Mt Elgon mushroom collection was done in Kapsokwon (chemweusus, Kongit and Bugaa) area. In this region local community, mainly the Kalenjins, collect and consume wild mushrooms.

3.3 Preparation of the mushrooms for analysis

At JKUAT all the mushrooms were cleaned off soils, mud, and trash. The fresh mushrooms for moisture and water soluble vitamin determination were kept in frozen storage.

To determine the effect of fruit body maturity on proximate, mineral and phytochemical composition, *oyster* was segregated into young and mature fruit bodies. The young fruit bodies were harvested five hours after pin head emergence while mature ones were fully open, a day after pin-head emergence. Oyster was chose on the basis of its being cultivated in JKUAT hence available at any stage of maturity.

To determine whether fruit body tissue had influence on proximate, mineral, water soluble vitamins and phytochemical composition, *oruka* mushroom was separated into caps (pilei) and stipes. The mushroom was chosen on the basis of its large size with distinct separate stipes and huge caps. It was also available in large quantity.

To determine if geographic location had influence on composition of the mushrooms, the following pairs of the same mushroom variety, *Obulando* vs *Olando* from Kakamega and Kisumu respectively and *Makunu* vs *Mariondonik* from Aberdares and Mt Elgon forests respectively were analyzed and compared.

The segregated and non-segregated mushrooms were cut into small pieces, dried in hot air oven at 70^0 C for 24 hours. The dry mushrooms were milled and stored in air-tight sample bottles. All the mushrooms were analyzed for proximate, mineral and phytochemical composition.

3.4 Determination of proximate composition of the mushrooms

Proximate composition was determined using standard methods. Lipids were determined by Soxhlet method; crude protein by semi-micro-kjeldhal method, total ash by incineration in a muffle furnace at 550^0 C for 8 hours and insoluble fibre by Hennenberg-Stohman method (AOAC, 2000). All determinations were done in triplicate using analytical grade chemicals. The percent soluble carbohydrates was determined by difference: 100-(lipid +protein +ash + insoluble fiber); on dry weight basis. The factor used in conversion of Nitrogen to protein content was 4.38 (Pathak *et al*, 1997).

3.5 Quantification of minerals.

The quantification of minerals, calcium, zinc, potassium, sodium, iron, copper and manganese was done by atomic absorption spectrometry using AAS (Shimadzu AA-6200) according to AOAC methods (AOAC, 2000). Phosphorus was determined using colorimetric method (AOAC, 1998).Using reference minerals (Fluka ppm ±2-4); standard curves (appendix 1f and h) for each mineral were prepared and used to determine the mineral concentration in the mushroom.

3.6 Determination of moisture and water soluble vitamins

The following mushrooms were selected for moisture content determination; *button, oyster*, *oruka* and *makunu ma mu*titu. The selection was based on quick availability in fresh state. Moisture content was determined by oven drying method, oven at 105^0 C until constant weight was obtained.

Water soluble vitamins were determined using the HPLC method described by Kimalasvi, (1985) with minor modifications. The incubation temperature was lowered from 90^0 C for 2 hours to 40^0 C for 16 hours. Weighed fresh samples and tissues (*Oruka* pilei and stipes) were macerated using motor and pestle and transferred into a 100 ml conical flask. To the macerate, 20ml 0.1 N sulfuric acid was added and the mixture put in a shaker maintained at 40^0 C for 16 hours. The pH was adjusted to 4.5 using 2.5 M sodium acetate solution. To hydrolyze the carbohydrates and proteins in the macerate, 0.3 g takadiastase and 0.5 g papain enzymes were added and the mixture incubated at 40^0 C for 16 hours. The mixture was centrifuged at 25^0 C and 10000 rpm for 15 minutes. The supernatant was filtered using whatman paper no.4 and then micro filtered using 0.45 µm micro-filter. Standard water soluble vitamins (Sigma 99 % and Fluka 98 % pure); B2, B6, B12, folate, niacin, pantothenic acid and ascorbic acid were each prepared in different concentrations, 40-200 ppm. From the dilutions, 30 µl each, was injected into the HPLC Shimadzu Model LC 10AvP, column: Supelco ODS (C18) 250 X4.6 mm, 5 µm and data processor: Shimadzu C-R7A plus.

The samples and standards were eluted with filtered and degassed mobile phase; methanol: water: acetic acid (40:59.5:0.5). The flow rate was maintained at 0.6 ml/min at 25^0 C. The retention time and concentration of reference vitamins were used to make the standard curves (appendix 1 c, d, e and g). Similarly 30 µl of the micro filtered mushroom extracts was injected into HPLC and eluted. Individual water soluble vitamin identity and content in the mushrooms were determined by comparing the retention times against those of standards and by calculation from the standard curves.

3.7 Determination of phytochemical compounds

To get the extract for phytochemical compounds determination, known weight (3-5 g) of the dried mushroom powder was mixed with 100 ml ethanol in a conical flask. The content was put on a shaker for 24 hours at room temperature. The liquid part was decanted and stored at 4^0 C. The solid was re-suspended in ethanol and procedure repeated. The liquid portions were combined, filtered using Whatman paper no. 4. The filtrate was concentrated in vacuum evaporator to about 10 ml, put in sample bottles and stored at 4^0 C.

3.7.1 Phytochemicals screening

Ethanolic extracts were subjected to preliminary phytochemical screening for the identification of various classes of active chemical constituents present as described by Harborne, (1998) with minor modifications.

3.7.1.1 Test for saponins

Foam test: To 1 ml of the extracts 5ml distilled water was added and shaken vigorously. Formation of foam that persisted for over 10 minutes indicated presence of saponins

3.7.1.2 Test for total polyphenols

Ferric Chloride test: To 1 ml of the extract, 2 ml of distilled water, 3 drops of 10 % aqueous ferric chloride ($FeCl_3$) and 3 drops of potassium ferrocyanide were added. Formation of blue or green color showed the presence of polyphenols

3.7.1.3 Test for anthraquinones

Weighed mushroom powder, 0.5 g, was boiled in 10 % hydrochloric acid and filtered while still hot. To the filtrate, 2 ml chloroform and 10 % ammonia solution

each were added. Formation of pink color in the aqueous layer indicated presence of anthraquinones.

3.7.1.4 Test for terpinoids

Water extract, 5 ml, was mixed with 2ml chloroform followed by sulfuric acid along the tube wall. Formation of brown colored ring at interface was a positive indicator.

3.7.1.5 Test for tannins

To 3 ml ethanolic extract was added 3 ml 10% ferric chloride ($FeCl_3$). Formation of blue/black color was a positive indicator.

3.7.1.6 Test for alkaloids

On silicagel-coated plates, 10 µl extract was spotted equidistant from each other and eluted with methanol-sulfuric acid solution. The dried plates were sprayed with Dragendroff reagent. Formation of red-brown coloration was a positive indicator.

3.7.2 Quantification of phytochemical compounds

3.7.2.1. Determination of total polyphenols

Phenolic compounds in the mushroom extracts were estimated by a colorimetric assay, based on procedures described by Barros *et al*, 2007 with minor modifications.

To 5 ml distilled water was added 0.5 ml Folin Ciocalteu's reagent. After 3 min, 1 ml 7.5 % sodium carbonate solution, 1ml extract were added to the mixture and made to 10 ml with distilled water. The mixture was kept in water bath maintained at 50 ^{0}C for 16 minutes. UV-Vis detector, (Shimadzu LC-10A) was used to read the absorbance at 765 nm. Gallic acid was prepared in different concentrations and the absorbance equally read at 765 nm. The data obtained was used to generate the

standard curve (appendix 1a) against which polyphenols in the mushrooms were calculated and expressed as Gallic Acid Equivalent (GAEs) / 100g dwb.

3.7.2.2. Determination of flavonoids

Flavonoids content in the extracts were determined by a colorimetric method as described by Barros *et al*, 2007 with minor modifications. To 1ml mushroom extract 0.3 ml 5% sodium nitrite and 4 ml distilled water were added and held for 5 minutes. To the mixture 0.3 ml of 10 % aluminium chloride was added and held for 6 minutes. Finally 2ml of 1M sodium hydroxide was added and the content made to 10 ml with distilled water. Using UV Visible spectrophotometer, the intensity of pink color was measured at 415 nm. Pure quercetin was prepared in different concentrations and absorbance read at same wavelength. The readings were used to make standard curve (appendix 1b), against which flavonoids in the sample were calculated and expressed as mg of quercetin equivalent (QE)/100 g dwb.

The same procedure was repeated but with catechin as standard in place of quercetin. Flavonoids in the sample were similarly calculated and expressed as mg catechin equivalent (CE)/ 100 g dwb.

3.8 Determination of radical scavenging activity (RAS)

The radical scavenging activity of the mushroom extracts was determined against 2, 2-Diphenyl-1-picryl hydrazyl radical (DPPH) method using UV visible spectrophotometer at 517 nm. Radical scavenging activity was measured by a modified method previously described by Cheung *et al*, 2003. The following concentrations of the extracts were prepared, 0.05, 0.1, 0.5, 1.0, 2.0 and 5 mg/ml in

ethanol. Ascorbic acid was used as the antioxidant standard at concentrations of 0.02, 0.05, 0.1, 0.2, 0.5 and 0.75 mg/ ml.

To I ml of the extract in a test tube, 3 ml ethanol was added followed by 0.5 ml 1mM DPPH in ethanol. Incubation was done for 5 minutes after which the absorbance was read. A blank solution was prepared containing the same amount of ethanol and DPPH. Ascorbic acid dilutions were equally treated; absorbance read and a standard curve generated using the data obtained. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation:

% RSA = [(ADPPH-AS)/ ADPPH] 100

Where: AS is the absorbance of the solution when the sample extract has been added

ADPPH is the absorbance of the DPPH solution.

The extract concentration providing 50 % inhibition (EC_{50}) was calculated from the graph

3.9 Determination of the effect of heat on phytochemicals

Two fresh mushrooms, *Button,* and *oruka* were chosen on the basis of being available fresh in large quantity. Each variety was divided into two portions. One portion was given a 1 minute dip in boiling water and the other portion a 1 minute steam treatment. The treated samples were cooled immediately with ice cubes, dried in hot air oven at 70^0 C, milled and extract prepared as described in section 3.3. The extracts were used in determination of total polyphenols, flavonoids and radical scavenging activity as described in 3.7.2.1 -3.8

3.10. Product Development

Fresh mushrooms were dried in a hot air oven maintained at 70^0 C to constant weight, milled and sieved using stainless steel sieve 710 µm aperture size. The fine fraction passing through constituted the mushroom flour used in product development.

Product development targeted to replace part of wheat white flour with mushroom flour. The white flour is highly refined and stripped of a proportion of nutrients. Mushroom flour, on the other hand is reported to be rich in protein, beneficial bioactive compounds and minerals of public health concern.

Several preliminary formulation trials were done before the final formulations were was made. Gluten dilution effect, the ability to retain form, effect on texture and the targeted increase in protein and minerals were all taken into account.

3.10.1 Development of wheat-mushroom flour noodles.

The composite meal was prepared according to the formulation in table 1. The product was based on the formulation by Nissin Food Co of Japan and whose products are in production and on sale in Jomo Kenyatta University. The new formula included white flour, wheat whole meal and mushroom flour. The formulation and processing procedure are presented in table 1 and figure 7 respectively.

Table 3.1: Formulation of noodles

		Product		
Ingredient		**1**	**2**	**3**
Whole wheat flour	%	100	75	40
Wheat white flour	%	0	0	40
Oyster mushroom flour	%	0	25	20
Sodium carbonate	%	0.2	0.2	0.2
Sodium phosphate	%	0.2	0.2	0.2
Salt	%	1.0	1.0	1.0
Water	%	50	50	50

NB. The flour (wheat and mushroom) constitute 100 %; other ingredients weights are calculated based on flour weight.

Processing Procedure

All ingredients were weighed into a mixer, water added and mixed for 15 min.

↓

The dough formed was compacted to 20mm thickness and rested for 10 min.

↓

Then sheeted via a series of sets of rotating rollers with diminishing gap to 0.9mm

↓

The 0.9mm dough sheet was passed through slitter/curler

↓

80g portions were scaled into frying steel bowls and fried for two minutes at 150^0 C

↓

The cooked noodles were removed and allowed to drain off the excess oil.

↓

After cooling, they were packaged in clear polythene bags

Figure 7: Flow chart for production of noodles

3.10.2 Sensory evaluation of wheat-mushroom noodles

Each formulation was coded with three-digit random numbers. Noodles were reconstituted by holding in boiling water for 5 minutes. The water was drained and the noodles served in small bowls. Sensory evaluation was done immediately

Sensory evaluation of the prepared noodles in relation to appearance, aroma, taste, texture and overall score was carried out using a questionnaire (Appendix 2). Twenty five semi-trained panellists were recruited from the faculty staff and graduate students for the exercise. .

A 7-point hedonic scale (1-dislike extremely to 7-like extremely) with equivalent intervals was used (Ihekoronye and Ngonddy, 1985). The assessment was done under natural light.

3.11 Development of wheat -mushroom flour soup

The soup developed was based on formulae obtained from literature with some modifications (Rai and Arumuganathan, 2008). Part of wheat flour and corn starch was substituted with mushroom flour. The formula and processing procedure are presented in Table 2 and Figure 8 respectively.

3.11.1 Sensory evaluation of mushroom soup

Each formulation was coded with a 3-digit random number. Hot soup was placed in small plastic bowls and sensory evaluation in relation to appearance, aroma, taste, texture/consistency and overall score was carried out using a questionnaire (Appendix 3).

Twenty five semi-trained panellists were recruited from the faculty staff and graduate students for the exercise. .

A 7-point hedonic scale (1-dislike extremely to 7-like extremely) with equivalent intervals was used (Ihekoronye and Ngonddy, 1985). The assessment was done under natural light.

Table 3.2: Formulation of mushroom soup

		Product		
Ingredient		**1**	**2**	**3**
Wheat flour	%	3.5	3.5	3.5
Modified corn starch	%	3.5	3.5	3.5
Oyster mushroom flour	%	0	2.5	0
Oruka mushroom flour	%	0	0	2.5
Skim milk powder	%	2.5	2.5	2.5
Cooking fat	%	2.0	2.0	2.0
Sugar	%	1.5	1.5	1.5
Salt	%	1.0	1.0	1.0
MSG	%	1.0	1.0	1.0
White onion powder	g	0.1	0.1	0.1
Black pepper	g	0.01	0.01	0.01
White pepper	g	0.01	0.01	0.01
Water	%	100	100	100

NB. Water constitutes 100 %.

Production procedure

All dry ingredients were weighed and thoroughly mixed in a non-stick cooking pan.

↓

The dry blend and water were mixed to a smooth paste.

↓

The mixture was slowly heated to boiling, and held for 10 minutes.

↓

The soup was removed, served in bowls and

↓

Sensory evaluation was done immediately

Figure 8: Flow chart for the production of mushroom soup

3.12. Development of wheat -mushroom flour biscuits

Development of biscuits aimed at a formulation with reduced fats and sugar but raised level of dietary fiber by substituting part of white flour with mushroom and whole wheat flours. Formulation and the processing procedures are shown in Table 3 and figure 9 respectively.

Table 3.3: Formulation of biscuits

		Product		
Ingredient		**1**	**2**	**3**
Wheat whole meal	%	100	75	35
White wheat flour	%	0	0	35
Oyster mushroom-flour	%	0	25	30
Margarine	%	10	10	10
Cooking fat	%	10	10	10
Sugar	%	15	15	15
Baking powder	%	1	1	1
Salt	%	0.5	0.5	0.5
Ammonium carbonate	%	0.3	0.3	0.3
Water	%	40	40	40

NB. Wheat and mushroom flour weight constitute 100 %, other ingredients weights are based on this.

3.12.1 Sensory evaluation of biscuits.

Each formulation product was coded with a 3-digit random number. Biscuits were placed in small plastic bowls, and sensory evaluation done in relation to appearance, aroma, taste, texture and overall score using a questionnaire (Appendix 4). Twenty five semi-trained panellists were recruited from the faculty staff and graduate students for the exercise.

Production procedure

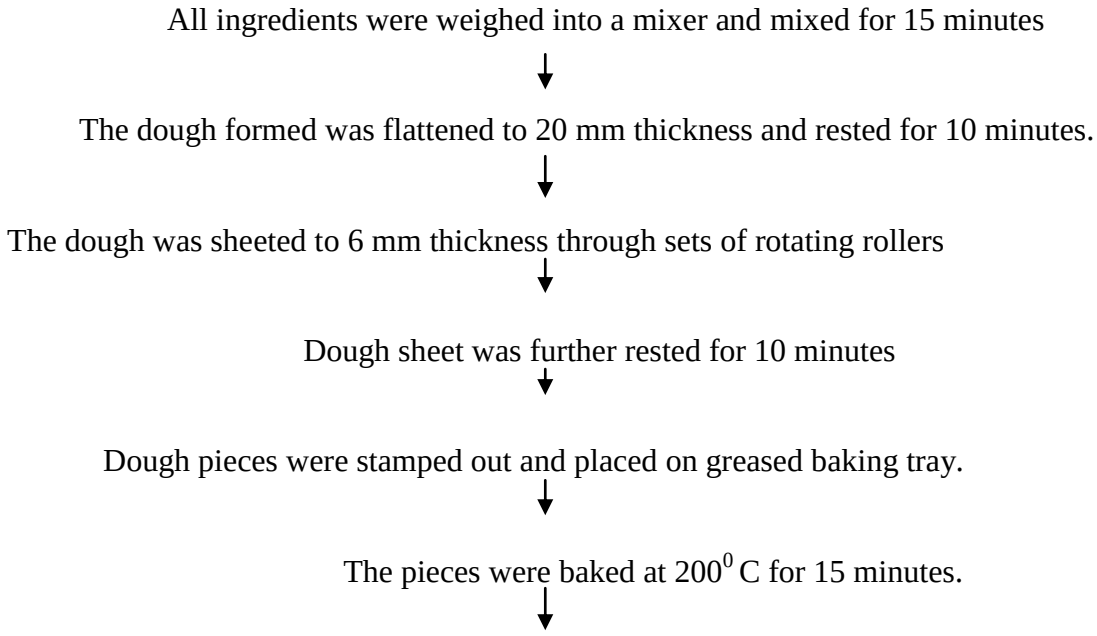


Figure 9: Flow chart for production of wheat meal-mushroom powder biscuits

A 7-point hedonic scale (1-dislike extremely to 7-like extremely) with equivalent intervals was used (Ihekoronye and Ngonddy, 1985). The assessment was done under natural light.

3.13 Nutrients in the developed products

Each of the noodles and biscuits formulations were analysed for protein, Iron and Zinc. These nutrients were chosen because of their significance in human nutrition. Protein malnutrition is common while Iron and Zinc are of public health concern in the country.

3.14 Data analysis

Descriptive analysis of data was done. Analysis of variance (ANOVA) between products was done using GenStat version 14 (Payne, 2006) and the means separated by calculating least significance difference (LSD). Correlation coefficient between total polyphenols and radical scavenging activity (RSA) was calculated

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Identification of mushrooms collected.

The mushrooms collected from various regions across the country are presented in Table 4.4.

Table 4.4: Mushrooms collected

Scientific name	Common(community)name	Region	Habitat
Agaricus bisporus	Button	Kiambu County	Cultivated
Preurotus florida	Oyster	Kiambu County	Cultivated
Termitomyces sp	Makunu (Kikuyu)	Aberdare Forest	Wild
Termitomyces sp	Mariondonik (Sabaot)	Mt Elgon Forest	Wild
-	Olando (Dholuo)	Kisumu County	Wild
-	Obulando (Luhya)	Kakamega County	Wild
Termitomyces sp	Oruka (Dholuo)	Kisumu West County	Wild
-	[Joga muhama (Giriama)]	ArabukoSokoke	Wild
Termitomyces sp	[Joga utuwe (Giriama)]	Arabuko Sokoke F	Wild
-	Malombo (Giriama)	Arabuko Sokoke F	Wild
Russula compressa	Mkundu wa nyani	Arabuko Sokoke F	Wild
-	Joga kadzonzo (Giriama)	Arabuko Sokoke F	Wild
Amanita zambiana	Rerema (Giriama)	Arabuko Sokoke F	Wild
-	Masikiro meruhe	Arabuko Sokoke F	Wild

Key: - Scientific name not yet known; Arabuko Sokoke F= Arabuko Sokoke Forest

Photographs of some of the mushrooms collected are presented in plate 4

A   B

 **C**  **D**

E   **F**

 **G**  **H**

Plates 4: Photographs of the mushroom collected

A Mkundu wa nyani; B, Masikiro meruhe; C, Rerema; D, Mariondonic/makunu; E, Oruka; F, Joga muhama; G, Kadzodzo; H, Malombo

The taxonomic identification of the mushrooms was incomplete therefore this report used local/common names for uniformity. Two cultivated mushroom varieties, white Button and white Oyster were included in the study for comparison purpose only

In coast region where most of the mushrooms were collected, the communities that use mushrooms as food are the Wadhta and Giriama living in the environs of the Arabuko Sokoke forest. Collection was done early in the morning mainly by women. Wadtha are culturally honey gatherers from the forest and are therefore more conversant with forest non wood products (FNWP) like the mushrooms. Little conservation of the wild mushrooms is done through drying, either because the locals are not trained or the volume collected is too low to warrant any conservation. Others like Orma consider mushroom a taboo; they do not eat at all.

A major problem encountered in collection is competition with wild animals like the baboons. It is common to find they started earlier and have eaten them, so one comes back empty handed. Encounter with dangerous animals like elephants is a reality which makes it very risky to the communities that would benefit from this freely available food. Entry to the forest is therefore highly restricted by Kenya Wildlife Service (KWS). The situation is slightly different in Aberdares forest where a few locals and immigrants collect and consume fresh wild mushrooms.

In western Kenya, Luos, Luhyas, Kisiis, Kalenjins and others use wild mushrooms for food. They not only collect but tsome also conserve by drying. The brown *obulando/ olando* can be found on sale in far off places like Gikomba market in Nairobi. The white *oruka* is not easily dried so it is consumed fresh at source.

Cooking method is similar for the fresh mushrooms in all the areas. Generally after thorough cleaning, the mushrooms may be size reduced and cooked in open pans

over low heat while stirring to evaporate most of the water. This is followed by addition of milk and a bit of salt to taste or it is then fried with seasoning and spices to taste. The cooked mushrooms are eaten with other starchy foods like *rice*, *ugali or chapati*.

The dry mushroom has to be reconstituted before cooking. After thorough cleaning, the mushrooms are soaked in clean water for several hours. It is then cooked by frying or boiling. Soda ash is traditionally added during the cooking to soften the mushrooms.

Use of bean trash ash extract is also practiced in cooking. After threshing the beans, the trash is burnt to ash. The ash mixed with water and allowed to stand for hours. This is filtered and the filtrate is mixed with mushroom during the cooking. This procedure is reported to soften the mushrooms and to hasten the cooking process. The brown mushroom is occasionally cooked with rice to make *mushroom rice pilau*; replacing beef during the cooking

4.2 Proximate composition of the mushrooms

Proximate composition of the mushroom collected is presented in table 5. The proximate composition values for all mushrooms analyzed range as follows: lipids, 1.6-10.3 %, insoluble fiber 4.5-16.3 %; ash 6.6-21.8 %; protein 16.6-35.9 % and carbohydrates 37.5-63.3 % (Table 5).

These values conform to documented figures of lipids 1.3-14.3 %; insoluble fiber 3.9 – 20.3 %; ash 17.4-33.6 %; proteins 14.0–60.3 %; carbohydrate 4.1-60.5 % of wild mushrooms (Manjunathan and Kaviyarasan, (2011); Okoro and Achuba, (2012) and Mallikarjuna *et al*, (2012). *Rerema* analyzed by Harkonen *et al*, (1995) in

Table 4.5: Proximate composition of the mushroom

Mushroom	Lipids%	Insol.fiber%	Ash%	Protein%	Carbs. %
Button	2.18±0.09	6.47±0.52	11.23±0.01	28.82±0.25	51.30±0.03
Oyster YFB	1.63±0.02	8.17±0.39	10.37±0.00	35.96±0.10	43.87±0.42
Oyster MFB	2.45±0.04	16.32±0.43	11.27±0.09	32.39±0.40	37.57±0.20
#*Olando*	6.38±0.19	6.66±0.177	12.29±0.01	29.43±0.74	45.24±0.65
#*Obulando*	6.88±0.00	9.47±0.283	6.6±0.04	29.97±0.66	47.08±0.15
**Makunu ma*	6.33±0.42	4.93±0.12	5.91±0.03	32.1±0.15	50.73±0.32
**Mariondonik*	7.62±0.07	8.57±0.15	6.44±0.37	35.94±0.51	41.43±0.34
Oruka-stipe	4.60±0.00	5.78±0.21	5.59±0.04	24.88±0.1	57.96±0.10
Oruka- cap	6.73±0.02	8.39±0.09	7.4±30.01	30.07±0.28	52.57±0.01
Joga muhama	10.3±0.28	6.76±0.17	10.82±0.54	31.68±0.01	40.44±0.10
Joga utuwe	4.57±0.00	6.47±0.20	7.49±0.20	19.2±0.26	62.27±0.25
Malambo	4.09±0.43	8.86±0.28	8.43±0.34	19.12±0.00	59.50±0.51
Mkundu wa n	4.94±0.10	5.95±0.12	7.35±0.38	22.74±0.00	59.02±0.68
J. kadzonzo	3.28±0.19	9.14±0.06	21.81±0.32	27.06±0.01	38.71±0.76
Rerema	7.61±0.08	4.52±0.24	11.28±1.05	26.16±0.05	50.43±0.56
Masikiro m	3.46±0.14	6.16±0.2	10.37±0.93	16.64±0.006	63.37±0.02

All values are expressed as means± SD on dry weight basis of triplicates, n=3

YFB= young fruit body; MFB=mature fruit body. Insol. fiber= Insoluble fiber

Olando of Kisumu same species as *Obulando* of Kakamega

**Makunu ma mutitu* of Aberdares is same species as *mariondonik* of Mt Elgon

'edible mushrooms of Tanzania' gave figures of lipids 15.7 %, ash 10.7 %, protein 27.3 % and carbohydrates of 27.3 %. The figures obtained in this research were lipids 7.6 %, ash 11.2 %, protein 26.1 and carbohydrates 50.4 %. in bracket. Except for the lipids and carbohydrates whose values are different, all other figure are comparable. *Button* and *Oyster* have been reported to have lipids as 1.0-9.4 %, fiber 1-16.2 %, protein 19.4-42.2 % and carbohydrates 26.1-53.3 % by Bano and Rajarathnam, (1986) and Tamar and Copur, (2010). The values obtained for these two species, lipids 1.6-2.1 %; fiber 6.4-8.1 %, protein 28.8-35.9 % and carbohydrates 43.8-51.3 % are within that range. The authors stated that ash is highly influenced by the growth substrate.

Effect of mushroom maturity on proximate composition

The maturity of the fruit body appeared to influence the proximate composition. This was reflected in data on Table 6.

Table 4.6: Effect of mushroom maturity on proximate composition

Mushroom	Lipids%	Fiber%	ash%	protein%	Carbohydrates%
Oyster Y	1.63±0.02 a	8.17±0.39 a	10.37±0.00 a	35.96±0.10 a	43.87±0.42 a
Oyster M	2.45±0.04 b	16.32±0.43 b	11.27±0.09 b	32.39±0.40b	37.57±0.20 b

Values in the same column followed by the same letter are not significantly different ($p \geq 0.05$)

The young fruit bodies (YFB) of *Oyster* have lower lipids, fiber and ash: 1.6 %, 8.1 % and 10.3 % respectively than the mature (MFB) bodies: 2.4 %, 16.3 % and 11.2 % respectively.

The reverse holds true for the protein and carbohydrates where the YFB have higher values, 35.9 % and 43.8 % respectively than the MFB, 32.3 % and 37.5 % respectively. This trend is in conformity to the reported figures by Barros et al, (2007). However the author gave values that were based on percent extract making it difficult to compare. Dikeman, (2005) reported that stage of maturity influence on proximate composition occurs in a non consistent manner. Crude fiber showed a big increase on maturity in *Oyster*, 8.1 % for YFB to 16.3 % in MFB. This was consistent with reported values by Manjunathan and Kaviyarasan, (2011). The increase is expected because insoluble fiber is composed of structural organic material, chitin, which provides support. As the mushroom gets large with maturity, the fiber is bound to increase. YFB have higher protein and carbohydrates than the MFB. Statistical data (Table 4.6) showed that all the nutrients in YFB and MFB were significantly different ($p \geq 0.05$).

Effect of mushroom tissue on proximate composition

Proximate composition for mushrooms seemed to be influenced by the tissue analyzed. The data obtained for *Oruka* mushroom cap and stipe is presented in Table 4.7. The figures showed that caps (pilei) have different proximate composition from the stipes of the same mushroom. The nutrients were higher in cap than in stipe except for the carbohydrate where the reverse was true. The trend is in conformity with report by Oboh and Shodehinde, (2009). The authors reported that stipe closer to cap has higher nutrients than further away. Statistical analysis (Table4.7) indicates that all the nutrients in the stipe and cap were significantly different ($p \geq 0.05$).

Table 4.7: Effect of mushroom tissue on proximate composition

Mushroom	Lipids%	Fiber%	ash%	protein%	Carbs.%
Oruka-stipe	4.60±0.00 a	5.78±0.21 a	5.59±0.04 a	24.88±0.1 a	57.96±0.10 a
Oruka- cap	6.73±0.02 b	8.39±0.09 b	7.4±30.014 b	30.07±0.28 b	52.57±0.01 b

Values in the same column followed by the same letter are not significantly different ($p \geq 0.05$)

Effect of geographical location on proximate composition of the mushrooms

Geographic location appeared to influence the proximate composition of a species. The result of analysis of *Mariondonik* and *makunu*, same species but collected from two different locations (Mt Elgon/Aberdare forests) gave different proximate composition values as shown in Table 4.8, The same pattern is shown by *olando* and *obulando* of Kisumu and Kakamega respectively. The data indicated that except for the protein in *Obulando/Olando* and ash in *makunu/Mariondonik* which were not significantly different ($p \geq 0.05$), all the other nutrients were significantly different ($p \geq 0.05$) in each pair. The data further indicated that proximate composition is not only species dependent but also geographic location influenced. The mushrooms from coast had lower protein values and higher soluble carbohydrates than those from elsewhere. It is likely that substrate composition and climatic conditions play a significant role in biosynthesis and accumulation of these nutrients.

Table 4.8: Effect of geographical location on proximate composition

Mushroom	Lipids%	Fiber%	ash%	protein%	Carbohy.%
Obulando	6.88±0.00 b	9.47±0.283 b	6.6±0.04 a	29.97±0.66 a	47.08±0.15 a
Olando	6.38±0.19 a	6.66±0.177 a	12.29±0.01 b	29.43±0.74 a	45.24±0.65 b
Makunu	6.33± 0.42 a	4.93± 0.12 a	5.91±0.03 a	32.1± 0.15 a	50.73±0.32 b
Mariondonik	7.62± 0.07 b	8.57± 0.15 b	6.44±0.37 a	35.94±0.51b	41.43± 0.34 a

Values in the same column followed by the same letter between each pair are not significantly different ($p \geq 0.05$).

Mushrooms in general are reported to have low lipid content (1.0-9.4 %) devoid of cholesterols (Bano and Rajarathnam, 1986). The authors have reported that the lipid content is influenced by substrate, species and the maturity of harvested fruit body. This is in agreement with the obtained lipids data on *oyster* YFB/MFB of 1.6/2.4 %.

The ash content of the mushrooms analyzed is consistent with reported values of 5-33.8 % by Okoro and Achuba, (2012), Adejumo and Awosanya, (2005) and kurtzman, (2005).

The protein content reported however depends on the factor used in conversion of nitrogen to protein. This study used 4.38 in place of 6.25 which exaggerates the protein content. Mushrooms are reported to have 10-20% non–protein nitrogen contained in the chitin and other non-protein compounds like amides (Nitschke, 2011). This 4.38 figure was arrived at by many researchers after taking into account total nitrogen (TN), amino acid nitrogen (AAN), amide-nitrogen (AN) and other

non-protein nitrogen (NPN). Figures of 3.99 were arrived at by Shinobu et al, (1995) and 4.7 by Mattila, (2002). Other values between 3.9 and 5.0 have been established on different mushroom species. The conversion factor calculation is based on amino acid nitrogen in relation to total nitrogen. The average value of 4.38 was recommended by several authors (Pathak *et al*, 1997).

Compared to several legumes which constitute major source of protein, mushrooms come out as a better source. Mushroom protein has been reported to be rich in essential amino acids including lysine which is limiting in cereals (Bano and Rajarathnam, 1986). On the average, pulses contain 15-25 percent proteins. However, the bioavailability is low due to a limiting amino acid (methionine), compact proteolysis-resistant structure of seed protein and presence of anti-nutritional compounds which affect digestibility of the protein (Brijesh *et al*, 2012). This fact coupled with resources required to produce pulses, land, fertilizers, tilling and time, make mushrooms production and consumption an attractive alternative.

Incorporation of mushroom in daily diets may address the problem of lysine deficiency.

In general it can be concluded that a mixture of young and mature fruiting bodies are best when used together to balance the nutrients. The stipe and trimmings generated during processing should not be discarded since they are also rich in nutrients. The results showed *Oyster* and *Mariondonik* were the best in terms of protein content.

4.3 Mineral content of the mushrooms

The minerals content of cultivated and wild mushrooms is presented in Table4.9 and 4.10.

Table 4.9: Macro elements in the mushrooms.

	Mineral mg/100g, dwb				
Mushroom	Ca	Na	K	P	Mg
Button	12.65± 0.09	432.35±1.10	4140.35± 0.01	93.26±0.11	6.15±0.48
Oyster YFB	15.80 ± 0.17	268.55±0.05	3839.60 0±.02	58.01±0.12	8,22±0.22
Oyster MFB	19.28± 0.35	385.55±0.07	4090.8±0.00	68.27±0.32	9.79±0.23
Makunu	7.01± 0.19	207.20±0.51	1421.35±0.02	59.26±0.01	8.05±0.07
Mariondonik	7.66±0.51	245.86±0.02	1450.55± 0.02	65.22±0.04	8.95±0.10
Olando	1.71±0.02	315.45±0.10	1972.5 ± 0.00	26.66±0.02	11.55±0.12
Obulando	2.59±0.16	323.22±0.85	4513.3 ± 0.01	70.78±0.02	9.05±0.32
Oruka –Stipe	22.72 ±0.24	383.65±0.80	1758.6 ± 0.00	62.45±0.07	4.92±0.07
Oruka -Cap	32.11± 0.24	376.30±0.92	3833.95 ± 0.01	85.38±0.11	5.82±0.13
Joga Muhama	31.93±0.05	350.90±o.55	1093.2 ± 0.02	56.86±0.00	32.76±0.15
Joga utuwe	1.56± 0.04	276.78±1.02	768.7 ± 0.12	32.84±0.03	23.65±0.13
Malombo	1.04± 0.04	164.20±0.69	575.5 ± 0.01	38.57±0.05	25.07±0.05
Mkundu wa n.	5.34 ± 0.11	129.45±0.01	587.0 ± 0.15	28.56 ±0.19	19.99±0.01
Joga kadzozo	1.71± 0.01	269.20±0.55	10.39 ± 0.07	1255.5±0.06	50.50±0.03
Rerema	25.39 ± 0.06	273.12±0.65	763.8 ± 0.02	61.43±0.01	35.48 ±0.41
Masikiro meru.	1.91±0.19	257.40±0.22	202.20±0.03	54.48±0.01	35.02±0.24

All values expressed as means, ± SD of triplicates on dry weight basis

YFB =Young Fruiting Bodies. MFB = Mature Fruiting Bodies

The figures obtained range as follows: Calcium 1.0-32.1 mg; Sodium 129.4-532.3 mg; copper 2.0-10.8 mg; manganese 4.4-19.8 mg; zinc 0.3-18.9 mg; Potassium 202.2-4140.3 mg; Phosphorus 26.6-1255.5 mg; Magnesium 4.9-50.5 mg and Iron 4.2-42.0 mg/100g on dry weight basis.

Of the major minerals, potassium occurs in the highest proportion in most of the mushrooms. The mineral values conforms to those reported for K, Ca, Na, and P whose values range from 59.3-4634 mg; 8.2–574.9 mg; 22.2–327.4 mg, and 100.5–769.9 mg/100g dry weight basis respectively in 5 wild and domesticated mushroom species analyzed in different locations in India and Nigeria (Mallikarjuna et al, (2012 and Okoro and Ochuba, 2012). Potassium content is 2-4 times higher than that of sodium. This has been documented as being good in management of hypertensive patients where potassium antagonizes the effect of sodium which contributes to hypertension (Bobek et al, (1995). Mushrooms are reported to have very high bio-accumulation of potassium and phosphorus when compared to substrate mineral composition. Potassium could occur in the range 20-40 fold higher in fruit body than in substrate (Kalac, 2012). The same does not apply to calcium or magnesium.

Iron, Zinc, Magnesium and manganese have been reported as 2.2-51 mg; 1.6-16 mg; 8.4-55 mg and 1.2-7 mg/100g dwb respectively by Okoro and Achuba, (2012).The values obtained are within that range. The Reference Dietary Intake for zinc and iron is 3-4mg/day (5.8-6.0mg/day for lactating and pregnant mothers) and 15-20mg/day respectively for healthy adults (FAO/WHO, 1998). These two minerals are of public health concern; their deficiency result in impaired growth in children, delayed sexual and bone maturation, defects in immune system and behavioral changes (O'Del, 1984). Bioavailability of non-heme iron and dietary zinc is greatly

Table 4.10: Micro elements in the mushrooms

Mineral mg/100g, dwb

Mushroom	Cu	Mn	Zn	Fe
Button	6.70± 0.45	6.55± 0.28	1.87± 0.02	6.57± 0.75
Oyster YFB	2.04± 0.01	6.13±0.25	0.39±0.02	5.95±0.50
Oyster MFB	3.45 ±0.64	7.35± 0.44	0.50 ±0.01	7.18±1.16
Makunu	2.89 ± 0.34	7.84 ± 0.14	0.48 ± 0.01	7.30±0.03
Mariondonik	3.50 ± 0.85	4.44 ± 1.20	0.52 ± 0.01	7.64±0.02
Olando	3.15 ± 0.91	9.30± 0.15	3.17 ±0.08	7.57±0.08
Obulando	6.14 ± 0.21	19.89 ± 0.43	1.22 ± 0.10	10.51±0.81
Oruka –Stipe	2.32± 0.71	6.10± 0.30	0.26 ±0.10	4.24±0.51
Oruka-Cap	3.13 ± 0.41	8.31 ± 1.01	0.68 ± 0.01	4.65±0.52
Joga Muhama	6.98 ± 0.12	13.85 ± 0.14	23.75 ± 0.07	42.09±0.32
Malombo	6.44± 0.09	11.40± 0.13	9.34± 0.08	24.79±0.19
Mkundu wa n.	5.98 ± 0.10	8.27± 0.11	6.22± 0.05	17.33±0.17
Joga kadzodzo	9.63 ± 0.42	9.63± 0.08	8.38± 0.07	33.88±0.23
Rerema	8.99± 0.15	7.46± 0.25	18.96± 0.09	16.75±0.23
Masikiro	10.83± 0.13	7.46± 0.09	12.36±0.23	40.51±0.07
meruhe				

All values expressed as means, ± SD of triplicates on dry weight basis.

YFB =Young Fruiting Bodies. MFB = Mature Fruiting Bodies

Table 4.11: Effect of geographic location on mushroom mineral composition

	Cu	**Mn**	**Zn**	**Fe**	Ca	**Na**	**K**	**P**	**Mg**
Makunu ma	2.89±0.34 a	7.84±0.14b	0.48±0.01a	7.30±0.03a	7.01±0.19 a	207.20±0.51a	1421.35±0.02a	59.26±0.01a	8.05±0.07a
Mariondonik	3.50±0.85a	4.44±1.20 a	0.52±0.01b	7.64±0.02 b	7.66±0.51a	245.86±0.02b	1450.55±0.02b	65.22±0.04b	8.95±0.10b
Obulando	3.15±0.91a	9.3a±0.15a	1.22±0.1a	10.51±0.81a	2.59±0.16a	623.2±0.85a	4513±0.01a	70.78±0.02a	9.05±0.32a
Olando	6.14±0.21b	19.89±0.43b	3.17±0.08b	7.57±0.08b	1.71±0.02b	615.5±0.10b	1972±0.001b	26.66±0.02b	11.55±0.12b

Values in the same column followed by the same letter between each pair are not significantly different ($p \geq 0.05$)

Table 4.12: Effect of mushroom maturity on the mineral composition

	Cu	**Mn**	**Zn**	**Fe**	**Ca**	**Na**	**K**	**P**	**Mg**
*Oyste*r MFB	3.45±0.64a	7.35±0.44a	0.5±0.01a	7.18±1.16a	19.28±0.35a	385.6±0.07a	4091±0.001a	9.79±0.23a	68.27±0.32a
Oyster YFB	2.04±0.00a	6.13±0.25b	0.39±0.02b	5.95±0.50a	15.8±0.17b	268.6±0.05b	3840±0.002b	58.01±0.12b	8.22±0.22b

Values in the same column followed by the same letter are not significantly different ($p \geq 0.05$)

Table 4.13: Effect of mushroom tissue on the mineral composition

	Cu	**Mn**	**Zn**	**Fe**	**Ca**	**Na**	**K**	**P**	**Mg**
Oruka stipe	2.32±0.71a	6.1±0.30a	0.26±0.01a	4.24±0.51a	22.72±0.24a	383.60±0.8a	1759±0.00a	62.45±0.07a	4.92±0.07a
Oruka cap	3.13±0.41a	8.31±1.01b	0.68±0.01b	4.65±0.52a	32.11±0.24b	476.3±0.92b	3834±0.01b	85.38±0.11b	5.82±0.01b

Values in the same column followed by the same letter are not significantly different ($p \geq 0.05$)

influenced by both dietary inhibitors and enhancers. The balance between absorption facilitators and inhibitors, along with the existing iron/zinc status of the individual determines the bioavailability of iron and zinc from individual foods or from a meal (O'Del, 1984). Amino acids (especially cysteine), ascorbic acid, citric acid, and fructose enhance iron absorption. Inhibitors of iron include phytate, polyphenols, high fiber, and oxalates (O'Del, 1984).

Promoters of zinc absorption include amino acids such as histidine and cysteine (Sandstrom, 1997). It is evident therefore that balancing nutrient intake is the key to promoting mineral bioavailability while avoiding mineral absorption inhibitors.

Effect of geographic location on mushroom mineral composition

Geographic location of mushrooms seemed to have an influence on mineral composition of the mushrooms. This is reflected in Table 4.11 where results for two mushroom pairs from different geographical locations are presented. The figures for *Mariondonik* and *Makunu* from Mt. Elgon and Aberdare forests respectively and *Olando and Obulando* from Kisumu and Kakamega Counties respectively clearly showed that Copper and Calcium in *Mariondonik* and *Makunu* were not significantly different ($p \geq 0.05$) while the other minerals were significantly different ($p \geq 0.05$) in both mushroom pairs.

Effect of mushroom maturity on the mineral composition

Mushroom maturity was found to influence the levels of mineral composition as shown in Table 4.12. Young fruit bodies (YFB) of *oyster* showed a lower mineral

content than the MFB across all the minerals analyzed. This corresponds with ash figures reported in Table 4.5. Statistical analysis of mineral composition in the young and mature *Oyster* mushroom is shown in Table 4.12. Micro minerals Copper and Iron were not significantly different ($p \geq 0.05$) while all other minerals were significantly different ($p \geq 0.05$) in the YFB and MFB. This would mean it is advisable to harvest the mushrooms before full maturity when the nutrients are balanced. However this is possible only for cultivated varieties.

Effect of mushroom tissue on mineral composition

Mushroom tissue seemed to influence mineral composition of the mushrooms. *Oruka* had higher mineral concentration in cap than in stipe across all the minerals analyzed. On dry weght basis (dwb) Potassium was 3833.9 mg/100g and 1758.6 mg/100g for cap and stipe respectively. This is consistent with reported values of potassium that range 3300-5005 mg/100g in cap and 1200-3800 mg/100g in stipe for three different varieties (Oboh and Shodehinde, 2009). Statistical analysis of mineral composition in *Oruka* stipe and cap is presented in Table 13. Copper and Iron were not significantly different ($p \geq 0.05$) in the stipe and pilei of *Oruka* while the other minerals were significantly different ($p \geq 0.05$). The figures seemed to suggest that Copper is synthesized early and it is not affected by geographical location, maturity or the mushroom tissue. Although many factors influence bioavailability of minerals, inclusion of mushrooms in our meals, and balancing on the factors that affect bioavailability, would contribute to a large extent to the body's daily requirements for most minerals.

4.4 Moisture content and water soluble vitamins in the mushrooms

Moisture content and water soluble vitamins values in fresh mushrooms are presented in table 4.14. Except the stipe, all the mushrooms had moisture content in excess of 80 %. Unless conserved, fresh mushrooms storage and marketability poses a big challenge to ordinary farmers who may not have refrigeration facilities. Storage of fresh mushrooms at 5^{o} C extends mushroom life to 4-5 days.

Riboflavin, folic acid and ascorbic acid were detected in all the mushrooms. Water soluble vitamins values per 100g on dry weight basis were B_2 0.7-6.4 mg; B_6 0.4-1.0 mg; B_{12} 1.1-1.7 mg; Folate 0-0.8 mg; Niacin 0.1-0.9 mg; pantothenic acid 0.8-1.2 mg and ascorbic acid 0.1-1.2 mg. The reported figures for vitamins for wild mushrooms were B_2 0.1-4.9 mg; B_{12} 0.5 mg; Folate 4.7-5.5 mg; Niacin 5.9-6.3 mg; pantothenic acid 2.3-2.8 mg and ascorbic acid 0.9-4.2 mg /100 g on dry weight basis (Egwin , (2011) and Chang and Miles (1991). Some of the figures obtained fall within the reported range while others are lower than the reported. Vitamin B6 was present in mature *oyster* and *oruka* cap while B12 was present in *button, oruka* and *makunu.* Niacine was detected in *button, oyster* and *makunu.* The concentration of vitamins seemed to vary in a non consistent manner. Neither the maturity nor the tissues show any pattern in detection or concentration of the different vitamins. Few researchers have included water soluble vitamins in their work, hence difficulties in comparison with documented figures.

The Reference Dietary Intake (RDI) for all the vitamins is given in Table 4.14. While one may not consume a lot of fresh mushrooms on daily basis, it is correct to state that inclusion of fresh mushrooms in the diets may contribute to RDI and thus

alleviate vitamin deficiency conditions. The actual daily requirement is influenced by age and gender. Lactating or pregnant mothers require higher amounts than the others (FAO/WHO, 1998). Folate is very significance to pregnant mothers because of the role it plays in stem cell differentiation. Pharmaceutically, folate has been implicated in treatment of anemia in pregnant women
(Qui, 2000).

Table 4.14: Moisture and water soluble vitamins in the mushrooms

	Mushroom						
Vit/moisture	*Button*	*oyster*	*oyster M*	*oruka* cap	*oruka* stipe	*Makunu*	DRI/day
Moisture%	91.80±0.00	93.05±0.10	91.24±0.0	88.80±0.01	76.98±0.10	80.54±0.02	
B_2	2.95±0.04	0.88±0.03	0.2.0±0.01	0.07±0.05	6.45±0.01	3.23±0.02	1.1-1.3mg
B_6	Nd	Nd	0.46±0.03	1.03±0.03	Nd	Nd	1.3-1.7mg
B_{12}	1.51±0.02	Nd	Nd	Nd	1.71±0.02	1.08±0.00	2.4-2.6 µg
Folate	0.89±0.01	0.14±0.01	0.46±0.03	0.03±0.02	0.83±0.01	0.56±0.01	400µg/day
Niacin	0.95±0.11	1.01±0.02	0.05±0.01	nd	Nd	0.98±0.02	14-16 mg
Panto.acid	Nd	1.26±0.01	0.87±0.03	nd	Nd	Nd	5-6 mg
Asc. acid	0.59±0.05	1.24±0.01	0.08±0.00	1.01±0.0	0.09±0.01	0.80±0.01	45-90mg

Vitamin values expressed as means, mg/100g ± SD of triplicates on dry weigh

Nd= not detected; Y= young; M= mature; Panto. acid = Pantothenic acid; Asc. = Ascorbic acid;

DRI=Dietary Reference Intake per day for a healthy adults. Source: FAO/WHO, 1998.

4.5 Phytochemical compounds detected in the mushroom

The phytochemical screening results are shown in Table 15. Phytochemical screening of the mushrooms revealed presence of saponins, polyphenols and terpenoids. Alkaloids, tannins and anthraquinons were absent in all the species.

Table 4.15: Phytochemicals compounds detected in the mushrooms.

Mushroom	Saponins	Polyphenols	Alkaloids	Tannins	Terpenoids	Anthraquinons
Button	+++	+	-	-	+	-
Oyster	++	+	-	-	+	-
Makunu-ma	+	+	-	-	-	-
Mariondonik	+	+	-	-	+	-
Olando	+	+	-	-	+	-
Obulando	++	+	-	-	+	-
Oruka	+++	+	-	-	+	-
J.. muhama	+	+	-	-	+	-
Joga utuwe	++	+	-	-	+	-
Malombo	+++	+	-	-	+	-
Mkundu wa n	+++	+	-	-	+	-
J. kadzonzo	++	+	-	-	+	-
Rerema	+++	+	-	-	+	-
Masikiro m.	+++	+	-	-	+	-

+ = present; ++= increasing concentration; - = absent

4.6 Total polyphenols TP), total flavonoids (TF) and radical scavenging activity (RSA)

Total polyphenols (TP), total flavonoids (TF) and radical scavenging activity (RSA) content of the mushrooms are presented in Table 4.16. The figures obtained for total polyphenols (TP) range between 210.5-1614.0 mg/100 g on dry weight basis. The figures conform to documented figures by Isabel *et al*, (2008) of 17-2640 mg/100 g for both wild and cultivated mushrooms. The total flavonoids (TF) (as quercetin equivalent) values obtained were 214.1-1695.4 mg QE/100g dwb; the reported figures by Isabel *et al*, (2008) range between 362-1245 mg QE/100 g dwb which is comparable. Flavonoids (as catechin) ranged 67.7-1204.7 mg CE/100 dwb. Catechin is reported mostly in tea and not mushrooms therefore making it difficult to compare. The total polyphenols content show a positive correlation with flavonoids, although not directly proportional. Mushroom with high TP have high TF as well.

High levels of flavonoids and polyphenols reflect a high RSA. The levels of these compounds are influenced by species, substrate on which mushrooms grew maturity and the part of the mushroom analyzed (Barros et al, 2007; Barbara et al, 2008).

Apart from polyphenols and flavonoids, RSA is influenced by other factors e.g. presences of H-donating groups like –NH or –SH. Other compounds which have RSA include ascorbic acid, tocopherols, and carotenoids. These compounds are reported to be present in mushrooms at different levels (Barros, 2007). This probably explains why there is no obvious trend that relates levels of TP, TF and RSA. The RSA level would therefore be influenced by the nature and levels of compounds extracted and their synergy (Barbara et al, 2008). The RSA figures

Table 4.16: Total polyphenols (TP), total flavonoids (TF) and radical scavenging activity (RSA)

Mushroom	TP. mg/100g	TF,QEmg/100g	TF,CE mg/100g	RSA,IC_{50}mg/100g
Button	460.42±1.02	801.34±0.50	297.45±0.10	156.83±0.89
Oyster MFB	675.56±0.97	890.87±0.90	445.72±0.65	61.86±0.56
Oyster- YFB	836.2±0.59	1129.75±0.33	585.50±0.11	62.64±0.32
Makunu ma mutitu	798.57±1.03	730.20± 0.55	442.40±0.90	68.62±0.48
Mariondonik	728.05±1.05	798.66±0.65	421.55±0.21	70.02±0.21
Olando	773.43±1.54	726.36±0.20	362.60±0.78	205.05±0.05
Obulando	432.66±0.41	740.77±0.14	370,45±0.67	185.10±0.14
*Oruk*a	788.52±0.45	979.64±0.60	498.54±0.12	76.65±0.57
Oruka- cap	1332.24±0.67	1511.08±0.85	1204.70±0.02	58.07±0.67
Oruka- stipe	872.57±0.90	648.20±0.79	323.96±0.35	59.59±0.60
Joga muhama	1543.22±1.22	921.30±1.02	138.95±0.55	68.29±0.45
Joga utuwe	1580.08±0.66	944.55±0.58	162.45±0.98	122.45±0.55
Malombo	1080.45±0.50	528.52±1.56	67.74±0.50	112.65±0.56
Mkundu wa nyani	947.95±0.75	463.50±0.46	298.75±0.90	116.25±0.85
Joga kadzodzo	1250.24±0.56	748.01±0.55	195.64±0.08	379.10±0.70
Rerema	1058.05±0.54	433.10±0.73	106.91±0.01	99.15±0.65
Masikiro meruhe	331.54±0.91	214.15±0.57	507.34±01	458.01±0.85

Values expressed as means, mg/100g ± SD of triplicates on dry weight basis (dwb)

TP. =Total polyphenols as Gallic Acid Equivalent (GAE); RSA=Radical scavenging activity; IC_{50}= Inhibition concentration for 50%

TF. = Total Flavonoids; QE= Quercetin Equivalent; CE= Catechin Equivalent

Correlation coefficient between total polyphenols and radical scavenging activity=0.82

obtained range between 58-458 mg/100 g on dwb. The reported values are in the range of 76- 1000 mg/100 g dwb in Portuguese mushrooms (Barros, 2007).

Effect of heat on phytochemical compounds and on RSA

Subjecting the mushrooms to heat affected TP TF and RSA levels. This is reflected by the figures in Table 4.17.

Table 4.17: Effect of heat on phytochemical compounds and on RSA

Mushroom	TP. mg/100g	TF,QE mg/100g	RSA-IC_{50} mg/
Button- untreated	460.42±1.02 c	801.34±0.50 c	156.83±0.89 a
Button-Steamed 1min	348.81±0.59 b	591.69±0.95 b	157.30±1.05 a
Button.boiling-water1 min.	210.51±0.26 a	533.32±0.36 a	163.62±0.75 b
LSD	1.392	1.306	1.808
Oruka untreated	788.52±0.45 a	979.64±0.60 a	76.65±0.57 b
Oruka- steamed 1min	1221.08±0.90 b	1695.44±0.55 b	59.82±0.87 a
Oruka- boiling water 1min.	1614.05±0.44 c	1832.02±0.15 c	60.38±0.55 a
LSD	1.267	0.955	1.357

Values in the same column followed by the same letter are not significantly different ($p \geq 0.05$).

In *button,* TP reduced to a larger extent in mushrooms submerged in boiling water than in the steam-treated mushrooms when compared to the untreated. In contrast, in *oruka* mushroom, the TP increased, far more in boiling water than in steam-treated mushrooms. Both trends have been reported (Choi, 2009). In the former case, heating may have destroyed the structure of some compounds thereby resulting in

reduction of both TP and TF as observed in *button* mushroom. It is also expected that boiling water would result in dissolution of some water soluble compounds as most phytochemical compounds are water-soluble. In the case of *oruka* mushroom, heat treatment may have increased extractability due to disruption of cell wall; thereby releasing bound compounds that may not have been extracted in the raw material (Choi, 2009). It is also suggested that thermal treatment may generate novel compounds that may have RSA properties. This may be the case for *oruka* where the boiling water dissolution of some compounds did not lower either the TP or TF.

Effect of mushroom maturity on phytochemicals and RSA

Phytochemical compounds and RSA figures for Oyster young(YFB) and mature (MFB) fruit bodies are presented in Table 18

Table 4.18: Effect of mushroom maturity on phytochemicals and RSA

Mushroom	TP. mg/100g	TF,QE mg/100g	RSA-IC_{50} mg/100g
Oyster-MFB	675.56±0.97 a	890.87±0.90 a	61.86±0.56 a
Oyster- YFB	836.20±0.59 b	1129.75±0.33 b	62.64±0.32 a

Values in the same column followed by the same letter are not significantly different ($p \geq 0.05$).

The young fruit bodies (YFB) have been found to have higher TP and TF than the mature fruit bodies (MFB) (Table 4.18). This is in conformity to reported figures of Barros *et al*, (2007), Barbra *et al*, (2008) and Oboh and Shodehinde, (2009). The authors suggested that the compounds in mature stages could be involved in

defences mechanism as a result of the aging process; hence reduced content on extraction. Statistical analysis, Table 18, showed that the TP and TF in both YFB and MFB were significantly different ($p \geq 0.05$), while RSA was not significantly different ($p \geq 0.05$). This affirms the suggestion that other non phytochemical compounds did take part in RSA or that some TP and TF compounds do not take part in RSA.

Effect of mushroom tissue on phytochemicals and RSA

Mushroom tissue seemed to influence TP, TF and RSA of the mushrooms. The results for *Oruka* cap and stipe analysis are presented in Table 19.

Table 4.19: Effect of mushroom tissue on levels of phytochemicals RSA

Mushroom	TP. mg/100g	TF,QE mg/100g	RSA-IC_{50} mg/100g
Oruka cap	1332.24±0.67 b	1511.08±0.85 b	58.07±0.67 a
Oruka stipe	872.57±0.90 a	648.20±0.79 a	59.59±0.60 b

Values in the same column followed by the same letter are not significantly different ($p \geq 0.05$).

The TP, TF and RSA obtained for the cap were 1332.2 mg, 1511.1 mg and 58.0 mg /100 g on dry weight basis respectively. Those for the stipe were 872.5 mg, 648.2 mg and 59.5 mg /100 g dwb respectively. Similar but lower figures have been reported for TP by Isabel *et al*, (2008) as follows. Cap 677-1066 mg/100 g and 400-760 mg/100 g dwb for stipe. When all factors that influence the nature and content of compounds responsible are considered, the differences are expected. Barros *et al*,

(2007) reported that although the stipe may have lower TP and TF, the RSA may be same or higher for stipe than that of cap. This is the case where RSA for cap and stipe in *oruka* mushroom was 58.0 mg and 59.5 mg /100 g respectively. Statistical analysis (Table 19) showed that TP, TF and RSA figures for *Oruka* cap and stipe were significantly different ($p \geq 0.05$). The stipes, however, should be processed to be used as food.

Effect of geographical location on phytochemicals and on RSA

Geographical location of mushrooms seemed to influence the levels of phytochemical compounds and RSA. The result of this analysis is presented in

Table 4.20

Although *Obulando* and *Olando* from Kakamega and Kisumu respectively are the same mushroom variety; the levels of TP, TF and RSA were significantly different ($p \geq 0.05$). Similar trend was found in *Makunu* and *Mariondonik* from Aberdares and Mt. Elgon forests respectively. Levels of TP, TF and RSA were found to be significantly different ($p \geq 0.05$) in the pair. The trend seemed to indicate that the substrate and climatic conditions influence the compounds synthesized.

Table 4.20: Effect of geographical location on phytochemicals and on RSA

Mushroom	TP. mg/100g	TF,QE mg/100g	RSA-IC_{50} mg/100g
Obulando	432.66±0.41 a	740.77±0.14 b	185.10±0.14 a
Olando	773.43±1.54 b	726.36±0.20 a	205.05±0.05 b
Makunu	789.57±1.03b	730.20±0.55a	68.62±0.48a
Mariondonik	728.05±1.11a	798.66±0.65b	70.02±0.21b

Values in the same column followed by the same letter in each pair are not significantly different ($p \geq 0.05$).

A general observation made was that all mushrooms from Arabuko Sokoke forest displayed high levels of total polyphenols than the rest from highlands. The flavonoids levels compared well with the mushrooms from other places. This would suggest that the geographic region of coast has influenced the synthesis and bioaccumulation of compounds different from elsewhere. The high temperatures and salt concentration may have influenced production of high levels of polyphenols to cope with environmental stress and probably pathogens.

4.7 Product sensory evaluation

4.7.1 Sensory evaluation of wheat meal-mushroom powder noodles.

Sensory evaluation for noodles is presented in Table 4.21. The appearance for the control formulation received the highest rating score and this was significantly

different ($p \geq 0.05$) from that of other formulations. Other attributes score for the products with mushroom flour were not significantly different ($p \geq 0.05$).

It was noticed however that increasing mushroom flour affected the texture and ability to retain form. High mushroom levels disintegrated the noodles strands to the extent that eating would require a spoon, rather than a fork. This was due to high dilution effect on gluten network by the high fiber in both wheat whole meal and mushroom powder. Noodles are a relatively new wheat product in the local market.

Table 4.21: Sensory evaluation for wheat -mushroom noodles.

Product/Attr.	Appearance	Aroma	Taste	Texture	Overall score
0% mush.	6.00±0.21 b	5.26±0.25 a	5.36±0.05 a	5.05 ±0.15 a	5.52±0.45 a
25% mush.	5.05±0.12 a	4.78±0.57 a	4.84±0.55 a	5.00± 0.12 a	5.15±0.70 a
20% mush.	5.03± 0.22 a	4.94±0.35 a	4.57± 0.20 a	4.42±0.10 a	4.94± 0.59 a
LSD	0.73	0.62	0.76	0.86	0.66

Values in the same column followed by the same letter are not significantly different ($p \geq 0.05$) Panellist=25; LSD= Least significance difference; Attr.=attribute; mush.= mushroom

Introduction of mushroom-enriched product would therefore be easy. If done commercially with a health implication aspect, it would have a high chance of acceptance and adoption.

4.7.2 Sensory evaluation of wheat –mushroom flour soup

Wheat-mushroom flour soups were subjected to sensory evaluation. The scores obtained are presented in Table 22.

The figures indicate that appearance in all the soup formulations was significantly different ($p \geq 0.05$). It was noted that although *Oruka* is white, the cooking made it brownish. This reduced the score for the appearance but did not lower its overall consumer acceptability score. The overall score for all the soup formulations was not significantly different ($p \geq 0.05$). This means there is potential for incorporating mushroom flour into soups.

Table 4.22: Sensory evaluation of wheat flour-mushroom soup

Product/Attr.	Appearance	Aroma	Taste	Texture	Overall score
0% mush.	5.74±0.50 b	5.74±0.25 a	5.84±0.15 a	5.68±0.11 a	5.58±0.75 a
2.5% *oyster*	5.15±0.05 ab	5.21±0.01 a	5.47±0.36 a	5.42±0.04 a	5.26±0.50 a
2.5% *oruka*	4.94±0.15 a	5.16±0.04 a	5.38 ±0.30 a	5.58±0.31 a	5.42±0.34 a
LSD	0.65	0.81	0.97	0.85	0.69

Values in the same column followed by the same letter are not significantly different ($p > 0.05$). Panellists=25; LSD= Least Significance Difference. Attr =Attribute

4.7.3 Sensory evaluation of wheat -mushroom flour biscuits

Wheat: mushroom flour biscuits, were subjected to sensory evaluation and the results are presented in Table 23. The figures indicate that the individual attributes in all the formulations were not significantly different ($p \geq 0.05$).

Table 4.23: Sensory attributes and score for wheat flour-mushroom flour Biscuits

	Attribute				
Product	Appearance	Aroma	Taste	Texture	Overall Score
0% mush.	5.450±0.20 a	4.60±0.53 a	5.150±0.33a	5.30±0.54 a	5.30±0.04 b
25% mush.	4.900±0.76 a	4.450±0.45a	4.400±0.23a	4.75±0.20 a	4.60±0.50 ab
30% mush.	4.800±0.36 a	4.100±0.22a	4.300±0.51a	4.45±0.10 a	4.40±0.68 a
LSD	0.69	0.71	0.81	0.82	0.70

Values in the same column followed by the same letter are not significantly different ($p \geq 0.05$). Panellists=25. LSD= Least significant difference

With every increase in proportion of mushroom flour, the biscuits appeared to get thinner and harder than the control. Detection of mushroom aroma and color change followed the same trend. However the effect did not affect the consumer acceptability of the other attributes and the scores were not significantly different ($p \geq 0.05$). The overall score for the three formulations was significantly different ($p \geq 0.05$). With training, the product has a chance of increased acceptability.

The palate cling and sticking to the teeth common in many commercial biscuit formulations were absent. This fact is beneficial in preventing dental caries brought by fermentation of sugars that stick on and between teeth. The children are the most affected because of frequency of biscuits consumption.

4.8 Products nutrients improvement

Nutrient improvement was evaluated in relation to protein, Iron and Zinc content in the products made. The three nutrients were chosen because of their importance in human nutrition. Proteins are a big challenge to many Kenyans in the low income group such that protein-calorie malnutrition is rampant. Similarly Zinc and Iron are

of public health concern because of the role played in growth of young children and young/child-bearing women. The results are presented in Tables 24 and 25.

Table 4.24: Effect of wheat: mushroom four mix on nutrients in noodles**.**

Wheat meal: mushroom	Protein %	Iron mg/100g	Zinc mg/100g
100:0	11.12±0.66 a	1.463±0.02 a	1.535±0.05 a
80:20	12.16±0.62 bc	1.537±0.05 b	1.573±0.01 b
75:25	13.42±0.46 c	1.537±0.02 b	1.621±0.01 c
LSD	1.308	0.03	0.01

Values in the same column followed by the same letter are not significantly different ($p \geq 0.05$). Nutrients were calculated on dry weight basis.

Table 4.25: Effect of wheat: mushroom flour mix on nutrients in biscuits

Wheat flour: mushroom	Protein %	Iron mg/100g	Zinc mg/100g
100:0	8.12±0.48 a	1.42±0.012a	0.69±0.05 a
75:25	9.70±0.57 bc	1.64± 0.02 b	0.78± 0.01 b
70:30	10.41±0.61 c	1.75±0.07 c	0.81± 0.01 b
LSD	1.033	0.03	0.05

Values in the same column followed by the same letter are not significantly different ($p \geq 0.05$). Nutrients were calculated on dry weight basis.

The figures (Tables 4.24 and 4.25) showed that increase in mushroom flour in the formulation brought an increase in protein, Iron and Zinc content. The increase of each nutrient was significantly different ($p \geq 0.05$) in all the formulations.

The consumer acceptance score for the three products developed showed that mushrooms can be incorporated into many palatable and nutritious products. Noodles and biscuits would be hardy for school children because they are light, dry and ready to eat. They can be eaten with a variety of accompaniments; either as a midmorning snack, lunch or after school snack. The products are quite appropriate for working class people too..

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

- This research work has demonstrated that both cultivated and wild mushrooms contain a wide range of nutrients and that they can be used in value-added product development. The null hypothesis was rejected.
- All the mushrooms contain high levels of proteins, insoluble fiber and almost all the minerals required for a healthy body. Iron and zinc which are minerals of public health concern are also available.
- Mushrooms were found to contain water soluble vitamins in significant proportions. Inclusion of fresh mushrooms in the diet would alleviate vitamins deficiency diseases common in the country.
- Phytochemical compounds were found to be present in all the mushrooms in abundance amounts. The compounds were also found to have radical scavenging activity.
- Wheat-mushroom flour blends produced noodles, biscuits and soup which received high score in terms of consumer acceptability.
- Nutritional quality of the developed products is higher than that of the control.

5.2 Recommendations and areas of further research

- This research largely worked on wild edible mushrooms from Arabuko Sokoke forest in Coast region. The country is endowed with many other varieties in the forest and elsewhere; these should also be characterized as well.
- Protein quality in terms of amino acids and amino acid score in wild mushrooms should be studied. This would provide true nutritional value of the mushrooms.
- Several edible mushroom species have antimicrobial properties and some are toxic at high levels. Wild mushrooms should be studied to determine these properties.
- Development of various value-added products should be embarked on as a matter of priority. This should target development of an elaborate value chain for commercialization extension programme.
- This research has found that all the mushrooms have polyphenols and high fiber content. Depending on the levels, they are known to interfere with other nutrients absorption from the small intestines. This aspect of mushrooms should be researched on.

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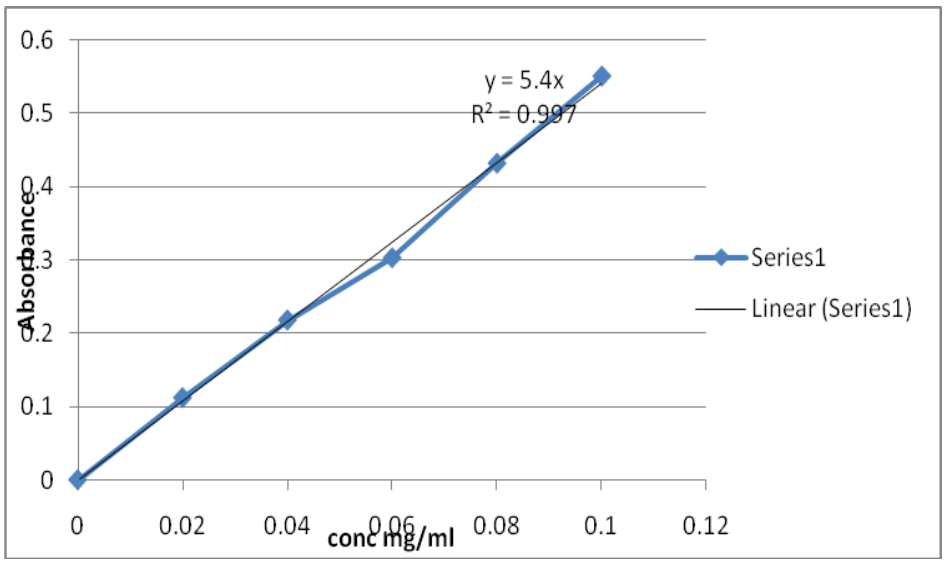
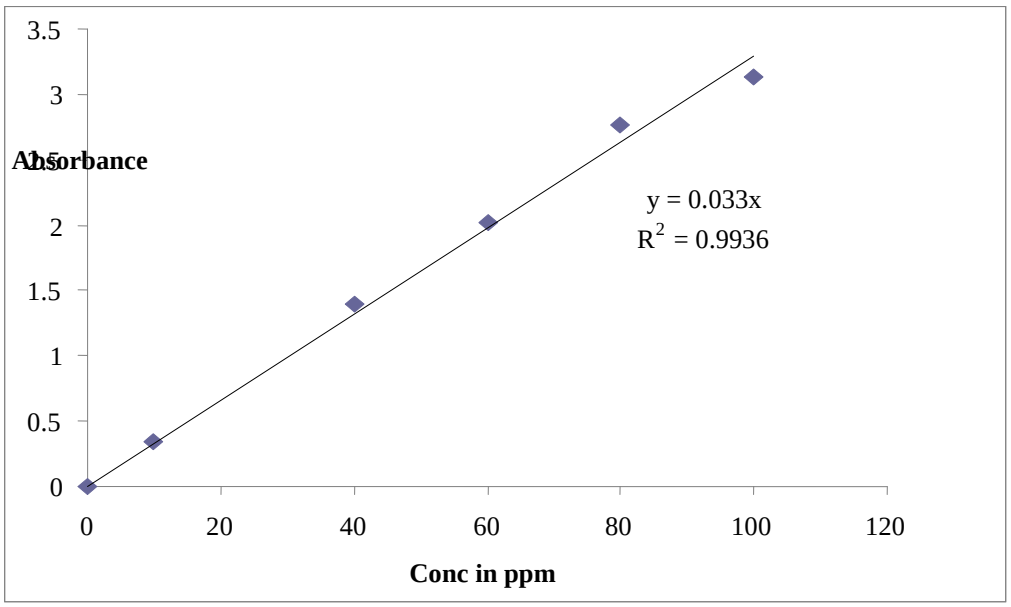
APPENDICES

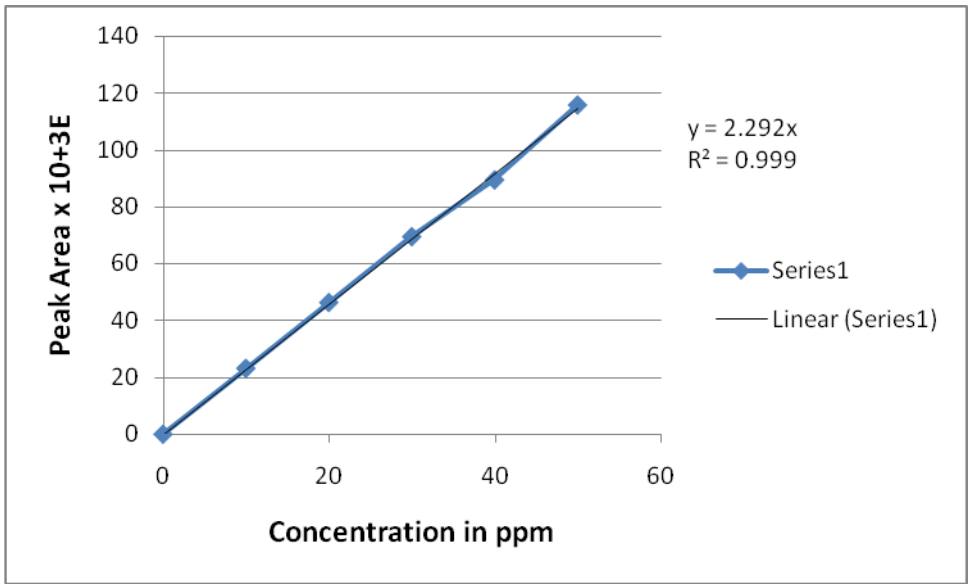
Appendix 1: Standard Curves



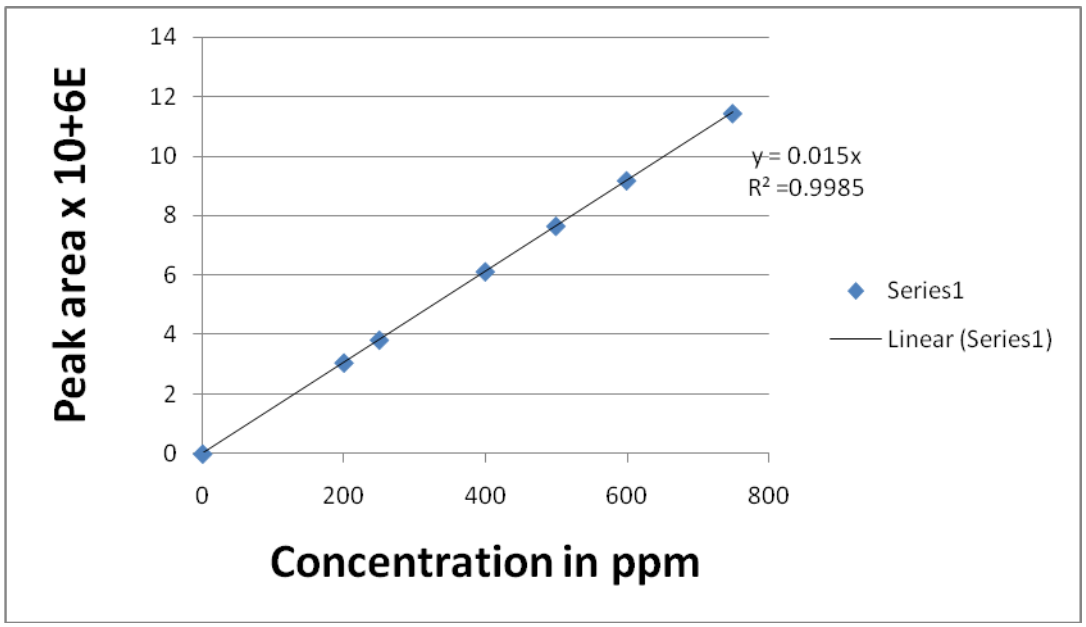
a) Total polyphenols standard curve



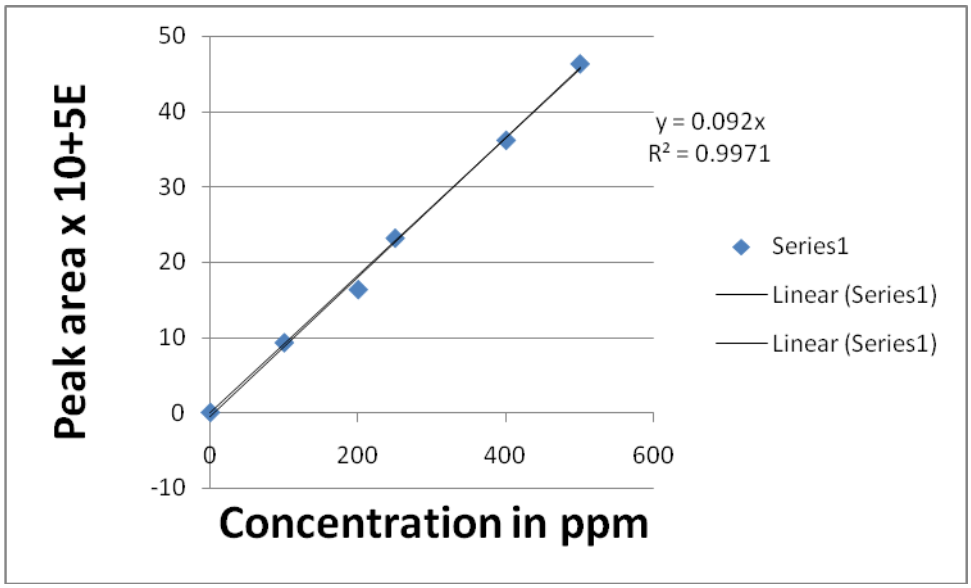
b) Flavovoids standard curve



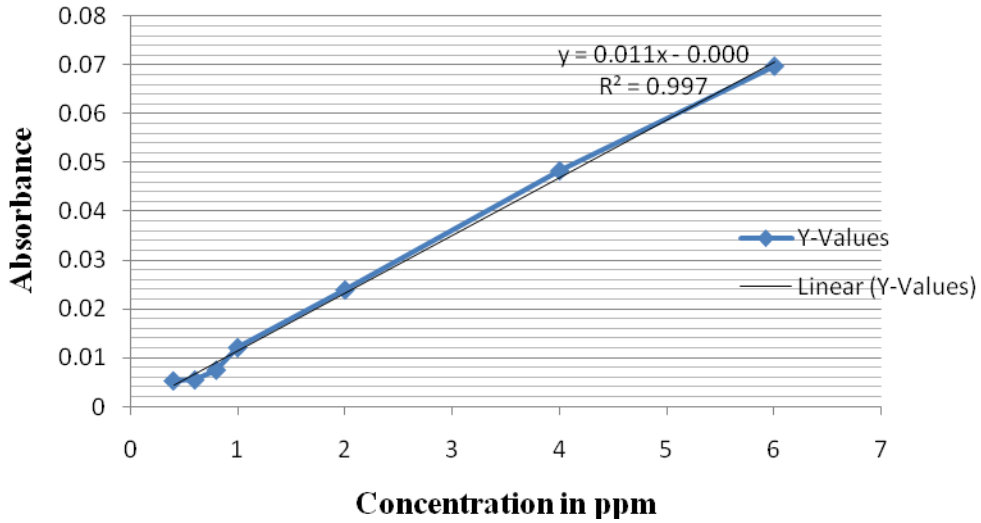
(c) Folic acid standard curve



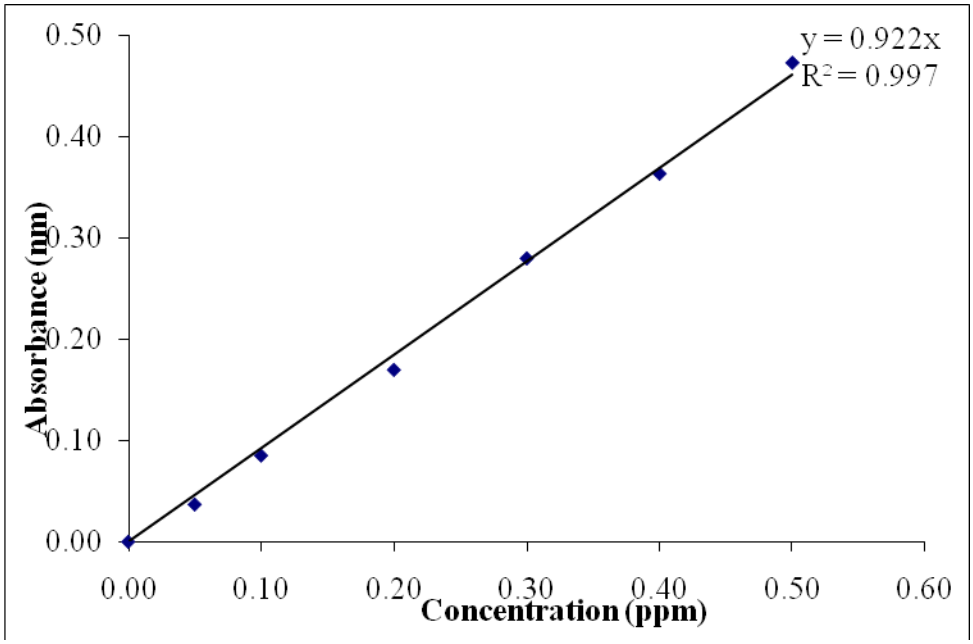
d) Riboflavin standard curve



e) Pyridoxin standard curve



f) Iron standard curve



Appendix 2: Sensory evaluation questionnaire for mushroom-wheat flour noodles

Instructions

You are provided with coded samples of **noodles** for sensory evaluation. Please assess each in terms of **appearance, aroma, taste, texture and overall score** by giving the appropriate score to each product and attribute in the table.

Use the score card provided.

Description **Score**

Like extremely……..……..7

Like moderately…….……6

Like slightly…….5

Neither like nor dislike……4

Dislike slightly…… 3

Dislike moderately……..…….2

Dislike extremely…….1

Sample code	Appearance	Aroma	Taste	Texture	Overall Score
740					
005					
589					

Give your comments/suggestions ……

Thank you for participating.

Appendix 3: Sensory evaluation questionnaire for mushroom-wheat flour soup

Instructions

You are provided with coded samples of **soup** for sensory evaluation. Please assess each in terms of **appearance, aroma, taste, texture/consistency and overall score** by giving the appropriate score to each product and attribute

in the table. Use the score card provided.

Description **Score**

Like extremely……..……..……..7

Like moderately…….……...6

Like slightly……..……5

Neither like nor dislike…….4

Dislike slightly…….…….…3

Dislike moderately…….……...2

Dislike extremely…….……1

Sample code	Appearance	Aroma	Taste	Texture	Overall Score
173					
502					
905					

Give your comments /suggestions……..

……

Thank you for participating.

Appendix 4: Sensory evaluation questionnaire for mushroom-wheat flour biscuits

Instructions

You are provided with coded samples of **biscuits** for sensory evaluation. Please assess each in terms of **appearance, aroma, taste, texture and overall score** by giving the appropriate score to each product and attribute in the table. Use the score card provided.

Description **Score**

Like extremely……..……7

Like moderately……..…..6

Like slightly…….……..5

Neither like nor dislike…….…....4

Dislike slightly……..3

Dislike moderately…….……..…….2

Dislike extremely……..1

Sample code	Appearance	Aroma	Taste	Texture	Overall Score
258					
534					
153					

Give your suggest/comments.....……

.....

Thank you for participating.