ANTIOXIDANT RETENTION IN SOLAR-DRIED MUSHROOMS AND ACCEPTABILITY OF MUSHROOM MILLET PORRIDGE BLEND AMONG HIV PATIENTS IN NAKURU COUNTY REFERRAL HOSPITAL, KENYA

BOSIRE MIRIAM MASIKO (Bsc. FND)

H60/33060/2015

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (FOOD NUTRITION AND DIETETICS) IN SCHOOL OF HEALTH SCIENCES OF KENYATTA UNIVERSITY

NOVEMBER, 2023

DECLARATION

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

Signature______________________ Date ___________________

Bosire Miriam Masiko **Reg. No.H60/33060/2015**
Department of Food, Nutrition and Dietetics
Kenyatta University

SUPERVISORS

We confirm that the work reported in this thesis was carried out by the student under our supervision as University Supervisors:

Signature ______________________ Date ______________________

Ann Munyaka (PhD)
Department of Food, Nutrition and Dietetics
Kenyatta University

Signature _______________________ Date _____________________

Juliana Kiio (PhD)
Department of Food, Nutrition and Dietetics
Kenyatta University

DEDICATION

This work is dedicated to my parents Joseph and Mary Bosire, my sisters Jacinta, Fridah, Luycer and Matildah Bosire and my nieces Aileen and Yvette for their love and support.

ACKNOWLEDGEMENTS

My appreciation goes to Kenyatta University's department of Food, Nutrition and Dietetics for providing a good study environment, space, materials and the equipped laboratories that enabled me to finish this work. Special appreciation goes to the lab technician John Gachoya for his guidance during the data collection phase of this study. More appreciation goes to my supervisors Dr. Ann Munyaka and Dr. Juliana Kiio for their guidance, timely feedback and assistance during the entire study period and the entire staff of the Department of Food Nutrition and Dietetics for providing guidance during the study. Very special gratitude goes to my entire family starting with my Dad Joseph Bosire, my Mom Mary Bosire and my sisters Jacinta, Frida, Luycer and Matilda for your financial backing, words of encouragement and undying support all through my study. I would also like to thank all my friends who helped me in one way or another during my study period.

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LIST OF ABBREVIATIONS

AIDS – acquired immunodeficiency syndrome

ANOVA – Analysis of variance

BHT – Butylated Hydroxytoluene

BL– Blanched

CSB – Corn Soy Blend

DW – Dry weight basis

HIV – Human Immune-deficiency Virus

HPLC – High Performance Liquid Chromatography

PEM – Protein Energy Malnutrition

PLWHAs – People Living with HIV and AIDS

RDA – Recommended Dietary Allowance

ROS – Reactive Oxygen Species

TPC – Total phenolic Content

UNBL – Unblanched

UV-VIS –Ultraviolet-Visible

OPERATIONAL DEFINITION OF TERMS

Acceptability	Determined using sensory evaluation scores from panelists.
Adults with HIV/AIDS	Both male and female adults between the ages of 25 to 40 years living with HIV/AIDS
Antioxidant retention	Value of antioxidants maintained in mushrooms after heat processing.
Antioxidants	A substance that when present inhibits or delays the oxidation of a substrate including the nutritional antioxidants; Beta-carotene, vitamin E, vitamin C and Total Phenolic content (TPC)
Enrichment	Addition of mushroom powder to millet flour to increase its nutritional content.
Free radicals	Molecules that exist independently, containing an unpaired electron in their structure making them highly reactive
Lipid peroxidation	A process whereby free radicals take electrons from lipids present in the cell membrane resulting in cell damage.
Oxidative stress	Body's inability to neutralize harmful effects of free radicals produced by cell functions in the body

ABSTRACT

The role of mushrooms in preventing and treating illness and promoting quality of life has gained increased awareness especially due to their high antioxidant content. Antioxidants are necessary for body protection against oxidative stress especially among people with lowered immune system, such as People Living with HIV/AIDS (PLWHAs). There are limited options of nutritional therapeutic foods available for use by PLWHAs. Millet is an underutilized indigenous crop in Kenya whose nutrient profile can be improved through blending with other foods. The purpose of this study was to establish the antioxidant (Vitamin E, Vitamin C, β-carotene and Total Phenolic Content) concentration and retention in fresh and solar-dried oyster mushrooms (*Pleurotus ostreatus*) and assess the acceptability of finger millet *(Eleusine Coracana)* porridge enriched with oyster mushroom among PLWHAs. The study adopted an experimental design with fresh mushrooms and pure millet flour as the control samples and mushroom enriched millet flour as the experimental samples. Fresh mushrooms were purchased in Juja sub-county, Kenya, transported using cool boxes and refrigerated at 4ºC in the Kenyatta University food chemistry laboratory. Millet samples were purchased from markets within Nairobi County, Kenya and transported and stored in hermetic bags. β-carotene and Total Phenolic Content of mushrooms and millet were analyzed using UV-VIS spectrophotometry at 450nm and 765nm respectively. Vitamin C and Vitamin E content were analyzed using reverse-phase high Pressure liquid chromatography (HPLC). Sensory acceptability of mushroom-millet porridge was determined using a 5-point hedonic scale questionnaire administered to 30 randomly selected participants (PLWHAs) from Nakuru County Referral Hospital. Independent T-tests and Analysis of Variance (ANOVA) were used to analyze nutrient content and sensory scores. The antioxidant nutrient content of fresh and solar dried mushrooms were: β-carotene- 13.73 ± 0.02 mg/100g dw, 1.21 ± 0.00 mg/100g dw ; Vitamin E- 23.23 ± 2.12 mg/100g dw, 0.96 ± 1.18 mg/100g dw; Vitamin C 37.25 ± 0.32 mg/100g dw, 5.60 ± 0.12 mg/100g dw and TPC 36.48 ± 1.50 mg/100g dw, 21.55 ± 0.24 mg/100g dw respectively. Blanching the mushrooms before solar drying increased the retention rate of the nutrient antioxidants in the solar dried mushrooms. There was a significant difference in the antioxidant content of fresh mushroom and solar dried mushrooms in all the tested nutrients ($p \leq 0.05$). Enriching millet flour with mushroom powder significantly increased β-carotene, Vitamin C and TPC concentrations ($p \leq 0.05$). There were significant differences between control porridge and mushroom enriched millet porridges on all sensory attributes apart from color ($p \leq 0.05$). The unblanched mushroom: millet porridge (60:40) blend was rated the poorest (2.97 ± 1.47) in all the sensory attributes. This study's findings demonstrate that oyster mushrooms are rich in antioxidants. However, solar drying the mushrooms without blanching significantly lowers the levels of the antioxidants. This study established that porridge made from millet flour enriched with mushroom is rich in β-carotene, Vitamin C and TPC; and is acceptable among PLWHAs. The consumption of porridge made from millet-mushroom blended flour should be promoted among PLWHAs not only because of protein content but also because of antioxidant content. Findings from this study are relevant to nutritionists, farmers, and policy makers as it shows the potential of mushrooms in enhancing diets, promoting health and income generation.

CHAPTER ONE: INTRODUCTION

1.1 Background to the study

There has been an increase in the popularity of cultivated mushrooms globally. Production of cultivated mushroom has undergone rapid growth of about 10 times in the past half century (Zhang, Geng, Shen, Wang & Dai, 2014). In addition, Royse (2014) has reported that global mushroom consumption has also undergone rapid increase from about 1kg per person in the late 1990s to approximately 4kgs per person in 2012. The most common mushroom species cultivated globally include: common mushroom (white and brown mushrooms), button mushrooms, shiitake, oyster mushroom and king oyster mushrooms (Reis, Martins, Barros& Ferreira, 2012).

According to Anchang (2014), mushroom farming in most African countries is at its infant stage with only south Africa, Kenya and Zimbabwe producing mushrooms at commercial level. In Kenya, mushroom cultivation has found a place among the small scale farmers with the most common mushrooms species cultivated being the button and oyster mushrooms (Gateri, Muriuki, Waiganjo & Ngeli, 2009). In Kenya, mushrooms have been utilized by local communities for a long time. Wild edible mushrooms were traditionally consumed in Western, Nyanza and the Coastal regions of Kenya (Musieba, 2013).

Apart from dietary uses, mushrooms have also been used for medicinal purposes for centuries (Feeney et al., 2014). According to, Sushila et al., (2012) there is a recent increase in the cognizance of the health benefits of mushrooms and their ability to provide biologically active substances with medicinal value. The aforementioned authors further outlined the properties of mushrooms as being "immune enhancement, cure and prevention of various diseases and improvement from life threatening diseases." These properties can have a role in promoting quality of life of individuals whose immune status is destroyed, such as in the case of people living with HIV and AIDs (PLWHAs). The immune boosting ability of mushrooms has been attributed to its high content in antioxidant compounds (Ramesh & Pattar, 2010).

Studies have reported high content of phenolic compounds and flavanoids in the oyster, brown, shiitake and button species of mushrooms (Mujić et al., 2011). However, Reis et al. (2012) stated that little information exists on the antioxidant properties of mushrooms species from Brazil, China, Spain, Taiwan and Thailand. Increased awareness of these properties prompted initiatives to develop pharmaceutical and nutraceutical products from mushrooms in Africa (Anchang, 2014). According to the aforementioned author, there are currently two products “immune assist 24/7” and “kay biotics” developed from mushrooms through these initiatives. These products are used to improve the immunity of HIV/AIDS patients in Ghana and Cameroon, respectively. Apart from being rich sources of antioxidants, mushrooms are considered healthy foods as they are low in calories but high in dietary fiber and proteins. The mushroom protein is complete as it contains all the nine essential amino acids required by the human body (Rathee et al., 2012). Mushrooms therefore have the potential of alleviating protein energy malnutrition (PEM) in HIV patients.

Millets are small seeded annual grains with good nutritional qualities (Bora, 2014). Saleh, Zhang, Chen, & Shen (2013) reported millet as good sources of essential amino acids, especially methionine. This is beneficial in alleviating Protein Energy Malnutrition (PEM) in patients, such as HIV patients. Millets are also good sources of the Vitamin B complex, phosphorus, iron and manganese (Sarita, 2016) which are important in enhancing immune function. Bora (2014) adds that millets are rich sources of phenolic compounds and antioxidants. These are important in boosting the immune status of individuals by reducing oxidative stress in the body. Apart from their nutritional properties, millets are known to contribute significantly to food security mainly in Asian and African countries attributed to their high tolerance to low application of soil nutrients, temperature fluctuations and drought resistance (Bhagavatula et al., 2013).

Interactions between antioxidants is important for protective effect against oxidative stress during food processing, storage, digestion and absorption of food by the body (Skibsted, 2012). Some of the most studied dietary antioxidants include Vitamin C, vitamin E and beta-carotene (Lobo et al., 2010). In recent years, studies on the antioxidant properties of polyphenols have increased (Scalbert et al., 2005). Vitamin C is capable of neutralizing reactive oxygen species (ROS) in the aqueous phase before lipid peroxidation is initiated (Pham-Huy et al., 2008). Vitamin E is a chain breaking antioxidant where it protects membrane fatty acids from lipid peroxidation (Samsam Shariat et al., 2013). B-carotene is reported to work together with vitamin E to provide protection to tissues rich in lipids (Pham-Huy et al., 2008).

1.2 Problem Statement

Mushrooms are rich sources of proteins, carbohydrates vitamins and minerals (Kokoti, 2015). Research has also shown mushrooms to boost the immune system of individuals with lowered immune status (Anchang, 2014). This may be attributed to the nutritional antioxidants present in mushrooms. However, there lacks extensive research on both wild and locally cultivated mushrooms in Kenya (Wandati et al., 2013). A study analyzing the mushroom value chain in Kenya showed that most farmers processed their fresh produce for sale (Odendo et al., 2014). According to Karanja (2015), Kenyan farmers also develop processed products such as sauces as well as solar- drying the surplus mushroom harvests for sale in the off season. The solar dried mushroom is ground into flour and used for various processes such as baking or added to porridge and soups. Oyster mushroom farming has gained popularity among small scale farmers in Nakuru county producing more than 100 kilograms of processed mushrooms per month and sell to individuals, hotels and restaurants within Nakuru town and it's environs (Kanjeru, 2013).

Millets are nutritionally dense, locally available, drought resistant but underutilized crops found in Kenya. In addition, Finger millet is one of the main food crops produced in Nakuru county (Muhunyu, 2008), despite this, it's consumption still remains lower than that of maize (Gewa et al., 2019) which remains a staple in the region. Millets are rich in essential amino acids, B vitamins, minerals and antioxidants that are important in promoting good nutrition and boosting the immunity (Bora, 2014; Saleh, Zhang, Chen, & Shen 2013; Sarita, 2016). However, studies have mentioned the lack of some of the natural antioxidants such as β-carotene and Vitamin C in millets (Asharani et al., 2010; Ramashia et al., 2019).

HIV and AIDS are still major public health concerns globally. There has been a decline in the trends of HIV prevalence from 7.1 % in 2007 to 4.9% in 2019 (Onyango et al., 2021) and a 48% decline in HIV related mortalities reported between 2005 and 2017 in Kenya. However, Kenya still remains to be among the top ten most affected countries in Africa accounting for approximately 80% of the global burden of HIV and Aids (Kharsany & Karim, 2016). Nakuru is ranked 9th nationally with a burden of 41,217 PLWHAs, 966 annual new infections and increasing incidences of HIV co-morbidities and mortalities

(Mumbi et al., 2019; Kibet et al., 2022). Despite being ranked after eight other counties, HIV and Aids still remains to be a major public health concern in the county

Most countries, including Kenya, have integrated nutrition interventions in the comprehensive care of PLWHAs (Beckett et al., 2016). This has seen the use of ready to use therapeutic foods such as corn-soy blend (CSB) porridge being used for adults living with HIV/AIDS. There is increased need to produce more locally available and cheaper products that can be used as alternatives to the CSB porridge, to reduce monotony of the therapeutic diets and to increase the utilization of local underutilized nutrient dense crops such as millet and mushrooms.

This study aims to provide information on the antioxidant properties of mushrooms and the effects of solar drying on the antioxidant properties of oyster mushrooms. It further sought to investigate the antioxidant nutrient composition and sensory acceptability oyster mushroom enriched fermented millet porridge among PLWHAs.

1.3 Purpose of the Study

The study aimed at establishing the antioxidant content of fresh and solar dried oyster mushrooms. It also sought to assess the acceptability of fermented millet porridge enriched with oyster mushroom among PLWHAs.

1.4 Objectives

1. To establish the antioxidant content (β-carotene, vitamin E, vitamin C and total phenolic content) of fresh and solar dried cultivated oyster mushrooms
2. To determine the retention of the antioxidants (β-carotene, Vitamin E, Vitamin C, and total phenolic content) in solar dried oyster mushrooms
3. To establish the antioxidant content of oyster mushroom enriched millet flour

4. To assess the sensory acceptability of oyster mushroom enriched fermented millet porridge among PLWHAs

1.5 Research Hypothesis

$H_{01:}$ There is no significant difference between antioxidant content (β-carotene, vitamin E, vitamin C and total phenolic content) of fresh and solar dried cultivated oyster mushrooms.

H_{02}: There is no significant difference in antioxidant content (β-carotene, vitamin E, vitamin C and total phenolic content) of millet flour and mushroom enriched millet flour.

H_{03}: There is no significant difference in the sensory acceptability of mushroom enriched fermented millet porridge and the pure fermented millet porridge among PLWHAs.

1.6 Significance of the Study

The study finding may be used to educate the public on the importance of mushrooms in the diet and the effect of solar drying on antioxidants in mushrooms. The research findings may also be useful to the health care personnel to advise on other products that can be used by HIV patients to promote their nutrition and health status. The product formulated during the study may add value to underutilized mushrooms and hence may contribute to increased utilization of local foods therefore enhance food security and income generation from value added products.

1.7 Study Delimitations

The study determined the levels of four antioxidants only (βeta-carotene, vitamin E, vitamin C and Total phenolic content) in the mushrooms. Other nutrients present in the mushrooms were not determined. The study also focused on analyzing the oyster mushroom species only. Since the study targeted to develop a therapeutic product for

PLWHAs, the study was limited to assess the acceptability of fermented mushroom/millet-based porridge on PLWHAs only.

1.8 Conceptual Framework

Studies have shown that mushrooms are rich sources of dietary antioxidants (Ramesh & Pattar, 2010) among other nutrients. However, Kamal & Kumar (2014) term mushrooms as seasonal and highly perishable. Processing and preservation is therefore important to ensure their availability throughout the year. Through solar drying, mushrooms become available for longer periods. This ensures reduced waste and increased consumption of mushrooms. Solar drying may however affect the retention of the antioxidants. Millets are locally available, drought resistant but underutilized crops in Kenya. They are reported to nutrient dense but have low concentration of the natural antioxidants. Enriching millet porridge with mushrooms enhances increased intake of mushrooms and hence improved nutrition and health outcomes among PLWHAs (Figure.1.1).

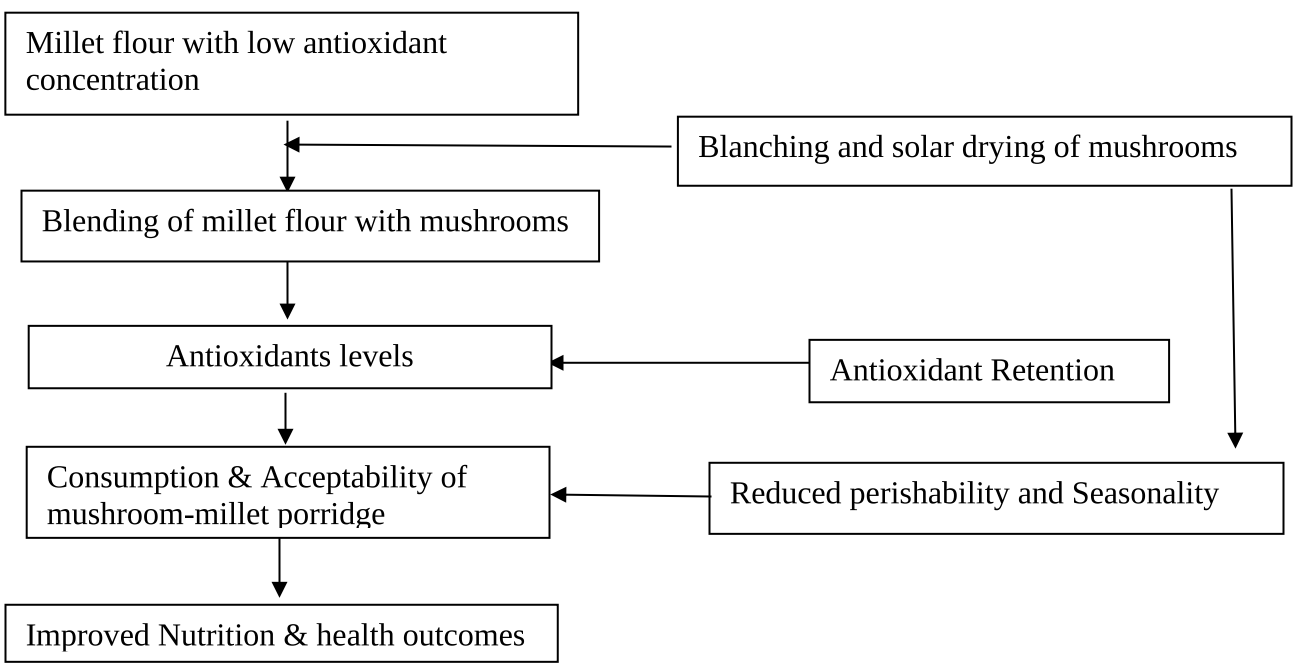

Figure 1.1: Conceptual framework antioxidant levels, effects of solar drying and acceptability of oyster mushrooms-finger millet flour blend Adapted from Piwoz & Preble, (2000)

CHAPTER TWO: LITERATURE REVIEW

2.1 Edible Mushrooms, nutrient and antioxidant profile

In the early days, mushrooms were mainly consumed due to their palatability and flavor (Ahmad et al., 2010). However, according to Ahmad and others, the present use of mushrooms is totally different from the traditional use due to the many researches done on their chemical composition. Mushrooms have recently been identified as reliable functional foods (Gogavekar et al., 2014). According to Rathee et al., (2012) consumers are currently more interested in foods that are considered healthy; aiming to promote their personal health and reduce chances of getting sick. "Edible mushrooms are rich in the essential nutrients such as carbohydrates, proteins, vitamins, minerals, dietary fiber and essential fats," (Adedayo et al., 2010). Their nutrient content varies among species and is highly dependent on their growth requirements.

Proximate composition of mushrooms is reported to be 92% water, 56% carbohydrate, 30% protein, 2% fat and 10% ash on dry weight basis (Singh, 2017). According to Nakalembe & Kabasa, (2013) mushrooms provide all the essential amino acids required by adults and in good quantities; this makes them an important food especially for the vegetarians. In addition, Valverde, Hernández-Pérez, & Paredes-López, (2015) reported that leucine, valine, aspartic and glutamic acid and glutamine are the most plentiful amino acids in mushrooms. Mushrooms contain very low amounts of fats mainly composed of linoleic, oleic and palmitic fatty acids (Pelin et al., 2013). Mushrooms hence provide a high protein less calorie diet which can be recommended for heart disease patients. In addition, their low starch levels make them ideal foods for overweight and diabetic patients. Edible mushrooms provide adequate amounts of the vitamins B1, B2, B12, vitamin C and Vitamin D (Valverde et al., 2015).

2.1.1 Oyster Mushrooms and their nutrient profile

Oyster mushrooms (*Pleurotus ostreatus*) also known as Pleurotus mushrooms, are a group of saprophytic fungi that grow wildly in both tropical and sub-tropical areas (Chirinang & Intarapichet, 2009). Oyster mushrooms can grow at moderate temperatures of between 20°C to 30°C, humidity of between 55-70% and on various agricultural waste materials used as substrates (Ahmed et al., 2013). In addition, Majesty, Ijeoma, Winner, & Prince, (2019) writes that *P. ostreatus* requires few environmental conditions and its fruiting body does not get attacked often by diseases and pests. Ijeoma and others further write that oyster mushroom species can be cultivated in a simple and cheap way. Given the flexible nature and ease of cultivating oyster mushrooms, they have been ranked as the second highest mushroom species cultivated among the edible mushrooms cultivated globally (Ahmed et al., 2013; Chirinang & Intarapichet, 2009). The cultivation of oyster mushrooms can therefore provide an alternative source of income as well as food for people in the country. According to Ahmed, Abdullah, Ahmed, & Bhuyan, (2013), the oyster mushrooms are a good source of carbohydrates, dietary fiber and amino acids. Table 2.1 presents ranges of proximate analysis results as reported by different studies. A study by Majesty et al., (2019) have reported the presence of Vitamin A, Vitamins B1, B2, B3, B6, B12 (Deepalakshmi & Sankaran, 2014), Vitamin C (Deepalakshmi & Sankaran, 2014), Vitamin D, Vitamin E and Vitamin K in oyster mushrooms. These mushrooms are also rich in the minerals such as iron, copper, calcium, phosphorus, zinc and manganese (Patil et al., 2010).

Table 2.1 Proximate composition of oyster mushrooms

	Range as reported by different studies
Moisture content (%)	83.24 – 91.45
Crude protein (db%)	22.61 – 28.85
Crude fat (% d.b)	2.47 – 5.01
Crude fiber (% d.b)	12.87 – 19.73
Total ash (% dw)	8.24 – 9.76
Carbohydrate (% db)	37.86 – 48.16

Table 2.1: Proximate composition of oyster mushrooms as reported by different studies (Li et al., 2017; Majesty et al., 2019; Tolera & Abera, 2017).

2.1.2 Antioxidant content of mushrooms

Recent years have seen mushrooms attract attention for their anti-oxidative properties. They are either used directly or as dietary supplements to help reduce oxidative stress in the body (Kozarski et al., 2015). Sánchez, (2016) reports that antioxidant compounds are found in the fruiting bodies and mycelium in mushrooms. The above author further states that each mushroom species is unique in the type of antioxidant components it has. Evidence shows that mushroom antioxidants can demonstrate their protective properties at different stages of the oxidation process and through different mechanisms (Kozarski et al., 2015). Mushroom antioxidants can be divided into two main types: primary antioxidants and preventive antioxidants.

One of the main antioxidants in mushrooms is phenolic compounds (Boonsong et al., 2016; Kozarski et al., 2015; Sánchez, 2016), majorly phenolic acids (Kozarski et al., 2015). The most prevalent phenolic acid is benzoic acid derivatives such as *p*-hydroxybenzoic, protocatechuic, gallic, gentisic, homogentisic, vanillic, 5-sulphosalicylic, syringic, veratric and vanillin (Kozarski et al., 2015). In vitro and in vivo assays have evidenced that polysaccharides in mushrooms to have significant antioxidant activities (Kozarski et al., 2015). Other antioxidants in mushrooms include flavonoids, carotenoids, tocopherols and ascorbic acid (Boonsong et al., 2016; Kozarski et al., 2015).

2.1.3 Antioxidant in Oyster Mushrooms

According to Gupta et al., (2017) the antioxidant potential of oyster mushroom can be attributed to its various characteristics, the most important being its ability to scavenge and reduce free radicals, chelate ferrous (Fe^{2+}) and cupric (Cu^{2+}) ions and to inhibit lipid peroxidation. Oyster mushrooms have been reported to be rich in antioxidants, and specifically vitamins and selenium which are important biological antioxidants (Singh et al., 2015). In addition, Chirinang & Intarapichet, (2009) write that phenolic compounds as the major antioxidant components in mushrooms. The fruiting bodies of oyster mushrooms show presence of several types of phenolic compounds such as: vanillic acid, naringin, chrysin, rutin, gallic acid, caffeic acid and cinnamic acid, homogentisic acid, ferulic acid, p-coumaric acid, p-hydroxybenzoic acid, protocatechuic acid and chlorogenic acids, (Singh et al., 2015; Strapáč, et al., 2017). Flavonoids, carotenoids and tocopherols, ascorbic acid, glycosides, lycopene, polysaccharides and ergothioneine have also been reported in *P. ostreatus* mushrooms (Chirinang & Intarapichet, 2009a; Gupta et al., 2017). Jayakumar, Thomas, Sheu, & Geraldine, (2011) further write that oyster mushrooms contain higher amounts of cystine, methionine and aspartic acid than other edible mushrooms. However, most researches have focused more on dietary value of edible mushrooms but there is relatively little information on the antioxidant activity and possible use of such mushrooms to reduce and neutralize oxidative stress.

2.2 Benefits of Antioxidants consumption among PLWHAs

Oxidative stress occurs in most human diseases and is suspected to have a significant effect in the pathogenesis of diseases (Akunueze et al., 2018). Human Immunodeficiency Virus has been reported to weaken the human immune system. Kashou & Agarwal, (2011), write that once an individual is infected by the virus, the body generates antibodies in an attempt to fight it off. However, the authors further write that this is a rate-limiting process and progression of the disease further compromises the body's immune system, leading to rise of opportunistic infection and AIDS. Similarly, Akunueze et al., (2018) write that HIV is the 'proximal agent for chronic diarrhea, fever, wasting, fatigue, pulmonary tuberculosis (PTB), sacorma and AIDS. In addition, Sundaram et al., (2008) writes that oxidative stress is seen to be higher in HIV positive patients receiving highly active antiretroviral therapy (HAART) as compared to those who are treatment naïve.

According to Akunueze et al., (2018), the HIV virus generates high levels of free radicals due to its effect on the different systems of the human body. The infection is believed to increase the oxidative stress in the body which in turn leads to an increase in opportunistic infections and disease progression to AIDS (Singh & Pai, 2015; Sundaram et al., 2008). Kashou & Agarwal, (2011) reports on a study done that evidenced a significant deficiency in Vitamin A, C and E antioxidants in HIV-positive children. The study suggests that an increase in oxidative stress due to free radicals overwhelmed the antioxidant body stores. Studies have reported on the need to use antioxidant reducing agents so as to suppress HIV expression and to reverse the mechanisms responsible for high opportunistic infections and disease progression to AIDS (Kashou & Agarwal, 2011). Since studies support that antioxidant deficiencies leads to rapid disease progression, researchers believe that dietary antioxidant supplementation will help reduce the viral load, restore the immune function and delay the disease progression in HIV positive patients (Kashou & Agarwal, 2011; Singh & Pai, 2015).

2.3 Finger Millets, antioxidant and nutrient profile

Millets are small to medium sized crops cultivated in the tropics and sub-tropical regions worldwide. They are classified under the grass sub-family *Panicoideae* (Kumar et al., 2018) together with maize and sorghum (Amadoubr & Le, 2013). They have been reported to be important foods in most underdeveloped countries because they can grow under diverse weather conditions (Bora, 2014), are resistant to insects and grow within a short period of time(Amadoubr & Le, 2013; Sarita, 2016). Millets are divided into major and minor millets. Nithiyanantham et al., (2019) lists the major types of millet as Pearl millet (*Pennisetum glaucum*), Foxtail millet (*Setaria italica*), Proso millet or white millet (*Panicum miliaceum*), and Finger Millet (*Eleusine coracana*) and lists minor millets as Barnyard millet (*Echinochloa* spp.), Kodo millet (*Paspalum scrobiculatum*), Guinea millet (*Brachiaria deflexa/Urochloa deflexa*), Little millet (*Panicum sumatrense*), and Browntop millet (*Urochloa ramose/Brachiaria ramose/Panicum ramosum*).

Millets have been reported to be nutritionally similar or superior to major cereals. According to Ashwani Kumar et al., (2018), millets contain gluten-free proteins, have a high fiber content, low glycemic index and are rich in bioactive compounds which give them additional benefits. The above-mentioned author further writes that the protein content of all types of millets is comparable, with a proximate composition of about 10%.

In addition, Sarita (2016) reported that millet proteins are rich in essential amino acids such as methionine and cysteine. Similarly, Ashwani Kumar et al., (2018) writes that finger millet protein is rich in essential amino acids such as methionine, valine and lysine and that of the total amino acids present in finger millet 44.7% are essential amino acids, an amount higher than the 33.9% recommended by FAO. Millets have also been reported to be high in the essential fatty acids; linoleic and oleic acids with several study findings have shown millets to be important in management of several diseases such as heart diseases, cancer, diabetes and celiac disease (Saleh et al., 2013). In addition, millets are good sources of the B vitamins, iron, manganese, magnesium and phosphorus (Saleh et al., 2013). However, millets have been reported to be extremely neglected and widely underutilized. Although millets have been reported to have anti-oxidative properties, studies have shown that they lack of some of the natural antioxidants. Asharani et al., (2010) reports that as much as finger millets provide good proportions of carotenoids, β-carotene could not be detected in varieties of finger millets tested using HPLC. Ramashia et al., (2019) also reported a lack of Vitamin C in millet flours.

2.4 Preservation of Mushrooms by solar drying

Mushrooms are highly perishable plants with a short shelf life. They survive only for 24 hours when stored under normal conditions and approximately for one week under refrigeration (Bala et al., 2009). Kamal and Kumar (2014) further indicate that the oyster mushrooms are more sensitive as compared to the other mushroom species and they start deteriorating within a day after harvest. The aforementioned authors state that this is caused by the high moisture content present in the oyster mushrooms. Some of the changes that show spoilage are changes in texture, moisture loss and enzymatic browning. Spoilt mushrooms are said to cause gastrointestinal discomforts when consumed (Kamal and Kumar, 2014). Because of their high perishability characteristic, mushrooms should be marketed and consumed soon after harvesting or processed for preservation.

Drying is one of the most effective methods used to preserve mushrooms (Tulek, 2011). Drying can be achieved through artificial methods or through direct sun drying. Most small-scale cultivators of mushrooms preserve their mushrooms through sun-drying. However, Bala et al., (2009) indicate that sun drying leads to unhygienic and poorly dried products because of the long time needed and overheating of the surface. The use of solar energy to preserve mushrooms has therefore been adopted in many mushrooms growing countries. Solar energy produces low heat suitable for drying mushrooms at low temperatures (Kumar et al., 2013). According to Tulek (2011), solar dried mushrooms still retain their sweet flavor. Drying of mushrooms has been seen to increase the contents of proteins, carbohydrates and phenolic contents and reduce the lipid content in the mushroom species '*Amanita zambiana'* Reid et al., (2016). In addition, Aishah and WanRosli, (2013) state that the powder from oyster mushrooms is rich in protein and low in fats. The dried mushrooms can be used as ingredients for various foods such as soups, pizzas, meats and rice dishes (Medany, 2014). Mushroom powder can be added to various recipes to improve the nutritional status of vulnerable populations. Traditional open sun drying and solar drying are still the most commonly used food preservation post-harvest methods used by small scale farmers (Udomkun et al., 2020), however, there is little research evidence on the nutritional and chemical properties of dried mushroom species other than the '*Amanita zambiana'*.

Figure 2.2: Simple drier used by small scale farmers

2.5 Food Blending with Mushroom Powder

Mushroom powder has been used in several studies to enrich wheat products and assessed for its addition in nutritional value of the foods. A study carried out by Mahamud et al., (2012) showed an increase in protein content of about 0.47% to 1.8%, in bread enriched with mushroom powder. Similarly, Singh & Verma, (2013) found that the protein content of the Indian food "ravaidli" increased as the level of fortification with mushroom powder increases. In the same study, carbohydrate and fat content of the food was seen to decrease as the level of fortification with mushroom powder increased. In addition, it was reported that protein content of biscuits made from wheat flour and mushroom powder increased by approximately 13.4% (Prodhan et al., 2015). The aforementioned authors further found that fat content and fiber content in the biscuits was also seen to increase with increase in mushroom powder used to bake them. All these studies show the effect of mushroom powder on the macronutrient properties of the foods.

However, there lacks adequate data on the effect of mushroom powder on the micronutrient content of the foods it is used to enrich. One study by Mane, Kale, Dhewale, & others, (2014) shows that there is a remarkable increase in potassium, riboflavin, niacin and thiamin content of Indian foods enriched with mushroom powder. In addition, Ondiek et al., (2019) stated that the fortification of maize flour with mushroom powder significantly increases the content of proteins, Iron, Zinc, Thiamine, Riboflavin, Niacin and Folate. Mushroom powder has been seen to increase contents of different nutrients such as minerals, fat, fiber and proteins in common cereals and cereal-based foods. There is need to find out the effect on antioxidant composition of foods after being fortified with mushroom powder.

2.6 Sensory Characteristics of Mushroom Enriched Foods

Food consumption of a product is highly determined by the sensory properties of the product. In the study where biscuits were enriched with mushroom powder, the mushroom biscuits obtained a lower score in terms of appearance and color (Prodhan et al., 2015). In the same study, normal biscuits got a higher score in the acceptability test as compared to mushroom biscuits. Wan Rosli, Nurhanan, & Aishah, (2012) reported that biscuits incorporated with 2% mushroom powder was accepted at a rate that is almost similar to that of normal biscuits. They further write that the acceptability scores decreased as the percentage of mushroom powder in the biscuits increased. Similarly, in a study by Mahamud et al., (2012) it was found that bread with 5% mushroom powder was better accepted as compared to that with 15% mushroom powder. The 5% mushroom powder did not significantly change the color, texture, and flavor of the bread. Similarly, a study by Ondiek, (2018) on sensory acceptability of mushroom/maize porridge formulation reported that the control was rated with better mean scores than the composite porridges. The above-mentioned author however reports that there was no notable difference ($P < 0.05$) between general acceptability scores given to the 90:10, 80:20 maize: mushroom porridge formulations and the control porridge. There is scarce information on the sensory acceptability of mushroom-millet food blends, this study therefore sought to assess the sensory acceptability of mushroom enriched finger millet fermented porridge among PLWHAs.

2.7 Summary of Literature Review

Mushrooms have been identified as rich sources of essential nutrients needed for the human body. Apart from their dietary and nutritional importance, mushrooms have also been acknowledged for their medicinal properties. Their nutritional and medicinal properties have greatly been studied. However, due to their highly perishable nature, fresh mushrooms are scarce in the market. Therefore, mushrooms are normally marketed soon after harvest or preserved or processed to reduce wastage. Preservation is done using two major methods, drying and freezing/refrigeration. Several studies have been conducted on the nutrient content of solar dried and refrigerated mushrooms. However, there is scarcity of data on the effect of solar drying on mushrooms grown and solar dried within the Kenyan settings. Mushroom powder from solar dried mushrooms has been used in the fortification of different foods, especially wheat and cereal based foods to increase their nutritional content. There is need for studies on the bio-fortification of local cereal based Kenyan dishes such as millets with mushrooms and their sensory acceptability among Kenyans.

CHAPTER THREE: METHODOLOGY

3.1 Study Design

The study adopted a single factor completely randomized experimental research design comparing the formulated product with the controls. It was conducted in two phases. In the first phase, laboratory analysis was conducted to determine the nutrient antioxidants composition of fresh (control) and solar dried cultivated oyster mushroom. Laboratory analysis was also conducted to determine antioxidant nutrient content in millet flour (control) and mushroom enriched millet flour. The second phase involved sensory evaluation of the acceptability of the mushroom enriched fermented millet porridge among PLWHAs.

3.2 Study Area

Acceptability tests were carried out at the Nakuru County Referral Hospital. Nakuru is ranked 9th nationally with a burde n of 41,217 PLWHAs, 966 annual new infections and increasing incidences of HIV co-morbidities and mortalities (Mumbi et al., 2019; Kibet et al., 2022) which is a public health concern. The hospital receives low- and middle-income patients from within and around Nakuru County and its environs.

3.3 Study Population

The sensory evaluation included PLWHAs attending the Comprehensive Care Clinic (CCC) at the Nakuru County Referral Hospital. Inclusion criteria included adults living with HIV and Aids who were between the ages of 20-60years and who were in the acute and chronic stages of HIV infection. This is because people from this age group are able to support themselves and decide for themselves what to eat. Only those willing to participate in the study upon informed consent were included. The study excluded adults whose sensory performances had been affected by the disease or ARV drugs. This was confirmed by the help of the departments' nutritionist and nurse through the participant's medical records. PLWHAs suffering from severe illness were also excluded.

3.4 Sampling

3.4.1 Food Sampling

Twenty kilograms of fresh cultivated oyster mushrooms were purchased from small scale mushroom growers in Kiambu County, Kenya. This was to allow for the purchase of freshly collected mushrooms direct from the farm. Growers in these places grow their mushrooms on agricultural waste and sawdust substrates. The fresh mushrooms were packaged in hermetic bags, transported, and refrigerated in the Kenyatta university food chemistry laboratory within 12 hours after harvesting awaiting analysis. The purchased mushrooms were sorted and washed.

Ten kilograms of finger millet **(***Eleusine coracana*) was purchased from githurai and city market which were randomly selected from open markets within Nairobi County. Five kilograms of the millet were purchased from each of the markets. The millet was transported and stored in hermetic bags in the Kenyatta university food chemistry laboratory at 27ºC. The millet was sorted, cleaned, and ground into flour which was subjected to nutrient analysis and sensory evaluation tests. The steps in food samples preparation are as described in the Figure 3.1 (Appendix A).

3.4.2 Sampling of Respondents for sensory analysis

Simple random sampling was used to select Participants for the acceptability study from the CCC of the Nakuru County Referral Hospital. Each patient attending the CCC was assigned a unique number and the excel random number generator was used to select 30 participants according to the guidelines for pilot affective and descriptive sensory tests (Civille et al., 1981).

3.5 Mushroom and millet processing steps.

3.5.1 Sorting

Sorting was done for both fresh mushrooms and millet grains to remove discolored, damaged food samples as well as dirt and extraneous matter. The fresh sorted mushrooms were then cleaned under cold clean running water.

3.5.2 Blanching of mushrooms

About half of the amount of the oyster mushroom samples was packed into a vacuum non-porous plastic bag and blanched at high temperatures (90^0C) over a short time (4 minutes). Vacuum bags were used to prevent loss of water soluble Vitamins such as Vitamin C. The samples were then cooled in cold water for 5minutes. According to Chege, Kuria, Kimiywe, & Nyambaka, (2014), blanching is important before drying of fresh vegetables for preservation of color and flavor, to prevent or minimize nutrient loss during drying as well as to extend storage life.

3.5.3 Drying

Both the blanched and the un-blanched mushrooms were solar dried at 45^0C-70^0C (Naik et al., 2006) until a moisture content of about 10% was attained (Kumari & Baskaran, 2004). The solar dryer used in this study was made of a wooden frame covered with UVA-treated polythene and insect nets for vents and a black sheet for the base. The mushrooms were ground to a powder using a standard blender and packed in sealed polythene bags to prevent moisture absorption and infestation by insects and molds. The polythene bags were stored in a cool, dark, and dry place awaiting analysis.

3.5.4 Enrichment of Millet Flour using Mushrooms

Mushroom (both blanched and unblanched) powder was added to millet flour at specific ratios of 20:80, 40:60 and 60:40 mushroom powder: millet flour respectively following procedures described by (Nguyen & Nguyen, 2018). The enriched millet flour was used for analysis and preparation of porridge for sensory analysis.

3.5.5 Fermentation and Cooking

One cup (250g) of the enriched millet flour was mixed with ½ cup of water in a jug to make a paste. The paste was then covered, stored in a warm place for 48 hours to allow the flour to ferment. This was done to increase the bioavailability of the nutrients (Assohoun et al., 2013). Once fermented, the various pastes were added at a ratio of one cup fermented paste to two cups of boiling water and cooked for 10-15 minutes to make a thick pap or porridge.

3.6 Research Instruments

The research used laboratory data sheets (Appendix B) to record data from the laboratory analysis. The 5-point hedonic scale questionnaire (Appendix C) was used to determine sensory evaluation for the fermented mushroom enriched millet porridge.

3.7 Nutrient Analyses

3.7.1 Determination of moisture content

Oven drying method number 925.10 was used to determine Moisture content (MC) of samples as described in AOAC methods (AOAC 2005). An electronic weighing balance scale (model NBY323/64 Avery East Africa) was used to weigh five grams of each sample and placed into pre-weighed dry crucibles. The samples were then heated in a hot air oven (Lab Tech model LDO-080H) at 105ºC for 3 hours. Samples were then cooled in a desiccator and weighing done at intervals of 30 minutes until a constant weight was achieved for three consecutive measurements. The final weight was noted and used to calculate the moisture content of the samples. Analysis was done in triplicate and mean values calculated to ensure accuracy. Moisture content was calculated as:

$$\%MC = \frac{(\text{Wght of crucible+ fresh sample})-(\text{wght of crucible+dry sample})}{\text{wght of fresh sample}} \times 100\ \%$$

3.7.2 Preparation of standards

3.7.2.1 Beta-carotene and Vitamin E standards

Beta-Carotene and Vitamin E standards were prepared according to Wakhanu (2014). Standards were prepared using 0.01 grams of beta-carotene (Sigma Chemicals) standards and analar crystalline (Aldrich chemicals) respectively. Exactly 0.01 grams of the respective standard crystals were added to 100ml volumetric flask and 10mls of n-hexane was added to the mark flask (100ppm). Different solutions of 20ppm, 40ppm, 60ppm and 100ppm were prepared using the n-hexane solution. A working standard of 10ppm was used to measure the peak absorbance. The obtained absorbance was then used to measure the concentration of the other solutions and prepare a β-carotene and vitamin E standard curve.

3.7.2.2 Vitamin C standard

Vitamin C standard amounting to 100mg was weighed using analytical balance into 100ml beaker and dissolved with 45ml of an extraction solution (3% Meta phosphoric acid (MPA) and 8% acetic acid prepared by dissolving 15g of MPA in 40ml acetic acid and 200ml of distilled water and made to 500ml mark using distilled water). The solution was transferred into a 100ml-volumetric flask rinsing the beaker three times with extraction solution and transferring the solution to the volumetric flask. The volumetric flask was then filled to the mark using the extraction solution and the concentration was equal to 1000ppm. Standards of different known concentration that is 10,20,40,60, 80 and 100ppm were prepared by diluting the stock solution with the extraction solution and used for preparing the standard curve.

3.7.2.3 Total phenolic compounds standard

Standards for the total phenolic content determination were prepared according to Waterhouse (2002). For Gallic acid stock solution, 0.5g of dry Gallic acid crystals (sigma chemicals) were put in a 100ml volumetric flask and dissolved in 10ml of ethanol. The resulting solution was then diluted using distilled water to volume.

To prepare sodium carbonate stock solution, 20g of anhydrous sodium carbonate was added to a beaker and dissolved using 100ml distilled water. The solution was brought to boil and cooled thereafter. After cooling, a few crystals of the sodium carbonate were added. The solution was left for 24 hours and filtered thereafter. Distilled water was added to the resulting extract and made up to 250ml.

Exactly 0, 1, 2, 3, 5 and 10mls of the Gallic acid stock solution were added to 100mls volumetric flasks. The solutions were then diluted to volume with distilled water to give phenol concentrations of 0, 29, 100, 150, 250 and 500mg/L of Gallic acid. From the above solutions, 0.04ml was added to test tubes. About 3.16mls of water and 0.2 mls of the Fiolin Ciocalteu reagent were pipetted and added to the test tubes. The resulting mixture was left for 5 minutes and 0.6mls of the sodium carbonate solution added and the mixture shaken well. The solution was left for 2 hours at 20^0C. Absorbance was measured using a UV-VIS spectrophotometer at 765nm.

3.7.3 Extraction and analysis of samples

3.7.3.1 Simultaneous Extraction and determination of Beta-carotene and Vitamin E

Extraction was done as described by Wakhanu, (2014). Five grams of each mushroom or the mushroom enriched millet flour was mixed with 50mls of acetone-hexane mixture with 0.1% Butylated Hydroxytoluene (BHT). The BHT acts as an antioxidant. The mixture was homogenized for 10 minutes using a mechanical shaker in a volumetric flask, centrifuged for 10 minutes, and decanted through a separating funnel. The supernatant was saponified using 25mls of 0.5M potassium hydroxide, shaken and allowed to settle for 30 minutes. The solution was washed using 100mls of distilled water and the extract dried by filtering over anhydrous sodium sulphate. The filtrate was concentrated in an evaporator at 45^0C and dissolved in methanol up to the 50ml mark in a volumetric flask.

Beta-carotene concentration was determined according to the AOAC 970.64 method (AOAC, 2005) using the UV-VIS spectrophotometer. The absorbance the standards and sample extract was measured at 450nm (Sathya et al., 2014; Scotter, 2011) in a UV-VIS spectrophotometer. Total concentration of β-carotene in the sample was calculated using excel computer software.

Vitamin E concentration in the sample was determined using High Performance Liquid Chromatography method described by Muturi, (2013). The extracted sample (20 μL) was injected into the Shimadzu HPLC system C18 Octadecyl-Silica (ODS 15cm × 46mm) column with a UV detector set at 286nm. HPLC grade hexane was used as the mobile phase at a flow rate of 1.5mls per minute. The corresponding peak area to the retention time of 15 minutes was used to calculate the sample concentration using standard graphs. Total concentration was calculated using excel computer software (Wakhanu, 2014).

3.7.3.2 Extraction and determination of Vitamin C (L-Ascorbic acid)

The method described by Vikram, Ramesh and Prapulla (2005) was followed. Three grams of mushroom or mushroom enriched millet flour samples were put in a 250ml conical flask and 10ml of 0.8% metaphosphoric acid added. The solution was then swirled and left to settle for approximately 10 minutes. The resulting mixture was filtered using Whatman filter paper and the extract placed in a 100ml volumetric flask. A solution was made to mark using distilled water.

Vitamin C was determined using the HPLC as described by Vikram et al.,(2005). Exactly 30µl of the extracted solution and standards were injected into the HPLC system. The ascorbic acid was separated using a C18 Octadecyl-Silica (ODS 15cm × 46mm) column with a UV detector set at 254nm. Metaphosphoric acid solution (0.08%) was used as the mobile phase at a flow rate of 0.5ml per minute. A standard curve was prepared using various concentrations of ascorbic acid solution prepared as described in section 3.7.2.2. The identification of ascorbic acid in the mushroom and fortified millet flour samples was based on the retention time of the ascorbic acid from the standard solutions while the quantification was based on the corresponding peak area. Total concentration was calculated using excel computer software.

3.7.3.3 Extraction and determination of Total Phenolic Content

Extraction of Total Phenolic Content (TPC) was done as described by Luvonga, (2012). Five grams of the samples were put in a conical flask and 100ml of 50% ethanol and methanol solutions added at 25^0C for extraction. Alumminum foil was used to cover the mixture and it was left to settle for 2 hours and the extract filtered. Phenolic Content was determined using the Folin-Ciocalteu method (Baba & Malik, 2015; Luvonga, 2012).

Exactly 0.04 ml of sample extracted was added to test tubes. Distilled water (3.16mls) and 0.2mls of Folin-Ciocalteu's reagent (Sigma Chemicals) was then pipetted and added to the test-tubes. Exactly 0.6mls of Sodium carbonate $NaCO_3$ (Sigma chemicals) was then added to the test tubes and the mixture shaken well. The mixture was left to stand for 1 hour in the dark. Absorbance was measured at 765nm using a UV-VIS spectrophotometer. Total phenolic content was expressed as Gallic acid equivalents (Baba & Malik, 2015) in milligrams per 100g (Wakhanu, 2014).

3.8 Acceptability Tests

Pure fermented millet porridge and fermented mushroom enriched millet from both blanched and unblanched porridge were cooked. The prepared porridges were put in white bowls, coded with random letters, and served to 30 untrained participants . Each participant was served with seven half- full bowls; one with pure millet porridge, porridge enriched with the three different concentrations of unblanched mushroom powder and porridge enriched with the three different concentrations of blanched mushroom powder. Warm water was provided for rinsing the mouth after tasting each sample. Data collection was done in a large room with wide windows to provide for social distancing and good ventillation to adhere with the Covid-19 regulations. Sanitisers and wash basins were also placed in the room to promote hygine. Using the 5- point hedonic scale (Appendix C), the respondents were required to rate the product's color, taste, flavor, and general acceptability using scores provided in the questionnaire.

3.9 Validity and Reliability of Tests

Analytical grade reagents were used in nutrient analysis. Nutrient content determination was done in triplicate and a mean and standard deviation calculated to ensure accuracy of tests. Reliability and precision was measured by reproducibility of results. This was calculated for 5 measurements of the sample for each test. Standards curves for the specific nutrients were drawn and R^2 values of 0.9995, 0.9987, 0.996 and 0.9985 were obtained for β-carotene, TPC, Ascorbic acid and Vitamin E, respectively.

3.10 Data Analysis and Reporting

Nutrient analysis was carried out in triplicate and mean values calculated. The data was analyzed using the SPSS statistical software. Independent T-tests were used to compare data on nutrient content of treated and untreated samples. Analysis of variance (ANOVA) was used to analyze sensory evaluation and acceptability data. The data was presented in form of tables and charts. The findings are presented in chapter four.

3.11 Logistical and Ethical Considerations

Ethical permits to conduct research were sought from the Kenyatta University Ethical Review Committee (Appendix G). Authority to conduct research was sought from the Kenyatta University graduate school. A Research permit was sought from National Commission for Science, Technology, and Innovation (NACOSTI) – NACOSTI/P/19/32526/27573 (Appendix H). Permit to do the research was also sought from the Ministry of Health as well as the Nakuru County Referral Hospital management. Informed voluntary consent was sought from the study participants.

CHAPTER FOUR: FINDINGS

4.1 Introduction

This chapter presents the study findings on nutrient analysis in raw mushroom and millet flour samples. Results on sensory evaluation carried out on porridge prepared from four different ratios of mushroom and millet flour are also presented. The results are organized as per specific objectives of the study namely: Nutrient antioxidant composition of fresh and solar dried mushroom samples; nutrient antioxidant composition of the composite flours from mushroom/millet formulations. Comparisons on nutrient antioxidant composition of plain millet flour with the composition of the different formulations of mushroom/millet blend and sensory evaluation of porridge from four different formulations, nutritional value of selected porridge samples.

4.2 Moisture content of mushroom and millet samples

Moisture content of the flour samples was determined using oven drying method. The fresh mushroom had a moisture content of 90.1% while the dried ones had a moisture content of 11.1%. These mositure contents are within 85% to 95% reported by Kumar et al., (2013) for fresh mushrooms and 9% to 13% reported by Siyame et al., (2021). The moisture content of millet flour was 13.5 % (Table 4.1), this figure was within the specified ranges of < 15% (Esilaba, 2021).

Table 4.1: Moisture content of different samples(%)

Sample	Fresh mushroom	Solar dried mushroom	Millet flour	20:80 Mushroom & millet flour	40:60 Mushroom & millet flour	60:40 Mushroom & millet flour
Moisture content	90.09	11.14	13.54	12.72	11.74	10.42

4.3 Antioxidant Nutrient content of fresh and blanched oyster mushrooms

The content of antioxidants in mushrooms is presented in Table 4.2. The vitamin C content of fresh mushroom was found to be 37.25 ± 0.32 mg/100g *dw* while the phenolic content was found to be 36.48 ± 1.50 mg/100g *dw*. The β-carotene content in fresh oyster mushrooms was found to be 13.73 ± 0.02 mg/100g *dw*.

Table 4.2: Antioxidant retention (%) of the blanched mushrooms

	Fresh Mushrooms mg/100g dw	Blanched Mushrooms mg/100g dw	Retention (%)	Independent T-Test (p-value)
B-Carotene	13.73 ± 0.02	11.10 ± 0.04	80.84	< 0.001
Vitamin E	23.23 ± 2.12	16.33 ± 0.09	70.30	0.030
Vitamin C	37.25 ± 0.32	37.08 ± 0.03	99.54	0.460
Phenolic Content	36.48 ± 1.50	30.12 ± 0.37	82.57	0.014

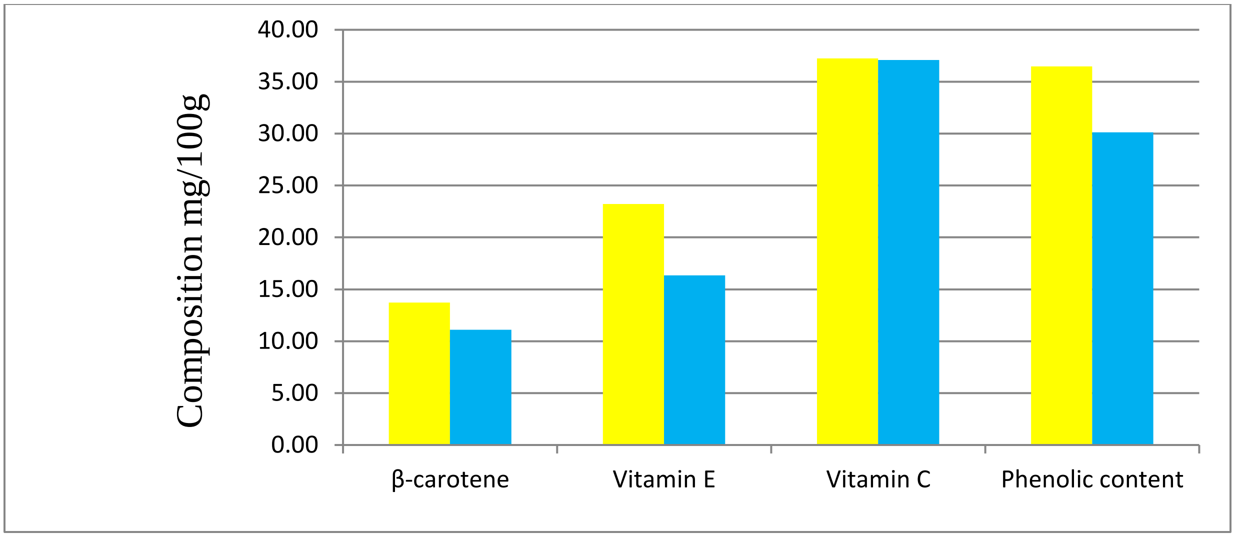

Figure 4.1: Antioxidant Nutrient content in fresh oyster mushrooms (mg/100g dw)

Independent T-tests were used to compare concentration of the antioxidants in the fresh and blanched mushrooms and the findings are shown in Table 4.2. β-Carotene, Vitamin E and TPC contents were notably reduced ($p < 0.05$) after blanching. However, blanching did not significantly reduce the Vitamin C content in the blanched mushrooms (Table 4.2). The highest retention (99.54%) of vitamin C was observed in blanched mushrooms. Blanching decreased the content of E in mushrooms with a retention of 70.3% of this vitamin being observed in blanched mushrooms relative to the fresh un-blanched ones (Table 4.2). Blanched mushrooms showed a β-carotene content retention rate of 80.84% with independent t-tests revealing that blanching significantly decreased the content of this vitamin (p value = < 0.001). Significant differences were also observed in the Vitamin E (p value = 0.030) and phenolic contents (p value = 0.014) contents when comparing unblanched fresh and blanched oyster mushrooms. In this study, there was no significant difference (p value = 0.460) in the vitamin C contents of fresh mushrooms and blanched mushrooms (Table 4.2).

4.4 Antioxidant nutrient content of blanched and non-blanched solar dried oyster mushrooms

The results on the content of oyster mushrooms solar dried before or after blanching are presented in Table 4.3. Independent T-tests were done to test the differences in concentration of the antioxidants in the unbvlanched and blanched dried mushrooms. All the nutrient antioxidant concentrations were significantly higher ($p < 0.05$) in the blanched solar dried mushrooms than in the unblanched solar dried mushrooms.

Table 4.3: Antioxidant Nutrient content in dried oyster mushrooms (mg/100g dw)

Antioxidant	Unblanched Dried	Blanched Dried	p-value (t-tests)
Beta-carotene	1.21 ± 0.00	10.90±0.01	< 0.001
Vitamin E	0.96 ± 0.18	13.62±1.07	0.002
Vitamin C	5.60 ± 0.12	29.45±0.00	< 0.001
Phenolic content	21.55 ± 0.24	26.12±1.39	0.027

4.5 Retention of antioxidants in blanched and non-blanched solar dried oyster mushrooms

In the curren t study, it was observed that the antioxidant nutrient contents of the solar dried mushrooms was lower than the fresh mushrooms as shown in Table 4.4. The table also shows the percentage retention of each antioxidant nutrient in solar dried mushrooms. Independent t-test was done to compare the difference between fresh and solar dried mushrooms. Solar drying was found to significantly reduce ($p < 0.05$) the concentration of nutrient antioxidants. Vitamin E had the lowest retention rate at 4.1% while the retention rate for the total Phenolic content was the highest.

Blanching the mushrooms prior to solar drying resulted in higher retention rates of antioxidants as shown in Table 4.4. Vitamin E showed the lowest retention rate (58.63%) with a significant difference (p value = 0.006) between the content of the blanched dried mushrooms and that of unblanched mushrooms. The rest of the nutrient antioxidants had considerably higher retention rates in mushrooms dried after blanching as compared to those dried without blanching.

Table 4.4: Comparison between antioxidant retention with unblanched and blanched solar dried mushrooms

	Unblanched dried oyster mushrooms		**Blanched dried oyster mushrooms**	
	Retention (%)	**p-value (t-tests)**	**Retention (%)**	**p-value (t-tests)**
B-carotene	8.79	< 0.001	79.39	< 0.001
Vitamin E	4.11	0.003	58.63	0.006
Vitamin C	16.10	< 0.001	79.06	0.001
Phenolic content	59.07	0.003	71.60	0.001

NOTE:

1. Percent retention rates show retention of the antioxidant concentration when fresh mushrooms are dried without blanching and after blanching.
2. Independent T-tests (p-values) show differences in concentration between fresh mushrooms and unblanched solar dried mushrooms and between fresh mushrooms and blanched solar dried mushrooms

4.6 Antioxidant content of mushroom-enriched millet flour

The antioxidant nutrient content of millet flour is shown in Table 4.5. Compared with the unblanched solar dried mushroom powder, millet flour showed a significantly higher content of β-carotene (p value = < 0.001). Vitamin E content was non-significantly higher in millet flour than in the unblanched solar dried mushroom (p value = 0.183). However, the solar dried mushroom powder showed significantly higher content of vitamin C and total phenolic substances compared to millet flour (p value = < 0.001). All the antioxidant nutrient contents in mushrooms dried after blanching were significantly higher ($p < 0.05$) than in millet flour (Table 4.5).

Table 4.5: Antioxidant content of solar dried oyster mushrooms and millet flour (mg/100g dw)

Nutrient	Millet flour (mg/100g dw)	Fresh Solar dried oyster mushrooms (mg/100 g dw)	Blanched solar dried oyster mushroom (mg/100 g dw)	ANOVA (p-value)
Beta-Carotene	1.27 ± 0.00[a]	1.21 ± 0.00[b]	10.90 ± 0.01[c]	< 0.001
Vitamin E	1.75 ± 0.29[a]	0.96 ± 0.18[a]	13.62 ± 1.07[b]	< 0.001
Vitamin C	0.10 ± 0.01[a]	5.60 ± 0.12[b]	29.45 ± 0.00[c]	< 0.001
Phenolic content	12.30 ± 0.12[a]	21.55 ± 0.24[b]	26.12 ± 1.39[c]	< 0.001

NB:

1. Superscripts with different letters in the same row indicate significantly different values at $p \leq 0.05$

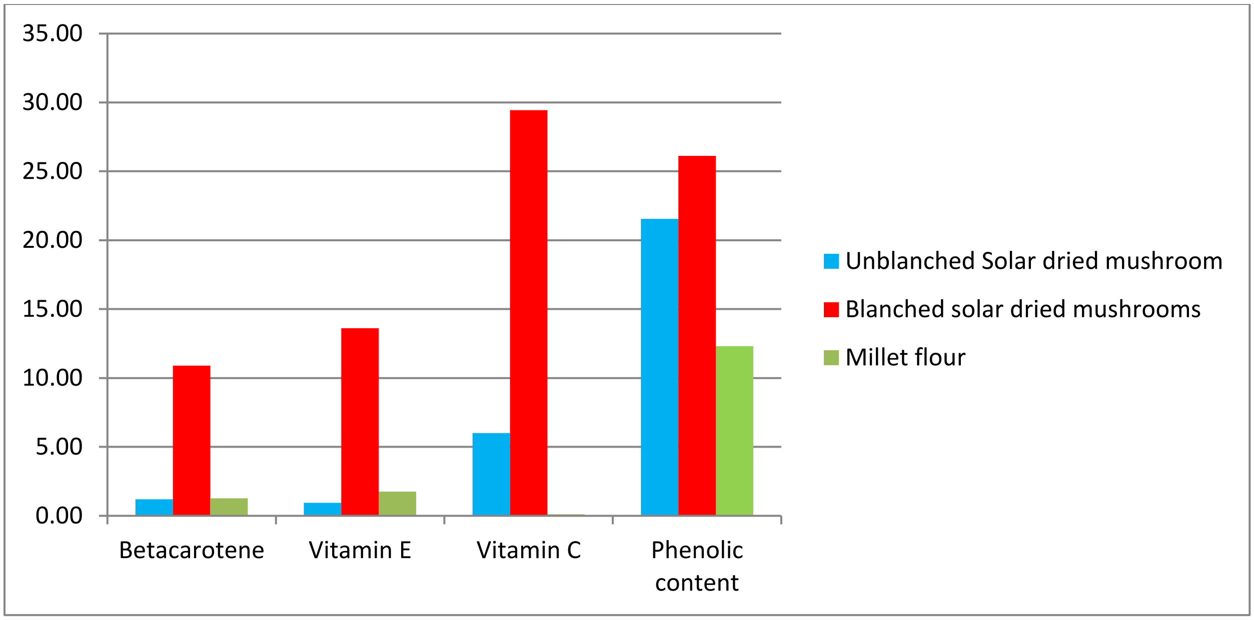

Figure 4.2: Comparisons between solar dried mushrooms and millet flour in antioxidant content

Different proportions of Mushroom powder: Millet flour formulations were prepared as described by Nguyen & Nguyen, (2018); 20:80, 40:60 and 60:40 formulations were prepared with both the unblanched and blanched mushroom powders and the nutrient antioxidant concentrations in the samples determined.

The concentration of β-carotene was observed to be highest in the 40:60 unblanched mushroom-millet formulation (1.35 ± 0.00 mg/100g *dw*) and lowest in the 20:80 unblanched mushroom-millet formulation. β-carotene in millet flour was higher than that found in the 20:80 unblanched mushroom-millet formulations. Vitamin E concentration of the unblanched mushroom powder- millet flour blend was found to decrease as the mushroom proportion in the millet increased with the 20:80 unblanched mushroom-millet formulations having the highest concentration and the 60:40 unblanched mushroom-millet formulations having the lowest concentration. On the other hand, Vitamin C was found to increase as the unblanched mushroom powder proportion was increased in the formulations with the 60:40 unblanched mushroom-millet formulation having the highest concentration at 0.38 ± 0.02 mg/100g *dw*. Phenolic content was also highest in the 60:40 unblanched mushroom-millet formulation at 14.73 ± 0.36 mg/100g *dw*. Both Vitamin C and phenolic content concentrations in the 20:80 unblanched mushroom-millet formulations were higher than in millet flour.

Blanched solar dried mushrooms were also used to enrich millet flour and their content of antioxidant nutrient analyzed. Similar to the composite flours made from with unblanched mushrooms and millet flour, β-Carotene was highest in the 40:60 blanched mushroom: millet flour formulation and lowest in the 20:80 blanched mushroom: millet formulation. Vitamin E content was highest in the 20:80 blanched mushroom: millet formulation at 19.08±0.75 mg/100g *dw* and the amount reduced as mushroom powder increased in the formulations. The content of Vitamin C and phenolic substances increased as the amount of blanched mushroom powder was increased in the composite flours with the 60:40 blanched mushroom: millet blends having the highest contents at 3.01±0.00mg/100g dw and 19.29±0.16 mg/100g dw respectively.

Table 4.6: Comparison between Antioxidant content in millet and mushroom enriched millet flour mg/100g dw

Antioxidant / Sample	Beta-carotene	Vitamin E	Vitamin C	Total Phenolic Content
Control	1.27 ± 0.003^{a}	1.75 ± 0.29^{a}	0.10 ± 0.01^{a}	12.30 ± 0.12^{a}
Unblanched 20:80	0.96 ± 0.00^{b}	1.63 ± 1.26^{a}	0.21 ± 0.02^{b}	14.63 ± 0.32^{b}
Blanched 20:80	8.45 ± 0.11^{c}	19.08 ± 0.75^{b}	2.59 ± 0.02^{c}	15.70 ± 0.33^{c}
Unblanched 40:60	1.35 ± 0.00^{d}	0.46 ± 0.01^{c}	0.33 ± 0.02^{d}	10.37 ± 0.15^{d}
Blanched 40:60	11.94 ± 0.02^{e}	16.52 ± 0.45^{d}	2.90 ± 0.01^{e}	16.54 ± 0.23^{e}
Unblanched 60:40	1.06 ± 0.00^{f}	0.44 ± 0.048^{c}	0.38 ± 0.01^{f}	14.73 ± 0.36^{b}
Blanched 60:40	10.98 ± 0.03^{g}	16.03 ± 0.06^{d}	3.01 ± 0.00^{g}	19.29 ± 0.16^{f}
Anova p-value	< 0.001	< 0.001	< 0.001	< 0.001

NB:

1. Superscripts with different letters in the same column indicate significantly different values at $p \leq 0.05$

Analysis of Variance (ANOVA) was used to compare the difference in concentration of nutrient antioxidants in millet flour and mushroom enriched millet flour. Results show that there was notable differences (p value = < 0.001) in all nutrient antioxidants between millet flour and the mushroom enriched millet flours. In addition, Post hoc LSD results show that the Beta-carotene and Vitamin C concentrations differed significantly in all the tested composite flour samples ($p < 0.05$). Vitamin E concentration was significantly higher (p <

0.05) in millet flour than in all the formulated mushroom-millet composite flours apart from the 20:80 unblanched mushroom - millet flour whose concentration was not significantly lower than the concentration in millet flour (p value = 0.800). Total Phenolic Content was significantly lower (p value = < 0.001) in the 40:60 unblanched mushroom-millet formulation (10.37±0.15 mg/100g *dw*) as compared to the control porridge flour. β-carotene composition was found to be significantly lower (p value = < 0.001) in the 60:40 unblanched mushroom millet composite flour (1.06 ± 0.00 mg/100g *dw*) than in millet flour (Table 4.6).

4.7 Acceptability of mushroom enriched fermented millet porridge among PLWHAs

A 5-point hedonic scale was used to evaluate the sensory attributes of the mushroom enriched millet porridge. Respondents were given samples of porridges made from millet flour blended with either unblanched or blanched mushrooms. Scores ranged from 1 (extremely dislike) to 5 (extremely like). The results are presented in Table 4.9. Significant differences were observed in sensory scores in the different sensory attributes in the four porridge formulations analyzed (0:100, 20:80, 40:60, 60:40) with regard to color, taste, aroma, consistency, flavor, and general acceptability. The control porridge (100% millet flour porridge) was scored higher in all the sensory attributes than all the porridge blends. There was an inverse relationship between the score rating and the amount of mushroom powder in the porridge in all the sensory attributes with the unblanched 60:40 mushroom: millet porridge blends scoring the least in terms of color (4.40±1.04), Taste (2.97±1.54), Aroma (3.13±1.52), Consistency (3.43±1.43), Flavor (3.00±1.50) and general acceptability (2.97±1.47). Porridge blends with blanched mushrooms received better scores than porridge blends with unblanched mushrooms in all the sensory attributes.

Post hoc LSD tests showed a substantial difference ($p < 0.05$) in the mean general acceptability scores between the control porridge and all the other porridge blends both blanched and unblanched. In all the sensory attributes, the control porridge received the highest scores. However, in terms of sensory scores for color, there was no notable difference ($p > 005$) between the control porridge and all the other porridge blends apart from the 60.40 unblanched mushroom powder: millet porridge blend (p value = 0.001). In terms of taste, the control had no significant difference ($p>0.05$) with all the porridge blends with blanched mushrooms powder. There was no significant difference between the control and the 20:80 blanched mushroom: millet porridge blend in terms of Aroma (p value = 0.058), consistency (p value = 0.074) and flavor (p value = 0.352). The control also had no significant difference with the 40:60 blanched mushroom: millet porridge blend in terms of flavor (p value = 0.295).

There was a significant difference in the scores between the 20:80 unblanched mushroom: millet porridge blends and the 20:80 blanched mushroom: millet porridge blend in terms of taste (p value = 0.042). Both the unblanched and blanched 20:80 mushroom: millet porridge blend mean scores differed significantly ($p < 0.05$) with the 60:40 unblanched mushroom: millet porridge blend mean score in all the sensory attributes including general acceptability of the porridge. The scores of the 20:80 blanched mushroom: millet porridge blend also differed significantly with the scores of the 40:60 unblanched mushroom: millet porridge blend in terms of taste (p value = 0.001) and flavor (p value = 0.015). There was no significant difference in the mean scores between the 40:60 unblanched mushroom: millet porridge blends and the 40:60 blanched mushroom: millet porridge blends in terms of color (p value = 0.838), Aroma (p value = 0.155), Consistency (p value = 0.608) and general acceptability (p value = 0.192).

A significant difference was found in the mean scores between the 40:60 unblanched mushroom: millet porridge blend and the blanched 60:40 blanched mushroom: millet porridge blends in terms of taste (p value = 0.016). The mean scores for consistency differed significantly ($p < 0.05$) among the 40:60 unblanched mushroom: millet porridge and the 60:40 unblanched mushroom: millet blend. The 60:40 unblanched mushroom: millet porridge blend differed significantly ($p < 0.05$) with the 40:60 blanched mushroom: millet porridge blend in all the sensory attributes apart from color. There was no significant difference in the scores between the unblanched and blanched 60:40 mushroom: millet porridge blends in terms of Color (p value = 0.308) and Aroma (p value = 0.124) only.

Table 4.7: Mean scores for sensory evaluation of porridge with mushroom samples by PLWHAs

Attribute / Sample	Color	Taste	Aroma	Consistency	Flavor	General acceptability
Control	4.93±0.25[a]	4.57±0.50[a]	4.57±0.68[a]	4.67±0.71[a]	4.40±0.97[a]	4.67±0.48[a]
Unblanched 20:80	4.77±0.63[ac]	3.80±1.16[bc]	3.73±1.11[b]	4.03±1.10[b]	3.70±1.21[bc]	3.63±1.03[b]
Blanched 20:80	4.87±0.35[ac]	4.33±0.55[a]	4.03±0.77[ab]	4.20±0.76[ab]	4.13±0.78[ab]	3.97±0.62[b]
Unblanched 40:60	4.63±0.49[abc]	3.43±1.31[cd]	3.50±1.25[bc]	4.00±1.05[b]	3.43±1.41[cd]	3.47±1.17[bc]
Blanched 40:60	4.67±0.66[abc]	4.17±0.83[ab]	3.90±0.61[b]	4.13±0.71[b]	4.10±0.71[ab]	3.80±0.81[b]
Unblanched 60:40	4.40±1.04[b]	2.97±1.54[d]	3.13±1.53[c]	3.43±1.43[c]	3.00 ±1.53[d]	2.97±1.47[c]
Blanched 60:40	4.57±0.68[bc]	4.07±0.69[ab]	3.57±0.90[bc]	4.00±0.98[b]	3.73±0.83[bc]	3.53±0.86[b]
Anova p-value	0.025	<0.001	<0.001	0.001	<0.001	<0.001

NB:

1. Values are means ± the standard deviation of all measurements (N=30)
2. Superscripts with different letters in the same column indicate significantly different values at $p \leq 0.05$
3. The lower the mean score the poorer the sensory rating (less acceptability) of the attributes

CHAPTER FIVE: DISCUSSION

5.1 Antioxidant Nutrient content of fresh oyster mushrooms

Oyster mushrooms have been reported to contain significant amounts of antioxidants such as phenolic compounds, Vitamin A, C and E among others (Chirinang & Intarapichet, 2009; Igile et al., 2020; Kortei et al., 2017).

Beta-carotene is a highly pigmented vitamin that is mostly found in fruits, grains, and vegetables. It is a major antioxidant that balances and counteracts the negative effects of free radicals (Kumari & Achal, 2008; Muthangya et al., 2014). The content of β-carotene in fresh oyster mushrooms observed in the current study was within the range of 3.1 - 36.3mg/100g dw reported in other studies (Jayakumar et al., 2009; Kumari & Achal, 2008; Muthangya et al., 2014) and are much higher than those (0.015 ± 0.001 mg/100 mg) reported by Ali et al., (2018). In addition, Jonathan et al., (2012) reported that they found no trace of β-carotene in oyster mushrooms cultivated on different agricultural wastes.

Vitamin E has been reported to protect cell membranes from reactive oxygen species and enhance immune response to diseases (Kumari & Achal, 2008). Igile et al., (2020) reported a content of vitamin E of 24.61 ± 0.60 mg/100g *dw* of in oyster mushrooms grown on rubber wood sawdust in Calabar, Nigeria whereas Jayakumar et al., (2009) reported 30.3 ± 0.08mg/100g in ethanolic extracts of oyster mushrooms both of which are in the same range of the value (23.23 ± 2.12 mg/100g *dw*) observed in the current study. A study by Reis et al., (2012) reported much lower (3.72 ±0.29 μg/100 g *fw*) amounts of vitamin E compared to the values obtained in the current study. On the other hand, Kumari & Achal,

(2008) reported much higher quantities (7.23 mg/g ± 0.111 mg/g fresh fruit body) in oyster mushrooms grown in wheat straw as a substrate.

Vitamin C acts as a free radical scavenger and it acts as the first line natural antioxidants. It is also reported to directly interact with radicals in the plasma therefore preventing damage to the red blood cell membranes (Muthangya et al., 2014). Oyster mushrooms have been reported to be good sources of Vitamin C. The content of vitamin C (37.25 ± 0.32mg/100g *dw*) in fresh oyster mushrooms observed in the current study were much higher than those reported by Obinna-Echem & Chukunda, (2018) 1.3-1.8mg/100g dw; Jonathan et al., (2012) 3.27-3.65mg/100g; Muthangya et al., (2014) 5.07-5.29mg/100g dw and Ali et al., (2018) 6.81mg/100g dw who studied Vitamin C concentration in oyster mushrooms grown on different substrates. The vitamin C content observed in the current study is within the range of values (27.88±0.05mg/100g, 25mg/100g and 36.3 mg/100g ± 0.0025 mg/g *fw*) reported by Igile et al., (2020) , Jayakumar et al., (2009) and Kumari & Achal, (2008) respectively.

Phenolic compounds are the major antioxidants in mushrooms (Chirinang & Intarapichet, 2009b; Kortei et al., 2017). The TPC value (36.48 ± 1.50mg/100g *dw*) obtained in the current study for fresh mushrooms was higher than the values (13.71 and 23.05mg/100g *dw*) reported by Sardar et al., (2017) on TPC in oyster mushrooms grown on sawdust and cotton substrates respectively but much lower than the values (4.27mg/g; 350.82-830.97mg/g; 2.21mg/g *dw*) reported by other studies (Dubost et al., 2007; Mohd Rashidi & Yang, 2016; Muthangya et al., 2014) respectively. In addition, a study by Srikram and Supapvanich, (2016) reported TPC values in the ranges of 9.78 – 567.65mg/100g *dw* in wild and cultivated mushrooms from North East Thailand.

Variances in the nutrient antioxidant concentrations findings from different studies has been attributed to differences in strains, cultivation substrates, location and nutrient supplements added during cultivation and analytical methods (Hoa et al., 2015)

5.1.1 Effect of Blanching on Nutrient antioxidants content in Oyster Mushrooms

Blanching is important before the processing of fruits and vegetables in various ways such as drying, freezing canning etc. (Wang et al., 2017). Blanching reduces microbial loads; de-actives enzymes and changes cell structure hence protecting the color, flavor and texture of a fruit or vegetable. In this study, all nutrient antioxidants were highly retained after blanching with Vitamin C being highest retained at 99.54% and Vitamin E being least retained at 70.3%. This can be explained by the fact that the nutrients did not come into direct contact with the boiling water and therefore prevented loss of solids which protects the nutrients (Guiamba & Svanberg, 2016).

There was no significant difference (p value = 0.460) in the Vitamin C content between the fresh and blanched mushrooms. Guiamba & Svanberg, (2016) found 100% retention of Vitamin C in vacuum packed (in closed plastic bags) High Temperatures Short Time (HTST) blanched mango puree samples. Munyaka et al. (2010) reported that blanching of broccoli inactivated enzymes and prevented the oxidation of ascorbic acid (vitamin C) to dehydro-ascorbic acid and resulted in high retention values of the vitamin. In addition, Liu et al., (2019) writes that non-contact blanching method was able to protect bioactive ingredients such as ascorbic acid in apricots because the samples were not blanched directly in hot water.

However, there were significant reduction in Vitamins E (p value = 0.030) and β-carotene (p value = <0.001) content when comparisons were made between unblanched and blanched mushrooms. These reductions may be attributed to thermal destruction since the samples were blanched in vacuum packed bags. These findings are in agreement with those of Sunmonu et al., (2021) who reported significant differences between fresh and blanched (steam and water blanching) tomatoes and pumpkin leaves in regard to their Vitamin A and E contents. The afore-mentioned authors attributed these reductions to isomerization, thermal destruction and oxidations of these vitamins. Eze et al. (2014) also reported a significant loss of Vitamin A and Vitamin E in African star apple after blanching and attributes this to oxidation and thermal destruction.

In the current study, significant differences (p value = 0.014) were also observed in the Total Phenolic Compounds (TPC) of fresh and blanched oyster mushrooms. There are contradictory reports on the effect of blanching on TPC. Bamidele et al., (2017) reported a significant increase in the TPC content of selected green leafy vegetables after blanching for 5 minutes. Dewanto et al., (2002) also report that blanching could increase the TPC content in vegetables. Similarly, Turkmen et al., (2005) found a significant increase in TPC of different vegetables after different cooking methods. The increase in TPC after cooking or blanching has been attributed to the release of bound phenolic acids and antinutrients hence increasing bio accessibility of TPCs and the reduction of enzyme mediated degradation (Bamidele et al., 2017). However, Bamidele et al., (2017) further reported that longer blanching times of green leafy vegetables significantly reduce the amount of TPC. Martínez et al., (2013) also reported a decrease in TPC in blanched turnip greens. Losses of TPC during blanching can be credited to thermal degradation of the phenolic compounds and oxidation (Bamidele et al., 2017; Irondi et al., 2017) and reduced extractability of the phenolic compounds due to formation of complexes with other nutrients (Awika et al., 2003).

5.2 Effect of solar drying on Retention of antioxidants content in Oyster Mushrooms

Oyster mushrooms have been reported to have a very short shelf life compared to other species of cultivated mushrooms because of their high moisture content, delicate nature, and proneness to bacterial and fungal contamination (Marshall & Nair, 2009). Due to this, drying is recommended to prolong their shelf life. In the current study the effect of blanching on the retention of antioxidant nutrients during drying was investigated. Blanching was also conducted to inactivate enzymes responsible for poor organoleptic qualities after drying. The results of the current study indicated that solar drying of mushrooms resulted in a significant ($p < 0.05$) decrease of the content of the various antioxidant nutrients examined in both fresh dried and blanched dried oyster mushrooms.

In this study, samples of fresh and blanched mushrooms were solar-dried. Other studies reported that blanching and pretreatments such as radiation with gamma rays (Kortei et al., 2017), chemical pretreatments with vinegar, lemon juice or other chemicals (Mutukwa et al., 2019; Raut, 2011) before drying will either increase or decrease nutrient antioxidant concentrations of especially Vitamin C and Total Phenolic Compounds (TPC) in solar dried oyster mushrooms. Different drying techniques also yield different concentrations of nutrient antioxidants in the mushrooms (Mishra et al., 2016). It is therefore important to consider such factors when choosing a drying and preservation method before drying mushrooms.

5.2.1 Effect of solar drying on retention of vitamin E in Oyster Mushrooms This study found that solar drying destroyed Vitamin E content in both unblanched and blanched mushrooms with 4.1% and 58.63% retention rates, respectively. These findings contradict with those of Seybold et al., (2004) who reported a substantial increase of between 51-73% of Vitamin E in tomatoes after a short term heating process. Similarly, Brown et al., (2020) also reported a 20% increase in Vitamin E concentration in meals re-heated up to 74ºC using microwaves for 8 minutes and conventional ovens for 45 minutes. Gliszczyńska-Świgło et al., (2006) also reported that Vitamin E concentration increased up to 1.7-fold after steaming and cooking broccoli in water. This increases have been attributed to better availability of the nutrient for extraction (Gliszczyńska-Świgło et al., 2006). This study's findings however agree with those of Bramley et al., (2000) who reported losses of between 15-40% of Vitamin E in the processing of oils that includes preheating and cooking of seeds and vegetables and in oils that have been used for deep frying foods In another study, Lokuruka (2011) reported a 30-40% loss of vitamin E during processing of soybeans into Tofu while Abushita et al., (2000) reported a 20-40% loss of vitamin E in the thermal processing of tomato paste. The significant loss of Vitamin E in this study could be associated to destruction of the Vitamin by oxygen and the presence of light and heat (Lokuruka, 2011; Okonkwo et al., 2011). The Vitamin E content in blanched dried mushrooms was significantly lower (p value = 0.006) than in the fresh mushrooms, this composition was however significantly higher (p value = 0.002) than that in the unblanched solar-dried mushrooms. The higher retention of Vitamin E in blanched dried mushrooms as compared to unblanched dried mushrooms is because of the inactivation of oxidative enzymes which decreases destruction during the drying of the mushrooms (Xiao et al., 2017). Similar to this study's findings Ramesh et al., (2001) found that blanching before drying increased the retention rate of Vitamin E in the drying of spice paprika. In addition, Korus, (2022) found that the Vitamin E composition in blanched dried kale is higher than that in unblanched kales.

5.2.2 Effect of solar drying on retention of β-carotene in Oyster mushrooms In the current study, β-carotene concentration decreased by 91.2% in the solar dried mushrooms compared with the fresh sample. Sablani (2006) reported that nutrient loss of as high as 80% can occur when vegetables are processed without inactivating the enzymes. Other studies have also stated that beta-carotene has very low retention rate after drying with nutrient losses from as low as 18% to as high as 86% in dried fruits and vegetables (Hiranvarachat et al., 2011, 2012; Zielinska & Markowski, 2012). Drying temperatures, pretreatments and drying time have been reported to have a significant effect on the retention of beta-carotene during drying of vegetables. Zielinska & Markowski (2012) concluded that beta-carotene retention decreased as temperature was increased while Hiranvarachat et al., (2012) stated that retention of the beta-carotene depended more on the pretreatment's pH rather than heat. Bechoff et al., (2009) reported that the drying technique had an effect on retention of beta-carotene where air-drying methods seemed to give better results than any other drying method. Solar drying, which is the method that was used in this study, could have led to the low retention rates due to the longer drying times and exposure to light associated with the method leading to oxidation of β-carotene which is relatively unstable in light (Mbondo et al., 2018).

Blanching can reduce carotenoid losses to less than 5% during drying (Sablani, (2006). In the current study, blanching the mushrooms before solar drying increased the retention of β-carotene rate by 70.6% relative to the retention in mushrooms solar-dried prior to blanching. Blanched solar-dried mushrooms had significantly (p value = <0.001) higher content of β-carotene than mushrooms that were solar drying prior to blanching. Similar to Vitamin E, better retention is due to the inactivation of the oxidative enzymes during blanching that make β-carotene susceptible to heat and light therefore minimizing loss of the carotenoids during drying (Xiao et al., 2017). Similar to this study findings, Gulzar et al., (2018) reported that blanching pretreatments of mango slices resulted in a significant positive effect on the retention of carotenoids during drying while Guiamba et al., (2018) also reported that severe degradation of β-carotene was noted in dried unblanched mango. Contrary to the reports of the aforementioned authors, Nascimento et al., (2009) indicated that blanched and unblanched dried sweet potatoes showed similar retention while blanched cassava showed lower retention of β-carotene than unblanched cassava during drying.

5.2.3 Effect of solar drying on retention of vitamin C in Oyster mushrooms

Vitamin C has been reported to be the least stable vitamin with a relatively low retention rate after drying during processing (Cernî, 2007). This has been attributed to its high sensitivity to high temperatures, irreversible oxidation process and leaching into water (Oliveira et al., 2016). Vitamin C is therefore used as an index to measure the nutrient quality of processes (Bonazzi & Dumoulin, 2011). In this study, solar drying of mushrooms resulted in vitamin C loss of about 86%. This loss of vitamin C is within the range of losses (62-90%) reported by other studies where the effect of solar drying on Vitamin C in fruits and vegetables was investigated (Mrkìc et al., 2006a; Oliveira et al., 2016; Shitanda & Wanjala, 2006). There seems to be no consensus in the relationship between drying temperatures and vitamin C losses as some studies have reported higher losses with higher temperatures (Pendre et al., 2012; Scala et al., 2011) while others have reported contradicting reports (Mrkìc et al., 2006). In comparison to covered drying, open sun-drying is reported to cause more losses of Vitamin C (Kandonga, 2019). Similarly, Oliveira et al., (2016) also reported that solar drying, the method used in this study, to be a more unfavorable drying option as compared to other drying technologies. Higher power levels and shorter drying times that can be achieved using methods such as microwave drying are recommended to ensure maximum Vitamin C retention.

Vitamin C is highly unstable in high temperatures susceptible to blanch-related leaching (Doseděl et al., 2021). In this study mushroom samples were first vacuum packed in sealed plastic bags before being blanched in hot water for a short time. This blanching method has been proven to increase the retention of Vitamin C by preventing leaching (Guiamba et al., 2018; Munyaka et al., 2010; Nyangena et al., 2019). In the current study, blanching mushrooms prior to drying resulted in a higher retention rate (79.06%) for vitamin C than that (16.11%) in mushrooms solar-dried prior to blanching. Guiamba et al., 2018 reported that apart from the loss that happens during blanching, there was no further degradation of vitamin C during the drying process of mangoes after they were blanched in closed plastic bags. Thermal degradation of Vitamin C during drying is associated with breakdown of the matrix structure caused by oxidation of vitamin C (Guiamba et al., 2018), vacuum blanching reduces leaching and oxidation of enzymes hence improving the retention of the vitamin during drying.

5.2.4 Effect of solar drying on retention of TPC in Oyster mushrooms

In regard to TPC retention, a loss of 41% due to thermal degradation was found in the current study. Other studies have reported that drying can either significantly increase or decrease TPC in fruits and vegetables (Hamrouni-Sellami et al., 2012). Bennett et al. (2011) reported that the amount of phenolic content in fruits and vegetables decreased during drying because of their susceptibility to oxidative degradation during drying. (Mbondo et al., 2018; Mongi et al., 2015) also reported that all drying methods including solar-drying resulted in a sharp decline in TPC of dried vegetables. Gąsecka et al., (2019) reported a 17% and 40% decrease in TPC in two different strains of mushrooms dried at 70ºC. Similarly, Liaotrakoon and Liaotrakoon (2018) report up to 25% loss in TPC in edible mushrooms during drying at high temperatures. This decline is also attributed to the degrading of phenols during drying and changes in the structure of the phenolic compounds (Gąsecka et al., 2019; R. J. Mongi, 2013). On the other hand, Jaworska et al., (2014) reported a 7-17% increase in TPC in mushrooms air-dried at a maximum temperature of 60ºC for up to 15 hours. Similarly, a study by Sim et al., (2017) also showed a notable increase ($p<0.05$) phenolic content in mushroom mycelia after vacuum drying. Oven drying reportedly had negative but non-significant ($p>0.05$) effects of TPC in mushrooms in the above-mentioned study. Significant differences in TPC were also reported in microwave-oven dried flowers and sun dried flowers with the latter providing lower retention rates (Hong-fang Ji, 2012). Choosing a suitable drying method has been stated to be vital in order to maintain phenolic content of vegetables during drying (Sim et al., 2017). Blanching the mushrooms prior to drying increased the retention rate of TPC to 71.60%. These findings are in agreement with those of Korus, (2011) who reported that losses in

TPC of blanched kales during drying were lower than those in non-blanched kale leaves. The increase in retention of the phenolic compounds can be attributed to inactivation of oxidative enzymes and the structural changes in the cell matrices that enhance release of extractable and non-extractable phenolic compounds (Kessy et al., 2016). Contradictory findings are reported by Kirakou et al., (2017), who indicated that solar drying without blanching recorded the highest retention of TPC in cowpea leaves while water blanching followed by drying recorded lower retention rates of cowpea leaves. Similarly, Akter et al., (2010) reported higher TPC in unblanched dried persimmons peels than in blanched peels. The lower amounts of TPC in blanched samples has been attributed to changes in the phenolic composition and contents that might occur during blanching such as leaching (Irondi et al., 2017).

5.3 Antioxidant Nutrient Concentration in Oyster Mushroom powder-- Millet Flour blend

Enrichment of cereal-based foods with mushroom powder to enhance nutritional quality without significantly affecting the sensory attributes of the food has been recommended by Aishah and Wan Ishak (2013). In the current study, enrichment of millet flour with mushroom powder increased the content of β-carotene, Vitamin C and TPC. The current study findings were in agreement with those of Tersoo-Abiem, et al. (2019), who reported a significant increase in protein, ash, mineral and vitamin content in *ibyer* (a traditional porridge made using millet flour in Nigeria) when it was enriched with mushroom powder. Similar results were reported by other authors (Aishah & Wan Ishak, 2013; Arora et al., 2018; Farzana et al., 2017; Mohammad, 2012). Ondiek et al., (2019) also reported a significant ($p \leq 0.05$) increase in the proteins, Iron, Zinc, Thiamine, Riboflavin, Niacin, and folate composition of maize porridge enriched with mushroom powder.

In this study, the β-carotene content of the porridge made from 20:80 unblanched mushroom powder: millet flour blend was significantly lower ($p \geq 0.05$) than that in the control. The findings however showed that the β-carotene content in the 20:80 blanched mushroom powder: millet flour blend was significantly higher (p value = <0.001) than that in the control. This can be attributed to the increased retention of β-carotene in dried blanched oyster mushrooms making vitamins more bioavailable. The β-carotene content was observed to increase and became significantly ($p \leq 0.05$) higher in the flour made from the 40:60 and 60:40 unblanched and blanched mushroom powder: millet flour blends. Similar findings on vitamin A increase in *ibyer* were reported by Tersoo-Abiem, et al. (2019). However, in the current study, the β-carotene concentration was observed to be lower in the porridge made from the 60:40 mushroom powder: millet flour blend formulation than in the 40:60 mushroom powder: millet flour blend with both blanched and unblanched dried mushroom powders. This observation is contrary to reports from other study findings where the β-carotene concentration was observed to increase with higher mushroom proportions in the food.

Similar findings to the current study on significant increase ($p \leq 0.05$) on vitamin C in mushroom enriched foods have been reported by Tersoo-Abiem, et al., (2019). The Vitamin C concentration of porridge seemed to increase with increase in the proportion of mushroom powder blended with millet flour. The concentrations were higher in the blends with blanched dried mushroom powder than in the blends with the unblanched mushroom powder. This can be attributed by the fact that retention rate of Vitamin C in the blanched mushrooms was higher than in the unblanched mushrooms making it more available during bio-fortification. In this study, vitamin E concentration in the porridge made from mushroom powder-millet flour blends was lower than that in the control flour. The concentration of vitamin E seemed to decrease as the amount of mushroom powder blended with millet flour increased. However, the findings show that there was no substantive difference ($p \geq 0.05$) between the control millet flour and the 20:80 unblanched mushroom: millet flour blend. There was a significant difference between the control flour and the 40:60 and 60:40 unblanched mushroom: millet flour blends. Blanched dried mushroom: millet porridge blends had significantly higher ($p < 0.05$) concentrations of Vitamin E than the control. Similar to the findings with the unblanched mushroom, the concentration of Vitamin E reduced as the amount of mushroom powder was increased in the blend. There are limited studies carried out on the effect of mushroom enrichment on Vitamin E concentration of food, the reduction in concentration of Vitamin E may be due to formation of food matrices that decrease the extractability and bioavailability of the vitamin (Borel et al., 2013).

In the current study, it was observed that blending millet flour with different proportions of either blanched or unblanched mushroom powder resulted in an increase in the TPC. Several studies that investigated the effect of mushroom powder on TPC of foods have reported results similar to this study. Arora et al. (2018) reported an increase in TPC in noodles once they were enriched with oyster mushroom powder. The above-mentioned author stated that the TPC content in the noodles increased with higher amounts of mushroom powder in the noodles. Similar findings were reported by several other authors, such as Arora et al., (2017) in mushroom enriched cakes and Vlaic et al., (2019) in mushroom enriched bread and Farzana et al. (2017) in mushroom enriched muffins. Chen et al. (2021) reported a non-significant increase in TPC of mushroom fortified cookies. In this study, the blanched mushroom powder: millet flour blends had higher concentrations of Total Phenolic Compounds than the unblanched mushroom powder: millet flour blends.

5.4 Sensory Acceptability of Oyster mushroom enriched millet porridge among PLWHAs

In the current study, the sensory mean scores of porridge were inversely related to the proportion of mushroom powder present in the porridge formulation. Therefore, the porridge made from the 60:40 mushroom powder: millet flour blend received the lowest sensory scores in all sensory attributes. This study's findings are in agreement with those reported by Ondiek, (2018) who reported that aroma and taste scores were inversely related to the amount of mushroom powder in a mushroom/maize porridge formulation. The color of a food product is an important influence in the choice of the consumer in purchasing or eating the food. According to Barrett et al., (2010) the "color appearance of any food products is among the first attributes encountered." In this study, the control porridge received the highest score ratings for color, followed by the 20:80 blanched mushroom: millet porridge blend. In terms of color, blanched samples received higher scores than the unblanched samples. Similarly, Ndife et al., (2019) also found blanched eggplants to have higher appearance scores than unblanched ones. However, there was no significant difference in the color scores given to the control and all the other porridge blends apart from the 60:40 unblanched mushroom: millet porridge blend.

Most panelists felt that the taste and the aroma of the porridge made from the 60:40 mushroom powder: millet flour blend were too strong for their liking. Similar findings were reported by Ondiek, (2018) who reported that panelists reported a 'very strong' taste and aroma in a maize: mushroom porridge blend with 30% mushroom powder. This dislike was worse in those who said that they had never eaten mushrooms before. Prescott, (2012) and Prescott & Murphy, (2009) reported that aroma can enhance or suppress the taste of a food product. Similarly, Gibson and Newsham (2018) noted that aroma is the biggest factor in the flavor of foods and drinks. Stevenson and Tomiczek, (2007) also reported that taste can affect the aroma of a food. Taste and aroma highly influence the flavor sensations of foods (Gibson & Newsham, 2018; Regueiro et al., 2017; Yin et al., 2017). In the current study, blanching mushrooms prior to solar drying increased the sensory scores of taste, aroma and flavor. Similarly Elfnesh & Kidane, (2011) found that sensory scores for flavor were higher in chips made from blanched potato slices than those from unblanched potato slices. In the current study, no notable difference was observed in the taste scores between the control and all the porridge blends with blanched mushrooms. Similarly, there was no significant difference between the flavor scores of control porridge and that made from the 20:80 and 40:60 blanched mushroom: millet flour blends.

Similarly, enrichment with mushroom powder reduced the sensory scores for consistency. Overall, high scores were rated for the porridge made from 20:80 mushroom powder: millet flour blend and 40:60 mushroom powder: millet flour blend. The porridge made from the 60:40 mushroom powder: millet flour blend received the lowest score on consistency. Information gathered from the remarks section of the tool showed sentiments from panelists indicating that mushroom powder thickened the porridge with the 60:40 mushroom powder: millet flour porridge being “too heavy that it was difficult to flow in a cup.” Just like the other sensory attributes blanching increased the scores given for consistency in the mushroom: millet porridge blends. However, there was no notable difference between the consistency scores of the blanched and unblanched 20:80

mushroom: millet porridge blends and also between the blanched and unblanched 40:60 mushroom: millet porridge blends in terms of consistency. There was however a notable difference between the consistency porridge made from the 60:40 blanched mushroom: millet flour and that made from and the 60:40 unblanched mushroom: millet flour blend.

Overall, the highest score for general acceptability was given to the control (Millet flour porridge). For the composite flours, the porridge made from the 20:80 blanched mushroom powder: millet flour blend received the highest score of 3.97 ± 0.62 (neither like nor dislike) while the 60:40 mushroom powder: millet flour blend received the lowest score of 2.97 ± 1.474 (slightly dislike). Taste, aroma and flavor were observed to have an influence on the general acceptability while color and consistency did not, a finding in agreement with the report of Ondiek, (2018). Aroma and taste are reported to be key determinants in the sensory effects of food choice and intake (Boesveldt & de Graaf, 2017; Gibson & Newsham, 2018). Similarly, Regueiro, Negreira, and Simal-Gándara (2017) reported that food flavor is among the main determinants of food choice and in most instances it supersedes other influencers of food choice.

CHAPTER SIX: SUMMARY OF FINDIGS, CONCLUSION AND RECOMMENDATIONS

6.1 Summary of the findings

The highest antioxidant concentration in fresh oyster mushrooms was TPC (36.48 ± 1.50 mg/100g dw) followed by Vitamin C (37.25 ± 0.32mg/100g dw), Vitamin E (23.23 ± 2.12mg/100g dw) then β-carotene (13.73± 0.02 mg/100g dw). Vacuum blanching the mushrooms significantly reduced the contents of β-carotene (p value = <0.001), Vitamin E (p value = 0.030) and Total Phenolic Substances (p value = 0.014). Vitamin C had the highest retention after vacuum blanching at 99.54% while Vitamin E had the least at 70.34%.

In unblanched solar dried oyster mushrooms TPC (21.55 ± 0.24 mg/100g dw) was the highest in concentration followed by Vitamin C (5.60 ± 0.12 mg/100g dw) with Vitamin E (0.96 ± 0.18 mg/100g dw) being the lowest. These concentrations were significantly lower ($p < 0.05$) than those of fresh oyster mushrooms. Similarly, antioxidant nutrient concentrations in blanched solar dried oyster mushrooms was significantly lower ($p < 0.05$) than that of fresh mushrooms, however vacuum blanching the oyster mushrooms prior to drying increased the retention rate of nutrient antioxidants in the mushrooms. In addition, vacuum blanched solar dried oyster mushrooms had significantly higher ($p < 0.05$) nutrient antioxidant concentrations than unblanched solar dried oyster mushrooms.

The content of both Vitamin C and TPC were higher in the blanched and unblanched solar dried mushrooms than in millet flour. Both these nutrients increased in concentration in millet flour once mushroom powder was added. The concentrations increased as mushroom powder was increased. βetacarotene concentration was notably lower in unblanched solar dried mushroom and in the 20:80 unblanched mushroom: millet blend than in millet flour. However, in blanched solar dried mushroom and the 20:80 blanched mushroom: millet blend, the β-carotene concentration was notably higher than that of millet flour. In both blanched and unblanched mushroom: millet blends, the β-carotene concentration in the 40:60 blends was higher than that in the 60:40 blends. Vitamin E concentration in millet flour was higher than in unblanched mushroom powder but not significantly. In blanched solar dried mushroom powder, Vitamin E was significantly higher than that in millet flour. Addition of mushroom powder to millet flour reduced the Vitamin E concentration in the blend and this decreased as the amounts of mushroom powder in the blend increased.

The mushroom enriched millet porridges were averagely rated with the porridge containing the highest amount of mushrooms receiving the lowest scores in all sensory attributes. Taste and aroma for the porridge made from the 60:40 unblanched mushroom powder: millet flour blend was not liked by majority of the participants. Overall, the porridge made from the blanched and unblanched 20:80 and 40:60 mushroom powder: millet flour blend seemed to receive better sensory rating with majority of the panelists stating that they slightly liked most of the sensory attributes. There were notable differences in all the sensory scores between the control porridge and the blended porridges apart from color. Prior blanching of the mushrooms before solar drying improved the sensory score ratings given to the porridge blends.

6.2 Conclusions

Fresh mushrooms are rich in nutrient antioxidants. However, blanching and solar drying significantly reduces the concentration of antioxidant nutrients in mushrooms. This can be improved by vacuum blanching the mushrooms before solar drying as it improves the retention of the nutrient antioxidants during solar drying. Enriching millet flour with solar dried mushroom powder increased the concentrations of majority of the tested antioxidants. Mushroom enriched fermented millet porridge is acceptable among PLWHAs. Enrichment therefore can go a long way in formulations of nutrient dense foods targeting specific categories of people such as PLWHAs using locally available but underutilized foods.

6.2.1 Conclusions on HypothesisThere was a significant difference in all the nutrient antioxidants (β-carotene, Vitamin E, Vitamin C and TPC) between fresh and solar dried oyster mushrooms. Therefore, the **first null hypothesis for the study was rejected.**

There was significant difference in the β-carotene, Vitamin C and TPC concentrations between millet flour and all formulations of mushroom millet flour. With only Vitamin E having no significant difference, **the second null hypothesis of the study was rejected.**

All the porridges made from different mushroom powder: millet flour ratios were rated on average scores in terms of general acceptability. A significant difference in the mean sensory scores between the control porridge and the composite porridges was observed. **Therefore, the third null hypothesis for this study was rejected.**

Generally, the 40:60 blanched mushrooms: millet formulation was the better formulation based on nutrient concentrations and general acceptability. Mushrooms and millets are both underutilized food products that are locally available in the country. They both have nutritional properties that can be very beneficial to the community in terms of improving the nutritional status, fighting malnutrition, and improving the immune status of PLWHAs and other vulnerable populations.

6.3 Recommendations

6.3.1 Recommendations for practice

- Blanching of mushrooms before solar drying should be taught and practiced by both small and large scale farmers to help in preservation of the antioxidant nutrients.

- Mushrooms are rich in nutrient antioxidants, blending mushroom powder with other food products to produce nutrient dense foods for different population groups should be encouraged and practiced

- Nutritionists across the country should promote consumption of mushrooms as part of their nutritional counseling among PLWHAs

6.3.2 Recommendations for policy

- Ministry of Agriculture and partners should come up with policies and incentives on farming and post-harvest handling of mushrooms due to the potential of mushrooms in enhancing diets, promoting health and income generation.

6.3.3 Recommendations for further research

- More studies should be done to determine other nutrients and antioxidants s that were not included in the present study.

- Research should be done to establish the concentration of the antioxidants in the cooked porridges and the anti-nutrient quality of the porridge to determine their bioavailability for absorption and utilization.

- More studies should be done on better ways to preserve mushrooms which will preserve their nutritional qualities and still be affordable and accessible to even the small-scale farmer.
- Intervention studies should be done to determine the effectiveness of using the mushroom-millet porridge in reducing morbidity rates among PLWHAs.

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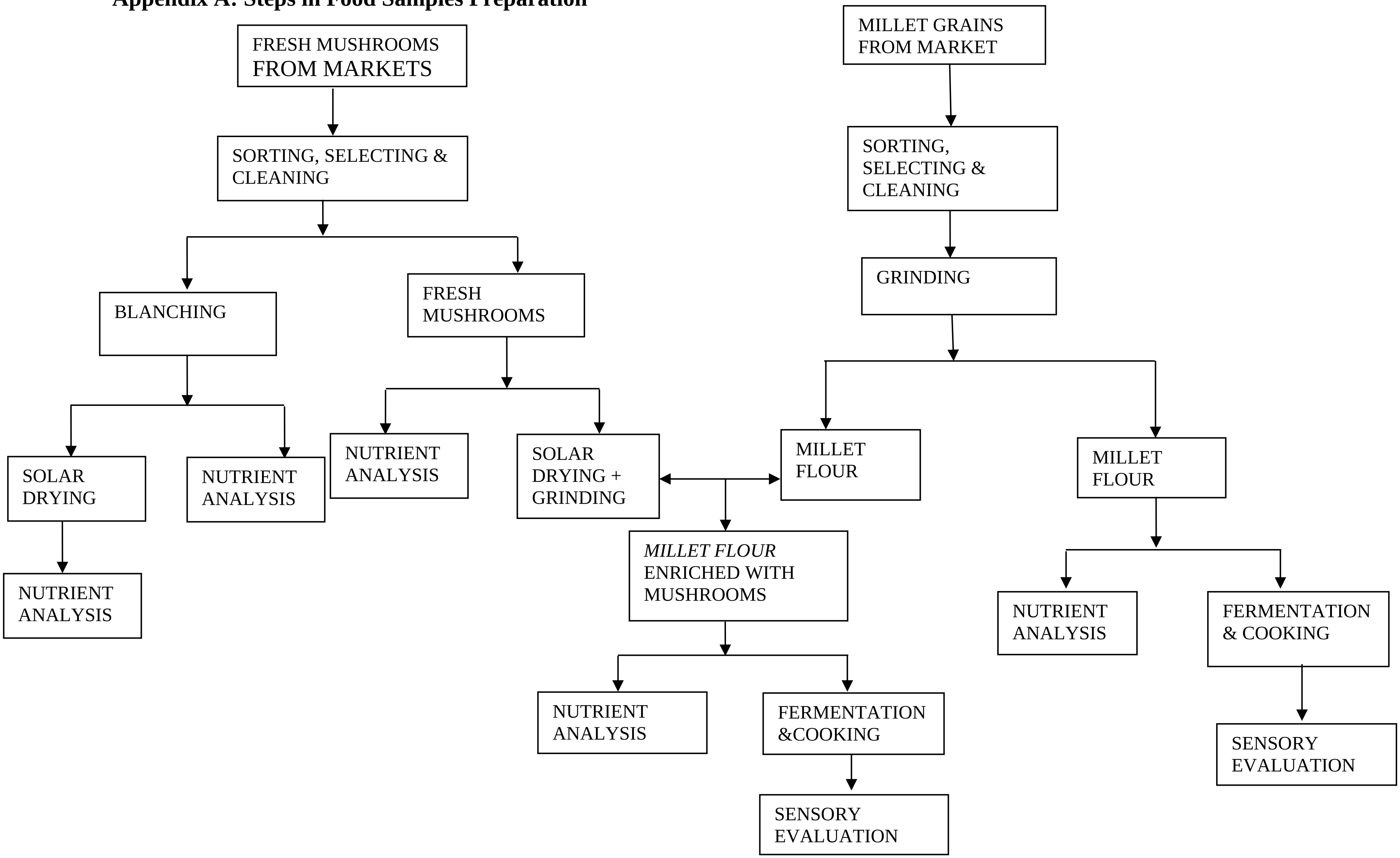
APPENDICES

Appendix A: Steps in Food Samples Preparation



Appendix B: Laboratory Data Sheets

Sample No:

Date:

NUTRIENT/ READING	Moisture content	Beta carotene	Vitamin E	Vitamin C	Polyphenols
Reading 1					
Reading 2					
Reading 3					
Mean ± SD					

SIGNATURES

Lab assistant:

Researcher:

Appendix C: Sensory Evaluation Questionnaire

Date............................ Time................ Age………. Sex………..

Instructions

You have been provided with four bowls each with four different types of porridges to carry out sensory evaluation. The four bowls have been coded differently. The goal is to express how much you like or dislike them. You have also been given water to rinse your mouth after tasting each porridge. Using the scale below, kindly express your attitude towards the product color, taste, flavor and general acceptability of each of the samples by inserting the appropriate score in the space provided. You can also give any comments about the products and please try to be as honest as possible. Thank you.

Description Score

Extremely Like 5

Slighlty Like……..4

Neither Like nor Dislike..3

Slighlty Dislike 2

Dislike extremely 1

ATTRIBUTE	**SAMPLES**			
SAMPLE CODES	**000**	**001**	**002**	**003**
Color				
Taste				
Aroma				
Consistency				
Flavor				
General Acceptability				

Remarks

…………………………………………………………………………………………………….

……………………………………………………………………………………………………..

……………………………………………………………………………………………………...

……………………………………………………………………………………………………...

Appendix D: Informed Consent Form

My name is Bosire Miriam Masiko. I am a Masters student at Kenyatta University. I am conducting a study on "Antioxidant Retention in solar dried mushrooms and acceptability of mushroom enriched millet porridge among HIV patients in Nakuru County Referral Hospital, Kenya" The information generated from this study will provide information useful in the nutritional education on importance of mushrooms. The study will also formulate a product that may contribute to food security and income generation from value added products.

Confidentiality

The sensory evaluation of the mushroom enriched millet porridge will be conducted at Nakuru County Referral hospital and any information given will be treated with a lot of confidentiality. The respondents names will not be recorded on the questionnaire.

Contact Information

If you have any questions you may contact Dr. Ann Munyaka on 0712-108087 or Dr. Juliana Kiio on 0725-999448 or the Kenyatta University Ethical Review Committee Secretariat on chairman.kuerc@ku.ac.ke,secretary.kuerc@ku.ac.ke,secretariat.kuerc@ku.ac.ke.

Participant's statement

The above information about my participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. My participation in this study is voluntary. I understand that my records will be kept private and that I can leave the study at any time.

Name of Participant……………………………………………………………………….................. ....

______________________________ ____________________________________

Signature or Thumbprint Date

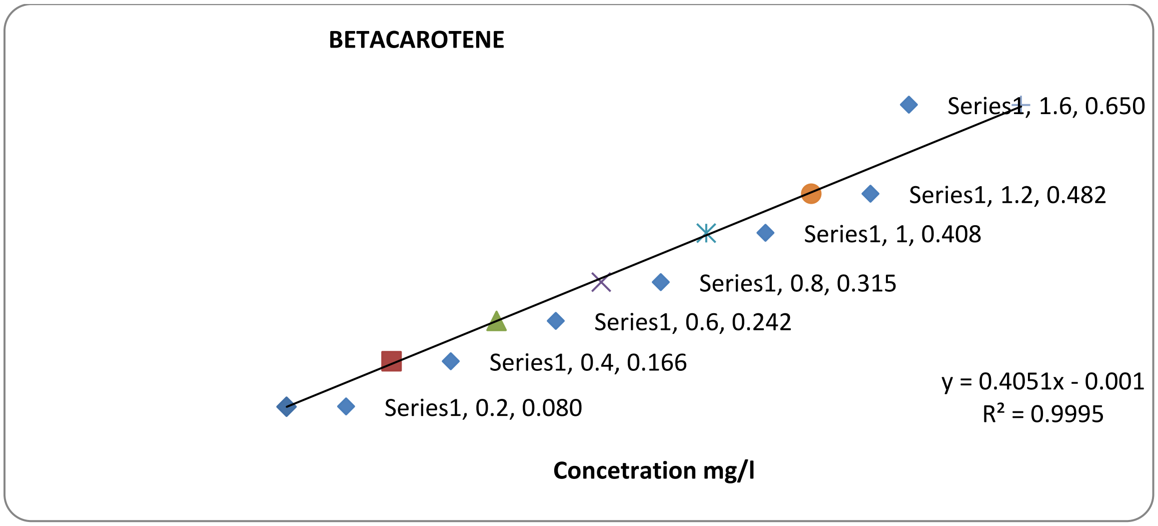
Investigators Statement

I, the undersigned, have explained to the volunteer in a language s/he understands the procedures to be followed in the study and the risks and benefits involved.

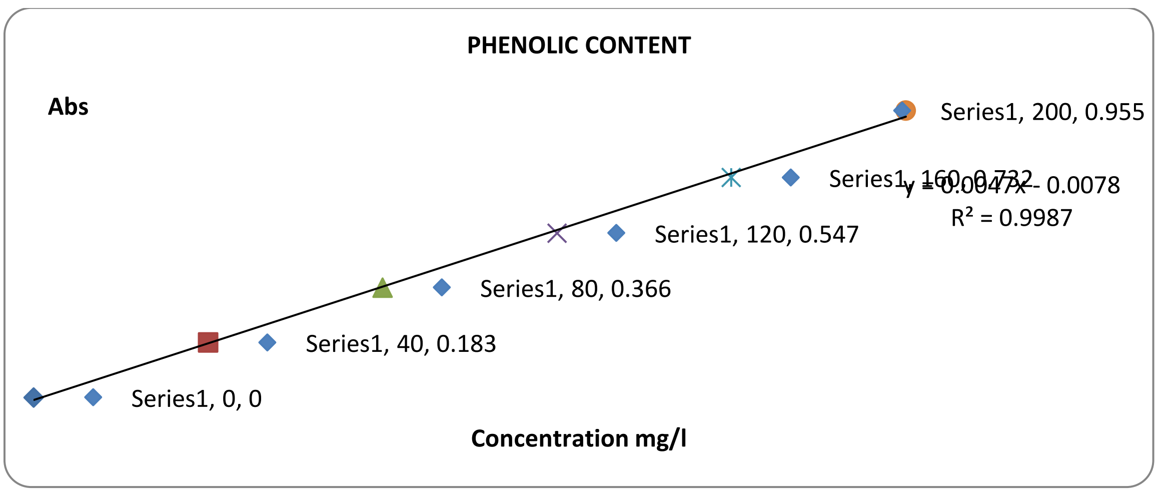
Name of interviewer……………………………………………………………………………………………

Signature or Thumbprint Date

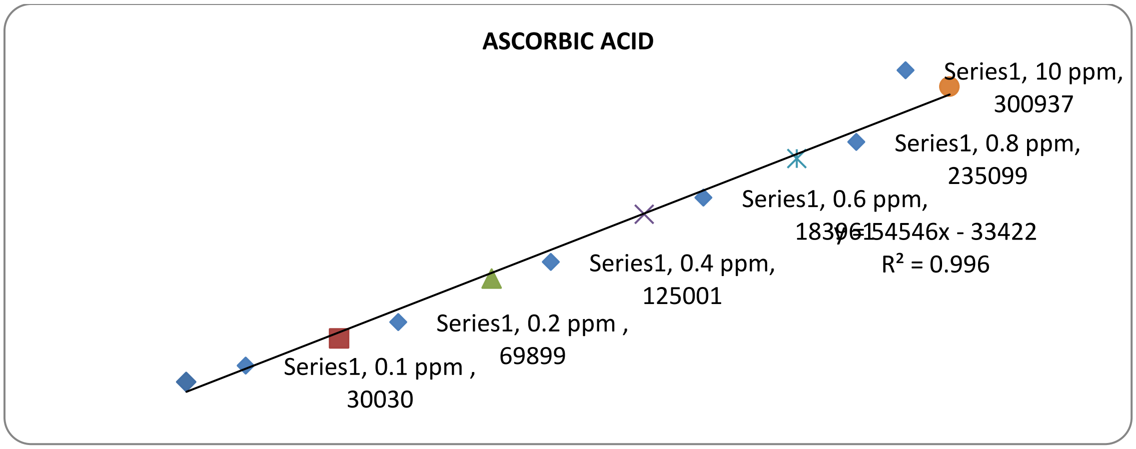
Appendix E: Standard Curves Equations and Correlation Coefficient R^2



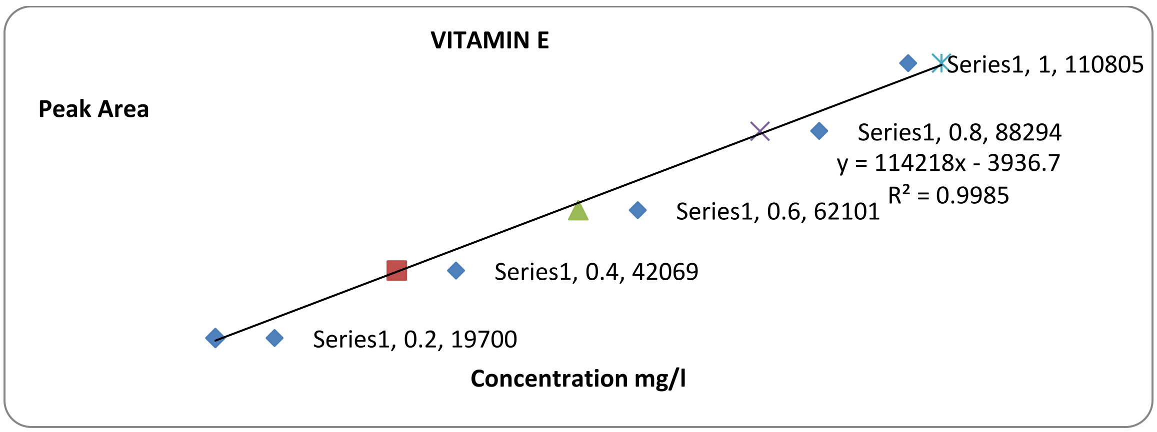
β-carotene standard curve of absorbance against concentration



Phenolic content standard curve of absorbance against concentration



Ascorbic acid standard curve of peak area against concentration



Vitamin E standard curve of peak area against concentration

Appendix F: Statistical Results of Post-hoc (LSD) for Sensory Data Analysis for unblanched and blanched mushroom:millet porridge blends

Tested attribute for sample formulations	**P values for different formulations**						
	0:100	**UNBL 20:80**	**BL 20:80**	**UNBL 40:60**	**BL 40:60**	**UNBL 60:40**	**BL 60:40**
0:100							
Color	-	0.308	0.683	0.067	0.103	0.001	0.026
Taste	-	0.004	0.372	0.000	0.127	>0.001	0.057
Aroma	-	0.003	0.058	0.000	0.018	>0.001	>0.001
Consistency	-	0.016	0.074	0.011	0.041	>0.001	0.011
Flavor	-	0.015	0.352	0.001	0.295	>0.001	0.021
General Acceptability	-	>0.001	0.006	>0.001	0.001	>0.001	>0.001
UNBL 20:80							
Color	0.308	-	0.540	0.414	0.540	0.026	0.221
Taste	0.004	-	0.042	0.161	0.161	0.002	0.308
Aroma	0.003	-	0.286	0.406	0.553	0.033	0.553
Consistency	0.016	-	0.522	0.898	0.701	0.022	0.898
Flavor	0.015	-	0.131	0.352	0.163	0.015	0.907
General Acceptability	>0.001	-	0.192	0.513	0.513	0.009	0.695
BL 20:80							
Color	0.683	0.540	-	0.154	0.221	0.005	0.067
Taste	0.372	0.042	-	0.001	0.524	>0.001	0.308
Aroma	0.058	0.286	-	0.058	0.635	0.002	0.097
Consistency	0.074	0.522	-	0.442	0.798	0.004	0.442

Flavor	0.352	0.131	-	0.015	0.907	>0.001	0.163
General Acceptability	0.006	0.192	-	0.051	0.513	>0.001	0.090
UNBL 40:60							
Color	0.067	0.414	0.154	-	0.838	0.154	0.683
Taste	>0.001	0.161	0.001	-	0.005	0.075	0.016
Aroma	>0.001	0.406	0.058	-	0.155	0.192	0.812
Consistency	0.011	0.898	0.442	-	0.608	0.030	1.00
Flavor	0.001	0.352	0.015	-	0.021	0.131	0.295
General Acceptability	>0.001	0.513	0.051	-	0.192	0.051	0.794
BL 40:60							
Color	0.103	0.540	0.221	0.838	-	0.103	0.540
Taste	0.127	0.161	0.524	0.005	-	>0.001	0.702
Aroma	0.018	0.553	0.635	0.155	-	0.007	0.236
Consistency	0.041	0.701	0.798	0.608	-	0.008	0.608
Flavor	0.295	0.163	0.907	0.021	-	>0.001	0.201
General Acceptability	0.001	0.513	0.513	0.192	-	0.001	0.296
UNBL 60:40							
Color	0.001	0.026	0.005	0.154	0.103	-	0.308
Taste	>0.001	0.002	>0.001	0.075	>0.001	-	>0.001
Aroma	>0.001	0.033	0.002	0.192	0.007	-	0.124
Consistency	>0.001	0.022	0.004	0.030	0.008	-	0.030
Flavor	>0.001	0.015	>0.001	0.131	>0.001	-	0.011
General Acceptability	>0.001	0.009	>0.001	0.051	0.001	-	0.027

BL 60:40							
Color	0.026	0.221	0.067	0.683	0.540	0.308	-
Taste	0.057	0.308	0.308	0.016	0.702	>0.001	-
Aroma	>0.001	0.553	0.097	0.812	0.236	0.124	-
Consistency	0.011	0.898	0.442	1.00	0.608	0.030	-
Flavor	0.021	0.907	0.163	0.295	0.201	0.011	-
General Acceptability	>0.001	0.695	0.090	0.794	0.296	0.027	-

Appendix G: Kenyatta University Ethical Review Committee Permits

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
kuerc.secretary@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844,
Nairobi, 00100
Tel: 8710901/12

Our Ref: **KU/ERC/ APPROVAL /VOL.1/241**

Date: 21st November, 2018

Bosire Miriam Masiko
P.O Box 43844-00100
NAIROBI

Dear Bosire ,

APPLICATION NUMBER: PKU/939/1996 "DETERMINATION OF ANTIOXIDANTS IN FRESH AND SOLAR-DRIED OYSTER MUSHROOMS AND SENSORY ACCEPTABILITY OF MUSHROOM-MILLET PORRIDGE BLEND AMONG ADULTS WITH HIV/AIDS, KENYA

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic "**Determination Of Antioxidants In Fresh And Solar-Dried Oyster Mushrooms And Sensory Acceptability Of Mushroom-Millet Porridge Blend Among Adults With HIV/AIDS, Kenya** received on 1st November, 2018 and discussed on 20th November, 2018

2. APPLICANT

Bosire Miriam Masiko

3. SITE

Kenya

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines and **APPROVED that the research may proceed for a period of ONE year from 20th November , 2018.**

Appendix H: Nacosti Research Permits

THIS IS TO CERTIFY THAT:
MISS. MIRIAM MASIKO BOSIRE
of KENYATTA UNIVERSITY, 16568-20100
NAKURU,has been permitted to conduct
research in Nairobi, Nakuru Counties

on the topic: DETERMINATION OF ANTIOXIDANTS IN FRESH AND SOLAR-DRIED OYSTER MUSHROOMS AND SENSORY ACCEPTABILITY OF MUSHROOM-MILLET PORRIDGE-BLEND AMONG ADULTS WITH HIV/AIDS, KENYA

for the period ending:
1st February,2020

Applicant's
Signature

Permit No : NACOSTI/P/19/32526/27573
Date Of Issue : 1st February,2019
Fee Recieved :Ksh 1000

Director General
National Commission for Science,
Technology & Innovation

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone +254-20-2213471, 2241349,3310571,2219420
Fax +254-20-318245,318249
Email: dg@nacosti.go.ke
Website: www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No. **NACOSTI/P/19/32526/27573**

Date: **1st February, 2019**

Miriam Masiko Bosire
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Determination of antioxidants in fresh and solar-dried oyster mushrooms and sensory acceptability of mushroom-millet porridge-blend among adults with HIV/AIDs, Kenya"*** I am pleased to inform you that you have been authorized to undertake research in **Nairobi and Nakuru Counties** for the period ending **1st February, 2020.**

You are advised to report to **the County Commissioners and the County Directors of Education, Nairobi and Nakuru Counties** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

DR. MOSES RUGUTT, PhD, OGW
DIRECTOR GENERAL/CEO

Copy to:

The County Commissioner
Nairobi County. 18/02/2019

COUNTY COMMISSIONER
NAIROBI COUNTY
P.O. Box 30124-00100, NBI
TEL. 341666

The County Director of Education
Nairobi County.

Appendix I: Kenyatta University Graduate School Permit

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Our Ref: H60/33060/2015

DATE: 17th September, 2018

Director General,
National Commission for Science, Technology
and Innovation
P.O. Box 30623-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MS. BOSIRE MIRIAM MASIKO – REG. NO. H60/33060/2015

I write to introduce Ms. Bosire Miriam Masiko who is a Postgraduate Student of this University. She is registered for M.Sc. degree programme in the **Department of Food, Nutrition & Dietetics.**

Ms. Bosire intends to conduct research for a M.Sc. thesis Proposal entitled, **"Determination of Antioxidants in Fresh and Solar-Dried Oyster Mushrooms and Sensory Acceptability of Mushroom-Millet Porridge Blend among Adults with HIV/AIDS."**

Any assistance given will be highly appreciated.

Yours faithfully,

KENYATTA UNIVERSITY OFFICE OF DEAN 01 NOV 2018 GRADUATE SCHOOL

PROF. PAUL OKEMO
DEAN, GRADUATE SCHOOL

Appendix J: Nakuru County and Hospital Permits

THE PRESIDENCY
MINISTRY OF INTERIOR AND
CO-ORDINATION OF NATIONAL GOVERNMENT

Telegram: "DISTRICTER" Nakuru
Telephone: Nakuru 051-2212515
When replying please quote

COUNTY COMMISSIONER
NAKURU COUNTY
P.O. BOX 81
NAKURU.

Ref No. CC. SR .EDU 12/1/2 VOL.1V/31

20th February 2019

TO WHOM IT MAY CONCERN

RE:- RESEARCH AUTHORIZATION
MIRIAM MASIKO BOSIRE

The above named student from Kenyatta University has been authorized to carry out research on **determination of antioxidants in fresh and solar-dried oyster mushrooms and sensory acceptability of mushroom-millet porridge-blend among adults with HIV/AIDs** at Nakuru Referral Hospital for a period ending 1st February 2020.

Please accord her all the necessary support to facilitate the success of her research.

J. B. KICHWEN
FOR COUNTY COMMISSIONER
NAKURU COUNTY

Miriam M. Bosire
P.O. Box 16568
Nakuru

11/03/2019

The Medical Superintendent.
Nakuru Level 5 Hospital
P.O. Box 71
Nakuru.

MEDICAL SUPERINTENDENT - NAKURU LEVEL 5 HOSPITAL
RECEIVED
11 MAR 2019
P.O. Box 71-20100, NAKURU

11/3/2019
- Research other Institutions for 2000/-
- To Report to I/C CCC for Advice.
MED. SUPT.

Dear Sir/Madam,

RE: PERMISSION TO CONDUCT RESEARCH.

My name is Miriam Bosire. I am an MSc student at Kenyatta University. I am currently conducting a research on "determination of antioxidants in fresh and solar dried oyster mushrooms and sensory acceptability of mushroom-millet porridge blend among adults with HIV/AIDS.

I would like to request permission to conduct the sensory acceptability test at the CCC department at the Nakuru level 5 Hospital.

Attached please find my proposal and letters from the Ministry of Education in Nakuru and NACOSTI

Yours Sincerely
Bosire Miriam

0726-466131