

**EFFECT OF MAIZE AND OYSTER MUSHROOM BLENDING RATIO ON
NUTRITIONAL, MICROBIAL, AND PHYSICO-CHEMICAL QUALITY OF MAIZE-
MUSHROOM COMPOSITE**

BY

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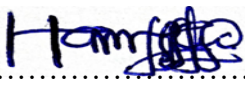
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DECLARATION

I, KILAMA GODFREY, humbly certify that the contents of this dissertation are original and have not been previously submitted for academic credit at any university or other higher education institution.

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APPROVAL

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DEDICATION

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ACRONYMS

ID	Iron deficiency
ZD	Zinc deficiency
VAD	Vitamin A deficiency
FAO	Food and Agricultural organization
WHO	World Health Organization
RUTF	Ready to Use Therapeutic Food
UNICEF	United Nations Children Emergency Fund
HIV	Human Immunodeficiency Virus
UNDP	United Nations Development Plan
GPS	Geographical Positioning System
AOAC	Association of official Analytical chemists
MC	Moisture Content
AAS	Atomic Absorption Spectrophotometer
RVA	Rapid Viscosity Analyzer
PV	Peak Viscosity
TV	Trough Viscosity
BD	Breakdown Viscosity
FV	Final Viscosity
SBV	Setback Viscosity
PT	Peak Time
PTempt	Peak Temperature
OAC	Oil Absorption Capacity
WAC	Water Absorption Capacity
BD	Bulk Density
CD	Compact Density
CBI	Compressibility Index

ANOVA	Analysis of Variance
GUREC	Gulu University Research Ethic Committee
CAO	Chief Administrative Officer
OFSP	Orange Flesh Sweet Potato
AOAC	Association of Official Analytical chemists
GPS	Geographical Positioning System

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ABSTRACT

complementary foods made from common starchy cereals like maize have been associated with high level of micronutrient deficiencies in children 6-24 months. Combining locally nutrient-dense underutilized foods like oyster mushrooms with starch-rich maize could enhance the nutritional quality of complementary foods. This study investigated how blending oyster mushrooms to maize flour would improve its nutritional, microbial, physico-chemical, and sensory quality. Refined maize flour was combined at 0% (control), 10%, 20%, 30%, and 40% (w/w) with flour derived from thoroughly cleaned blanched, and sun-dried oyster mushrooms. The nutritional, microbiological, physical-chemical, and sensory qualities of the composites were assessed using standard procedures. With all ratios of mushroom, the nutritional quality of the composites was significantly improved. For example, adding 10%, 20%, 30%, or 40% oyster mushrooms to maize flour increased the protein content from 6.67% to 8.74%, 6.67% to 11.04%, 6.67% to 14.23%, and 6.67% to 17.87%, respectively. Ash and fiber content percentage increment ranged from 0.31 to 2.48% and 0.22 to 3.04%, respectively. The composite's mineral composition had percentages increment ranging from 2.7 to 8.88% for iron, 1.9 to 6% for zinc, 26.03 to 55.33% for calcium, and 15.24 to 51.11% for magnesium. The physico-chemical quality of the composites was better, but the microbiological quality was negatively affected. Despite bioavailability limitations, theoretical nutritional calculations showed that consuming 250g/day of a blend that contains 10% mushroom contributes roughly 75% of the iron and 187% of the zinc requirement of children 6-24 months. The sensory evaluations of the composite-made porridges revealed poor ratings for the color, flavor, aftertaste, and aroma, but had no impact on the acceptability of the porridges made from blends containing 10% mushroom. The viscosity and water absorption of the composites, in particular, favorably impacted caregivers' acceptability, whereas the capability to absorb oil

decreased caregivers' acceptability. For children 6-24 months who rely on maize porridge as a complementary food, this study suggests combining oyster mushroom with maize flour to increase the nutritional content of the flour mixture.

Key words: children 6-24 months; maize-mushroom composite; microbial quality; micronutrient deficiency; nutritional quality; physico-chemical quality

CHAPTER ONE: INTRODUCTION

1.1 Background

Malnutrition affects more than half of the world's population, and the majority of cases are found in low-income nations (Haq et al., 2021). In low-income countries, children are the most vulnerable population, and undernutrition is responsible for 50% of all child fatalities (Adoko et al., 2021). Undernutrition such as stunting, wasting, and underweight affect mostly children aged 6-24 months and account for 21.3%, 6.9%, and 5.6% of all malnutrition cases worldwide, respectively (FAO et al., 2020). Additionally, two billion individuals worldwide suffer from micronutrient deficiencies (Campos Ponce et al., 2019). Compared to developed countries, Sub-Saharan countries account for greater cases of micronutrient deficiency (720 million) (Bhutta et al., 2013). Micronutrient deficiencies like iron deficiency (ID) (approximate 24.8%) and zinc deficiency (ZD) (4-73%) (Li et al., 2019), and Vitamin A deficiency (VAD) (more than 127million children) (Ssentongo et al., 2020) are the most detrimental forms of malnutrition in sub-Saharan African. ID and ZD have been listed among the most prevalent severe cases micronutrient deficiencies among children aged 6-24months (Barffour et al., 2019). In fact, ZD prevalence varies from 20 to 69% in Uganda alone, with northern Uganda accounting for 70% of this prevalence (Bitarakwate et al., 2003; GAIN and UNICEF., 2019) while ID prevalence among children is 53% (Nankinga et al., 2019) of with Northern Uganda accounting for 45% of this (Obai et al., 2016).

Micronutrients such as iron and zinc are important biological cofactor, regulators of numerous enzymatic control physiological processes (Sazawal et al., 2018). Lack of essential micronutrients, however, negatively impacts children's growth and development. For instance, lack of zinc leads to stunt growth of children aged 6-24 months and also result in to biosynthesis of hepcidin

molecules (Barffour et al., 2019). Hepcidin molecules lead to iron deficiency due to downregulation of iron homeostasis and hence interfere with the bioavailability of iron (Harika et al., 2017). As result of such, ZD is closely linked to iron deficiency (Philipo et al., 2021). Additionally, More than 18% of instances of lower respiratory tract infection, more than 11% of cases of diarrhea, and more than 10% of cases of malaria have been linked to ZD (Philipo et al., 2021). On the other hand, iron deficiency lead to preventable iron deficiency anemia which is linked to poor cognitive, neurophysiological and psychomotor development among children (Lozoff, 2007). Undernutrition in Sub-Saharan Africa is known to reach its peak at 6-17 months when a child is introduced to complementary foods (Nassanga et al., 2018). This is primarily attributed to low energy density food, narrow range of dietary options, indecorous food handling, indecorous preparation of food, and indecorous feeding practices (Alowo et al., 2018).

Nevertheless, by the age of 6 months, breast milk alone is insufficient to provide a child's daily physiological nutritional needs (Aber et al., 2018); and hence adequacy of complementary foods given to children become paramount (Barrera et al., 2018). Adequate complementary foods facilitate proper growth and development of children (Buzigi et al., 2020). However, in sub-Saharan Africa, most children aged 6-24months are introduced to less nutrient-dense complementary foods when they are 6 months old, which are typically cereal-based (Oladiran and Emmambux, 2020). This has led to poor nutritional outcomes (Bhutta et al., 2013). This is mostly because cereals like maize, which are frequently used in complementary feeding, lack two critical micronutrients such as zinc and iron for healthy growth and development, and certain essential amino acids like lysine and tryptophan (Buzigi et al., 2020). As a result, infants who regularly consume maize as complementary foods are at risk for micronutrient deficiencies (Ekesa et al., 2019). Therefore, there is need to put more emphasis on the important of fortifying maize-based

complementary foods and strongly recommend feeding infants on fortified foods (Tzioumis and Adair 2014; Kajjura *et al.*, 2019; Zuma et al. 2018; WHO, 2009).

To boost intake of micronutrients, several methods, including fortification and supplementation, have been used to improve the amount of key essential micronutrients, notably zinc and iron, in food (Brown et al., 2007; Bulletin, 2004; Ohanenye et al., 2021). The common food vehicles that have been employed for fortification include biofortified staples crops (Tarini et al., 2021), and ready-to-use therapeutic foods (RUTFs) like plumpy nut and citadel (WHO, 2013). It is crucial to note that children who exhibit severe acute malnutrition are given supplemental products like zinc and iron supplementation tablets, which are utilized as a curative regimen (Gupta et al., 2020; Horton S., Begin F., Greig A., 2008; Tarini et al., 2021; Wessells et al., 2018). This suggests that it is only seldom utilized and that it would be difficult to adopt in household daily diet. Additionally, a lack of supplies and poor compliance have consistently hampered the success of medicinal supplementation (Gemede, 2020, Adoko et al., 2021). In addition, because of their high cost and limited availability, other fortified products are out of reach for households in resource-constrained environments (Erokhin et al., 2021; FAO et al., 2020). Biofortification is one of the most cost effective way of improving micronutrient content in the food vehicle used and hence it is benefit can easily reach those in resource constraint settings (Buzigi et al., 2020). However, more expensive enhanced seeds and skepticism among consumers over the safety of genetically modified crops have hampered access to micronutrients through biofortification (Adoko et al., 2021; Low et al., 2017). As a result, there is a need to develop preventative measures that utilize readily available staple foods for acceptance and sustainability (Lenters et al., 2013).

Several studies have recommended the use and use of traditional processing techniques for household foods, including malting, germination, fermentation, roasting, and blending, to enhance

nutritional quality of starch cereals with a focus on increasing intake of micronutrients (Ekpa et al., 2019; Nkhata et al., 2018; Ramashia et al., 2019). Combining foods from various food groups generates food composites that are simple to use as complementary foods and improves nutritional quality through complementarity (Nassanga et al., 2018). For instance, previous studies on maize-soya bean composites (Abiose *et al.* 2015), maize-groundnut composites (Temba *et al.*, 2017), cereals-legumes composites (Bintu et al., 2015), all found a significant increase in the protein and energy content (0.8kcal/g) of the composites. However, majority food composite formulations prioritize improving protein and energy content compromising on micronutrient levels that are needed to meet the nutrient requirement of children aged 6-24months (Haile and Getahun, 2018), and affordable food composites targeting micronutrient such as zinc and iron is barely available to the rural poor. Therefore, it is necessary to combine locally accessible materials that are a rich source of zinc and iron as essential micronutrients to close the gap of micronutrient insufficiency among children between the ages of 6 and 24 months.

Oyster mushrooms (*Pleurotus ostreatus*) are a delicacy in Uganda, especially in the rural areas, and are rich in important macronutrients like protein and micronutrients like zinc (8.0%) and iron (14.28%) (Salami et al., 2017). Nonetheless, they are underutilized in Uganda for reducing malnutrition. Oyster mushrooms are nutrient-rich food sources which can be utilized to enhance quality of maize-based complementary foods to create nutrient-rich complementary food composites that are simple to incorporate into daily household nutrition practices mitigate micronutrient deficiencies like ID and ZD. Oyster mushroom consumption has been linked to enhanced intake of micronutrients including zinc and iron as well as better cognitive function (Feng et al., 2019). Therefore, this study seeks to investigate the extent of using oyster mushroom to improve quality of maize-based complementary food.

1.2 Statement of the problem

In Uganda, micronutrient deficiencies are still common. Iron deficiency accounts for 53% of cases, while zinc deficiency accounts for 20% to 69% of cases. Specifically, Northern Uganda contributes the highest proportion of these deficiencies amongst children 6-24 months (Aber et al., 2018) in which 45% and 70% children are iron deficient anemic and zinc deficient respectively (Obai et al., 2016). Poor feeding account for the persistently high level of micronutrient deficiencies. Additionally, low purchasing power and a lack of dietary variety in resource constraints areas could have exacerbated the prevalent of micronutrient. These determinants drive households in such localities to use what is available and accessible as a complementary food, which are mainly plant-based origins such as cereal and legumes (Abeshu, Adish, et al., 2016; Myeza et al., 2015; Onyeneke et al., 2019). Intrinsic starch characteristic of cereal-based foods such as maize affect their physico-chemical quality, and hence when turned into flour produce high viscous porridge which cannot easily be consumed by children aged 6-24 months. Therefore, in order to adjust it to the appropriate consistency (Forsido et al., 2019) large volume of water is added (Oladiran and Emmambux, 2020) which reduces solid content and nutrient density. As a result, the children's capacity to satisfy the minimum daily requirements for nutrients, particularly micronutrients, is seriously hampered. Concomitantly, important micronutrients such as zinc and iron are severely lacking in cereals like maize (Ekesa et al., 2019). An efficient and long-lasting method of enhancing the nutritional condition of rural poor people in resource-constrained environments is to use locally accessible food sources. However, majority of existing complementary food composites have targeted enhancing protein and energy (Adoko et al., 2021) without much or no focus on enhancing micronutrient levels.

Due to its naturally high zinc and iron content (Anyakudo and Adeniji, 2020), oyster mushrooms (*Pleurotus ostreatus*) have the potential to be combined with maize flour to create complementary foods. However, the amount of inclusion required to meet the nutrient needs of children between the aged 6-24 months is yet unknown. On the other hand, Food processing, such as blending, changes the composition of the components, which impacts the nutritional value and physico-chemical qualities (Ejigui et al., 2007), and microbial quality (Adoko et al., 2021). Consumer sensory preference and acceptability are influenced by blending. It is unknown how adding oyster mushrooms as a complementary food to improve its quality may affect those qualities in maize. Therefore, the purpose of this study is to determine whether blending oyster mushrooms to maize flour can enhance the quality of a composite made of maize and mushrooms.

1.3 Objectives:

1.3.1 General Objective

To evaluate how blending oyster mushroom flour to maize flour affects the nutritional, microbiological, and physico-chemical qualities of the composite made of maize and mushrooms.

1.3.2 Specific Objectives

- I. To determine the effect of blending maize flour with oyster mushroom flour on the nutritional composition of the maize-mushroom composites
- II. To determine the effect of blending maize flour with oyster mushroom flour on microbial quality of maize-mushroom composites
- III. To determine the effect of blending maize with mushroom on physico-chemical characteristics and sensory acceptability of maize-mushroom composites

1.4 Hypotheses.

- I. Blending maize flour with oyster mushrooms flour has no effect on the nutritional quality of the composite.
- II. Blending maize flour with oyster mushrooms flour has no effect on the microbial quality of the composite.
- III. Blending maize with oyster mushroom has no effect on Physico-chemical characteristics and consumer sensory preference and acceptability of maize-mushroom composites.

1.5 Significance of the study

Through the creation of a complementary composite containing both maize and oyster mushrooms, the current study offered helpful information regarding the potential of underutilized oyster mushrooms in reducing zinc insufficiency in children aged 6-24 months. The food sector will then be able to produce new food products based on oyster mushrooms by using this solid evidence to support the promotion of mushroom production and consumption as a healthy food.

1.6 Scope of the study

This research studied the effect of blending ratio of maize and mushroom on nutritional, microbial, and physico-chemical quality of maize-mushroom composite. Particularly, the study focused on enhancing quality of complementary foods in Northern Uganda. The independent variable in this research was the blending ratios of maize and mushroom while the dependent variable was the quality of maize-mushroom composite. Quality of maize-mushroom composite was analyzed in three dependent variables, which include; nutritional, microbial, and physico-chemical quality. The study focused on children 6-24 months in Northern Uganda to reduce malnutrition among them.

1.7 Study limitation

The increased level of total minerals in the different maize-mushroom composites indicate that these composites would contribute to the children's overall micronutrient intake. For instance, calcium, zinc, iron, and Magnesium levels were found to be greater in foods containing mushroom. This is plausible due to high concentration of calcium, magnesium, iron, phosphorus, and zinc in mushroom. However, the amount of these nutrients was determined in the flour not in the porridge, therefore, the impact of food preparation on the composition of these nutrients was not examined. Additionally, the results obtained in this study is only applicable to the mushroom cultivated in the northern Uganda particularly Gulu since nutritional composition of mushroom varies with geographical location.

CHAPTER TWO: LITERATURE REVIEW

2.1. Significance of Complementary feeding in promotion of positive nutritional outcomes

Good nutrition during infancy and early childhood (0-24 months) is crucial for children to develop to their full potential since it is a "essential/critical window" for a child's health, development, and growth (Aber et al., 2018; WHO, 2005). Common childhood ailments including diarrhea, nutritional deficiencies, and stunted growth in children are most prevalent during the critical window (Geremew Yohannes et al., 2020). Additionally, it is quite difficult to reverse stunting that was obtained at critical window when the children turn two (Abeshu, Lelisa, et al., 2016; Moura et al., 2022; Ogunlesi et al., 2014). Therefore, at the beginning of 6 months, complementary feeding needs to be prompt and satisfactory in term of amounts, consistency, frequency, and using a wide variety of foods supplemented with continues breastfeeding until 24 months or beyond (UNICEF et al., 2011). Complementary foods should be prepared with the appropriate texture for the child's age and served in sufficient quantity and safe manner, and feeding should be done responsively as described in psychosocial care guidelines (Geremew Yohannes et al., 2020). Children aged 6-24 months will need adequate complementary foods that are prepared and delivered in a safe and suitable manner in order to meet their changing nutritional needs as a result of significant changes in nutrient intake at 6 months.(Dewey, 2013). Therefore, adequate and safe complementary foods should be administered to children aged 6-24 months to facilitate proper growth and neurological stage of development (Nassanga et al., 2018). In addition, as children age, the viscosity (1-3 p.a.s) of the food should also be increased gradually with respect to child's requirement and abilities (WHO, 2012). As a result, infants can be fed on mashed, pureed, and semi-solid foods starting at 6 months, and can be fed on mashed or finely chopped foods between 9 and 11 months, as opposed to 12 months, when they can be fed on the same types of foods as consumed by the rest of the

family members (WHO, 2012). As recommended to meet the daily nutrients needs (3mg/day for zinc, and for iron it is 11mg/day and 12.5mg/day for age range of 6-11 months and 12-24 months) (Abeshu, Lelisa, et al., 2016) of children aged 6-24 months, complementary foods should be given a frequency of 2-3 times and 3-4 times a day for breastfeeding infants of 6-8 months and 9-24 months, respectively (WHO, 2005).

Complementary feeding is still a problem for children aged 6-24 months in many developing nations (de Pee, 2015). In India, for example, only 7% of breastfed children aged 6-24 months exceeded the minimum requirements for a diet, even though 54.5% of children aged 6-8 months had received any complementary foods the day before (Senarath and Dibley, 2012). In Nigeria, just 21% of breastfed children consume the recommended minimum of supplemental foods (Ogunlesi et al., 2014). Furthermore, only 4.2% of breastfed children aged 6-24 months in Ethiopia consume a diet that is at least satisfactory (Solomon et al., 2017). The challenges that arise during complementary feeding vary depending on the location, but many of them are universal (Abeshu, Adish, et al., 2016; Forsido et al., 2019). They frequently exhibit poor feeding habits and inadequate nutritional standards for homemade complementary foods (UNICEF et al., 2011). In fact, in developing nations complementary feeding practice is characterized by improper timing (either too early or too late introduced), infrequent feeding, and inadequate feeding techniques, poor hygiene standards, and poor infant care procedures (Ogunlesi et al., 2014). In addition to these, the complementary food is of poor nutritional quality, with insufficient variety, inappropriate consistency (food is too thin or thick), insufficient amounts of essential vitamins and minerals, particularly vitamin A, iron, zinc, and calcium, and insufficient amounts of calories for non-breastfed and breastfed infants (Oladiran and Emmambux, 2020). The children's growth and nutritional state are negatively impacted by the low quality and lack of variety in the foods

available. Therefore, in order to improve the nutritional quality of complementary foods, there is need to use local nutritious food sources like oyster mushroom.

2.2. Challenges associated with adherence to the recommended complementary feeding practices

For children to develop properly and grow to their full potential, adherence to the recommended complementary feeding practices for a healthy diet is essential. The right timing to start, the right amount of food for the child's age, a variety of diet, the right meal frequency, the right consistency and texture of the meal, hygiene practices, responsive feeding, and the right feeding during illness are all indication of adherence to the recommended complementary feeding practices (UNICEF et al., 2011). Adequate and appropriate complementary feeding practices provides opportunity for the promotion of ideal growth and development throughout the first 24 months of life. Early breastfeeding initiation, exclusive breastfeeding during the first two years of life, the timely and safe introduction of complementary feeding (with appropriate texture and consistency) coupled with continued breastfeeding up to 24 months or beyond are all significant parts of infant and young child feeding practices (WHO, 2012). The prevalence of malnutrition and death at 24 months of age is estimated to decrease by 17% as a result of proper complementary feeding practices , which may also prevent 6% of under-five fatalities annually (Forsido et al., 2019). Improper complementary feeding practices and infectious disease is the primary cause of micronutrient deficiency in the first 24 months of life which is attributed to failure to adhere to recommended complementary feeding practices (Oladiran and Emmambux, 2020).

In order to satisfy the changing demands of the developing infant, WHO recommends that a child should be breastfed exclusively for the first 6 months of life (Jama et al., 2020). Unfortunately, only a small percentage of infants are properly fed, though. In fact, only 25% of infants receive

the recommended level of dietary diversity, and only 41% of infants worldwide are believed to be exclusively breastfed for the first 6 months (Jama et al., 2020). In developing nations, inappropriate complementary feeding practices are most prevalent (Gizaw et al., 2017). Recent TDHS, (2016) (Tanzania Demographic and Health survey) data revealed that 26% of children aged 6-24 months received a minimum recommended diverse diet, 39% of children aged 6-24 months received minimum recommended meal frequency, and 59% of infants were exclusively breastfed for 6 months. In Uganda particularly Lamwo district, Aber et al., (2018) reported that more than half (55.8%) of the children were given less than the recommended amount of food. The author further reported that only 40.1% of all study respondents were adherent to recommended complementary feeding practices. The challenges associated with adherence to recommended complementary feeding practices vary depending on the location, but many of them are universal. These challenges include education level, attitude, and knowledge of the caregivers, high cost of standardized complementary foods, limited diversity, that collectively hamper adherences to recommended complementary feeding practices (Abeshu, Lelisa, et al., 2016; Forsido et al., 2019; Nassanga et al., 2018). However, in developing nations, high cost of standardized complementary foods and narrow range of dietary diversity are the mainly challenges which hampered adherence to recommended complementary feeding practices in resource constraint settings (Dewey, 2013; Oladiran and Emmambux, 2020). These determinants drive caregivers to use what is available and accessible as complementary foods (Oladiran and Emmambux, 2020), which are mainly plant-based origin; and such complementary foods are less nutritious and barely meet the daily requirement for key micronutrients such as zinc and iron (Langyan et al., 2021). On top of that, conditions under which starchy complementary foods are prepared and stored, expose children aged 6-24months frequently to infections (Baluka et al., 2015). Similarly, always little care and

attention are given to micronutrient requirement of children aged 6-24months which are needed for the selection of nutritious foods (Nsiah-Asamoah et al., 2019). Additionally, caregivers are not knowledgeable enough on simple food processing to provide these children with the proper nutrients (Oduor et al., 2019).

Therefore, improving the micronutrient content of maize—specifically its zinc and iron content—as a complementary food with nutrient-rich oyster mushrooms may have the potential to solve a number of problems with children's micronutrients in Northern Uganda.

2.3. Strategies to improve adherence to complementary feeding practices among children 6-24 months

The child's physiological and nutrient needs are hardly met with complementary feeding diet which is mostly plant source-based gruels and porridges because they are less nutrients dense (Geremew Yohannes et al., 2020). Therefore, in addition to plant-based foods, the complementary feeding diet should contain multiple servings of various animal source and/or fortified foods per week (Moura et al., 2022). Unfortunately, many households in developing nation primarily those in resource constraint settings lack the wherewithal to afford such a diet (Alowo et al., 2018). In addition, the distance to and frequency of the market days, the inability to keep fresh foods cool (a requirement for food safety), and the availability of time to prepare and feed small children frequently at balanced meals are all practical barriers to meeting the requirements for dietary diversity (de Pee, 2015). Therefore, in addition to enhancing breastfeeding, food selection, meal frequency, hygienic preparation, and feeding practices for infants and young children to the fullest extent possible, the nutrient content of complementary foods frequently requires further improvement, particularly for nutrients like vitamin A, iron, zinc, iodine, calcium, and essential fatty acids.

Fortification and supplementation are the two main methods for supplying these extra nutrients (Adoko et al., 2021) to children aged 6-23 months. Fortification can be done in a variety of ways. These ways include by blending a small amount of powder at home or spread to a home-cooked meal like fortified blended food or infant porridge (Bégin et al., 2001; Irakiza et al., 2021a). However, for the latter, the levels of fortification are not developed to fulfill the requirements of children aged 6-24 months, while they can help with nutritional intake, they are not adequate to completely prevent micronutrients deficiency (de Pee, 2015). For this age range, supplements are frequently restricted to a single nutrient (vitamin A or D, iron), and they can only be given as drops or syrup. Transporting syrup is cumbersome, and high-dose supplements, like vitamin A capsules, must be administered under medical staff supervision. These factors severely limit the frequency of administration, which is a problem for nutrients that should be consumed more frequently because they cannot be dosed at high levels and/or the body cannot store them for long. For this reason, fortification solutions are favored for supplying several micronutrients.

Using fortification techniques, local food resources can be used to increase the consumption of micronutrients like zinc and iron in children aged 6-24 months (Abeshu, Lelisa, et al., 2016). Items including sugar, oil, and grains have been employed as frequent food fortification vehicles (Bégin et al., 2001; Program, 2003). These crops are either biofortified (Garg et al., 2018) or blended to produced commercialize food composite like RUTFs (Potani et al., 2021). Agronomic bio-fortification, plant breeding, genetic manipulation, and other traditional methods are all examples of biofortification, which is a nutrition-sensitive agricultural intervention (De Brauw et al., 2018). These methods are designed to enhance consumption of important micronutrients like zinc and iron (Van Der Straeten et al., 2020). As it targets traditional staple crops like zinc-fortified beans, iodine-fortified fertilizer, and beans high in iron, bio-fortification is more effective at the

household level (GRAIN, 2019). Due to its sustainability and cost-effectiveness, bio-fortification can easily benefit the underprivileged in rural areas (Philipo et al., 2021). However, many are wary of the safety of biofortified crops, especially those that are genetically engineered, which discourages the adoption and consumption of bio-fortified foods (Covic et al., 2017). Furthermore, the quantity of biofortified food required to satisfy the daily need of the nutrient in biofortified foods is difficult to meet due to the necessity to vary diets. Therefore, it is necessary to develop additional techniques to boost the consumption of micronutrients from bio-fortified crops. Additionally, because households in resource-constrained environments have limited purchasing power, other items (RUTFs) are expensive and difficult for them to afford (Gupta et al., 2020a).

The development of locally accessible food resources to enable complementary feeding programs to increase the intake of micronutrients by children aged 6-24 months of a low-income home has been encouraged and emphasized by numerous research (Tadesse and Gutema, 2020). Numerous locally accessible food sources have varying quantities of nutrients; consequently, with proper selection and blending, these food sources result in products that are nutrient dense and have improved nutritional quality (Nassanga et al., 2018). The complementarity of nutrients from multiple sources accounts for this improved nutritional quality. As a result, correctly preparing and administering food composites may have the ability to supply a variety of macronutrients and micronutrients that are necessary for healthy growth (Araro et al., 2020). Therefore, the food composite should be created to increase nutrient consumption in question since standard nutrition methods are unable to provide enough of these nutrients to meet the Daily Allowance (RDA) requirement. For instance, the maize-soybean-groundnut-fish composite study by Amagloh et al., (2015) revealed a PDCAAS (Protein Digestibility Corrected Amino Acid Score) of 0.85. Furthermore, according to Mensa-Wilmot et al., (2001) methionine is the limiting essential amino

acid in all of the blends, with PDCAAS ranging from 0.44 to 0.51 for complementary foods made of maize, cowpea, and soybeans. Ejigui et al., (2007) examined blend of germination- or fermented maize with peanut or cowpea (70:30). Although the protein content of the blends improved, the authors further found that the amino acid score remained below 1.0, indicating that the blends had inadequate protein quality since the criteria for the amino acids were not properly met. Cowpea, peanut, or sardine were combined with corn or sorghum (MOSHA and VICENT, 2005), and despite the composites' higher protein content, the authors found that the total essential amino acid content was between 349 and 406 mg/g, which was higher than the required level (339 mg/g protein). However, a critical examination of the literature revealed that the majority of food composite formulations focused on boosting protein and energy content at the expense of micronutrient levels necessary to meet the nutritional needs of children aged 6-24 months, and that affordable food composites aimed at micronutrients like zinc and iron are hardly ever accessible to the rural poor. Nonetheless, while adding legumes to cereals increases the protein content of complementary porridge, composite porridges frequently have high viscosity, which results in porridge with poor energy levels. For example, for a complementary porridge of roasted soybeans-maize composite, Ejigui and Desrosiers, (2011) reported a higher viscosity (range of 4000-4500cP) than the recommended range of 1000-3000cP. The viscosity of acha and soybean complementary porridge was also observed to be higher than 6000cP at 10% solids content (Oladiran and Emmambux, 2020). Therefore, to bridge these gaps, compositing cereal such as maize should be done using local rich food source like oyster mushroom with low inherent starch compared to legumes. Therefore, research on the addition of oyster mushrooms to maize-based complementary foods to produce products targeting zinc- and iron deficiency in children 6-24months becomes novel.

2.4. Nutritional composition of white maize and oyster mushroom

White maize is a common food in many parts of the world, especially in sub-Saharan Africa, and is consumed by people of all ages (Nuss & Tanumihardjo, 2010). In regions with a high prevalence of micronutrient deficiencies, maize is widely grown and consumed (Galani et al., 2020). Maize provides energy mainly with little proteins and micronutrients contents (Bathla et al., 2019). Dried maize grains contains moisture (less than 11%), crude protein (7.77-14.60%), carbohydrates (69.7-74.5%), fats (3.2-7.1%), fiber (0.8-3.2%) (Ullah et al., 2010). Vitamin A (β -carotenoids), B-complex, C, K have been found present in significant amounts in maize (Bathla et al., 2019). As seen in Table 1, maize also contains potassium, which has diuretic effects, and selenium, which helps the thyroid gland's function. Unfortunately, the critical amino acids lysine and tryptophan, which the body needs from the diet, are absent from maize. The majority of micronutrients, such as iron, zinc, calcium, magnesium, and phosphorus, are insufficient in maize. As a result, people and communities who rely solely on maize have micronutrient deficiencies.

Table 1: Nutritional composition of typical white refined maize flour

Nutritional parameter	Content
Energy (Kcal/100g)	361
Moisture (g/100g)	10.9
Dry weight (g/100g)	89.1
Crude Protein (g/100g)	6.9
Crude fat (g/100g)	3.9
Total carbohydrate (g/100g)	76.9
Crude fiber (g/100g)	4.6
Vitamin A (mcg/100g)	0
Thiamin (mg/100g)	0.246
Total folate (mg/100g)	25
Niacin (mg/100g)	1.9
Iron (mg/100g)	2.4
Calcium (mg/100g)	7.0
Zinc (mg/100g)	1.7

Source: Adapted from Harvest Plus Food Composition table 2010 (Hotz et al., 2012)

As can be seen from the Table 1, maize has a poor nutritional quality. Combining maize-based complementary foods may improve the micronutrient content of maize flour, which is important for preventing micronutrient deficiencies in areas where maize is a major staple crop or in areas where it is widely consumed. Although maize flour is used in a variety of products, including bread and complementary flours, limited information on how to combine maize flour with other dietary sources for nutritional improvement necessary to reduce zinc deficiency in children aged 6-24 months.

Mushrooms belong to the kingdom of fungi, which feed saprophytically on decomposing organic waste to grow and thrive. Due to its high nutritional content, edible mushrooms have been utilized as a delicacy and supplementary food in African tribes for a long time (Valverde *et al.*, 2015), which also characterize it as a functional food (Aror *et al.*, 2017). The most popular type of mushroom that is highly consumed is the oyster mushroom (*Pleurotus ostreatus*) (Valverde *et al.*, 2015; Mayanja & Tipi, 2018). Prior to the development of science in the mushroom industry, humans ate a variety of edible forest mushrooms due to their flavor and health advantages (Agahar-Murugkar and Subbulakshmi, 2005). However, further research has made it possible to grow mushrooms (Lakhanpal and Rana, 2005). As a result, this type of farming has eliminated the seasonality of edible forest mushrooms and provides year-round oyster mushroom output. Oyster mushrooms are now more readily available and accessible to people as a result. Due to its high rate of return and low production cost, mushroom farming has recently attracted more young people interested in entrepreneurship (Gateri, 2012) despite not being widely used in domestic agricultural production (Okhuoya *et al.*, 2010). Concomitantly, compared to cereals, oyster mushrooms are so nutrient-dense that they contain all the essential amino acids (Spim *et al.*, 2020). Compared to grains and legumes, oyster mushrooms have a 28% higher protein content (Tolera &

Abera, 2017). In addition, the oyster mushroom is a dense food source of vitamins such as niacin (21%), riboflavin (18%), pantothenic (11%), vitamin B6, and vitamin A. More so, the fiber content of oyster mushrooms is higher (Oluwaseun *et al.*, 2020) compared to maize and legumes. By lowering microbial attacks, the higher fiber content may lower the moisture level of oyster mushroom flour, increasing the flour's long-term storage durability. Furthermore, oyster mushroom is inherently richer in unsaturated fatty acid with less saturated fatty acid (Siyame et al., 2021). Additionally, the ratio of phytate to calcium, iron, and zinc in oyster mushrooms is 0.05M, 0.01M, and 0.05M, respectively, which is less than 2.50M, 0.40M, and 10.00M of their critical values. As a result, micronutrients like calcium, zinc, and iron are more bioavailable than they are in legumes (Majesty et al., 2019). In addition, the oyster mushroom has high content of mineral such as potassium, calcium (38.12mg/100g), magnesium, iron (14.28mg/100g), zinc (8.00mg/100g), phosphorus, and copper (0.54mg/100g) (Zhang et al., 2014). The zinc content (8.00mg/100g) is higher than that of maize (0.30mg/100g) and soya bean (1.15mg/100g) (Bamidele and Fasogbon, 2020). Oyster mushrooms therefore contain more zinc than the 3 mg per day recommended by the WHO for children between the ages of 6 and 24 months. Based on the nutritional profile of oyster mushrooms, it can be used to enhance the nutrients of supplemental porridge made with less nutritious maize flour to satisfy the needs of children between the ages of 6 and 24 months (Ishara et al., 2018). Oyster mushrooms are nutritionally adequate, but their addition to maize flour to create a complementary composite of maize and mushrooms, as will be investigated in this study to develop nutrition-sensitive products to increase the intake of micronutrients like zinc and iron by children aged 6-24 months old, is less well known.

2.5 Effect of variation of ingredient on the nutritional quality of foods

The addition of ingredients inevitably alters the nutritional quality of food. Although not general, this alteration is dependent on the type of nutrient (Ilhamto et al., 2014). According to study in 2020, nutritional compositions was significantly impacted except moisture content of a composite made from cowpea flour, orange-fleshed sweet potatoes, and cassava flour (Olaniran et al., 2020). The amount of each item blended determines how much the macronutrient and micronutrient levels have changed (Desalegn et al., 2015). For instance, the amount of energy, iron, and fat in the composite increased considerably in soy biscuit after blending the biscuit with mushroom were (Farzana & Mohajan, 2015). Similar findings were found in a different study, where the authors stated that the proximate composition of maize-soybean-peanut- moringa oleifera powder, except crude fat and carbohydrates, increased significantly when various food ingredients were combined in the right way (Shiriki et al., 2015). According to recent data, adding honey larvae (*Apis mellifera*) to the staple grain (maize) greatly increased the composite's content of lipids (14.3%), protein (12.56%), and calories (427.18 kcal/100 g) (Mekuria et al., 2021). It has also been shown that proximate composition can be improved for micronutrients that are significant for public health. For example, in order to improve the nutritional value of the composite flatbread, sweet potatoes with 30% orange flesh potato were found to provide enough vitamin A to meet 83.3% of the RDA for preschool children (Kidane et al., 2013). In a different study, adding vegetables and skim milk powder to millet-based composite flour considerably enhanced the amount of zinc and vitamin A present, increasing their respective contents to 4.1 mg/100g and 161 gRAE/100g from 15.5 gRAE/100g (Tumwine et al., 2019). The addition of various ingredients may either have a positive or negative effect on the nutritional quality of foods. Positively, the addition of oyster mushroom flour to wheat flour significantly enhances the composition of fiber, protein, zinc, and

iron of the bread. However, from a negative perspective, fat, protein, energy and protein reduced significantly in orange flesh sweet potato enriched pieces of bread instead (Kidane et al., 2013). On the basis of the aforementioned effects of the addition of different ingredients, it is clear that the effects of ingredient variation are not uniform in terms of nutritional content but rather depend on the blending ratios and kind of ingredients. As a result, this study's focus will be on the effect of adding oyster mushrooms to the maize-based supplementary formula rather than extrapolating from existing research.

2.6. The effect of variation of ingredients on the microbial quality of foods

With longer storage times, composite products' nutritional and sensory quality continues to deteriorate (Mmari et al., 2017). Microbial attack is mostly account for the composite quality's deterioration (Rawat, 2015) with the microbes principally bacteria (coliform) and fungi (yeasts and moulds) (Lorenzo et al., 2018). Therefore, the load of these microorganisms should be below the typical maximum limit for each microbe for the food products to be considered microbiologically acceptable. Variation in ingredients has an impact on how microbially healthy food items are, and it mostly depends on the amount of blending, the types of ingredients, and the length of storage. For example, combining maize and honey larvae yields a composite that is safe for microorganisms (Mekuria et al., 2021). When samples of flatbread were combined with orange-fleshed sweet potatoes, a similar outcome was attained and a significant amount of coliform, mold, and yeast were found to be microbiologically acceptable (Woreda, 2015). However, a different finding was shown with a composite made of cassava, bananas, iron-rich beans, and orange-fleshed sweet potatoes, which had a shorter shelf life (Adoko et al., 2021). According to the findings of the aforementioned investigations, variation of ingredients have different effects because of their different types, blend ratios, and storage times. However, there is

no information on how and how much the addition of oyster mushrooms affects the microbiological storage stability of complementary foods made from maize, the information needs to be guaranteed experimentally.

2.7. Effect of variation of ingredients on physico-chemical properties and consumer sensory preference of food composites

Variation of ingredients impacts physico-chemical properties of complementary food (Dongmo et al., 2020). In the light of the present study, Physico-chemical properties of interest are viscosity, bulk density, compact density, compressibility index, pasting characteristic, water and oil absorption capacity of porridge. The alteration of physico-chemical properties is largely blending ratios-and types of ingredients-dependent (Desalegn et al., 2015). The quality of these physico-chemical characteristics is a key factor in determining how well-liked foods are by customers. When it comes to food composite products, combining traditional malted legumes with maize flour greatly reduces the viscosity and stiffness of the composite (Ejigui et al., 2007). Similarly, compounding maize-based complementary food with sweet potato apparently decrease the viscosity of the composites (Amagloh et al., 2013). Blending fermented maize with soybeans lead to a creation of a composite that has higher viscosity and less shear thinning behavior than the control (maize flour) (Ejigui and Desrosiers, 2011). On the other hand, cereal-based complementary composite porridge blended with finger millet and maize flour has lower firmness, water and oil absorption capacity but the incorporation of 10% of cassava in the composite formula boosts the firmness, water and oil absorption capacity of the porridge (Calvin and George, 2018). Additionally, the bulk density, compact density, and pasting qualities of maize-wingled composites significantly decreased with the addition of 10% wingled termites. According to the findings of the aforementioned studies, different ingredients have different effects on food,

depending on their types and blend ratio. However, there is no information on how and how much the addition of oyster mushrooms affects the physico-chemical properties of complementary foods made from maize, which will be studied in this study and thus should be determined experimentally. Nonetheless, the change in physico-chemical quality of food composites alter consumer sensory preference of food composites (Bintu et al., 2015; Calvin and George, 2018; Ekpa et al., 2019).

Color, aroma, taste, mouthfeel, and thickness are the fundamental organoleptic characteristics that influence a product's overall acceptability among consumers (Bayarri et al., 2011). The organoleptic quality greatly depends on food ingredients and food processing technology applied (Desalegn et al., 2015). Overall consumer's acceptability of food products may be affected heterogeneously by food ingredient variation. Incorporation of wheat flour with mushroom and soy flour positively impacted the consumer preference for those sensory characteristics (Farzana and Mohajan, 2015). In another study, flavor pancakes with different ratios of OFSP and Iron-rich beans were significantly higher compared to the original pancake (Adoko et al., 2021). In addition, For crust color and crumb appearance, soy wheat bread samples did not significantly differ from whole wheat bread samples (Ndife et al., 2011). However, texture, flavor and overall acceptability were altered significantly (Ndife et al., 2011). From the discussion above, the impact of ingredient addition on customer preference cannot be generalized and instead depends on the sensory qualities. For instance, study conducted in 2014 reported that the sensory rating for taste, texture, color except appearance were higher for yoghurt compounded with baobab flavor compare to the rating of yoghurt without the flavor (Chipurura et al., 2014). This implies that when examining consumer sensory preference, greater focus should be placed on each sensory attribute as opposed to overall acceptability.

The effect of ingredient addition could depend on the type of final product. One study in 2015 reported that flat-breads blended with OFSP were liked and accepted by caregivers of pre-school children in term of all sensory characteristics (Woreda, 2015). In contrast, a different study found that adding wheat flour to pasta formulations together with 30% broad bean flour had no impact on customer preference for any organoleptic qualities (Giménez et al., 2012). The apparent impact of ingredient addition on the sensory qualities of food items depends on the components and the desired base product, it is crucial to note from the literature. Therefore, it follows that the impact of oyster mushroom addition on consumer desire for complementary foods based on maize cannot be inferred from literature.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The study was conducted at Gulu University, Gulu-Uganda with geographical positioning system (GPS) coordinate of 2°47'14.39" N 32°19'0.60" E. Gulu is one of the cities in Uganda, located in the Northern part of the country, approximately 333km from Kampala (capital city of Uganda), and has a GPS coordinate of 2°46'54.0"N32°17'57.0"E.

3.2 Study design

Quantitative design was employed whereby laboratory analysis employed to examine parameters such as nutritional composition, microbial load and physico-chemical properties. Analysis on nutritional composition and microbial load was carried out on the flour, and physico-chemical properties was done on porridge of maize and the composite flour. For sensory evaluation, cross-sectional study design was conducted in Gulu District to obtain information on consumer sensory preference and acceptability of the composite.

3.3 Sample collection and preparation

For this study, 20kg of white maize grains were purchased from local markets in Gulu's Laroo division and packed in a sterilized polythene bag. The maize grains were hand-sorted in a Turpline, winnowed three times in a Tarpaulin, and packed in a polythene bag. Following that, it was ground to 0.25mm particle size using a 20HP mill, and the refined flour was packed in a sterile polythene bag for composite formulation. Maize was chosen as a staple crop because it is widely consumed in Northern Uganda and is frequently used as a complementary food (Ekpa et al., 2019).

For oyster mushrooms, 50kg of fresh oyster mushrooms were purchased from a local producer in Gulu who adheres to high-quality production standards. The mushroom was sorted, cleaned to

remove soil particles, sliced in a Tarpaulin with a knife, and then blanched in a three-stove using firewood glow. Blanched oyster mushrooms wrapped in a square of cotton cloth and suspended in hot steam of boiling water in a drum for 3 minutes. Blanching deactivates enzymes in oyster mushrooms, and hence reducing perishability of mushroom in terms of flavor, color, and texture. After blanching, the oyster mushrooms were placed in a Turpline and sun-dried for seven days to reduce the moisture content to less than 10%. Following that, the dried mushrooms were placed in a polythene bag and ground to a particle size of 0.25mm in a 20HP mill. As a result, it was packed in a sterilized polythene bag and stored at room temperature (20-25⁰C) before being used in composite formulation. Oyster mushroom was chosen because it is consumed by the rural poor of northern Uganda, has a high level of micronutrients, and is popular among consumers because it is delicious (Majesty et al., 2019)

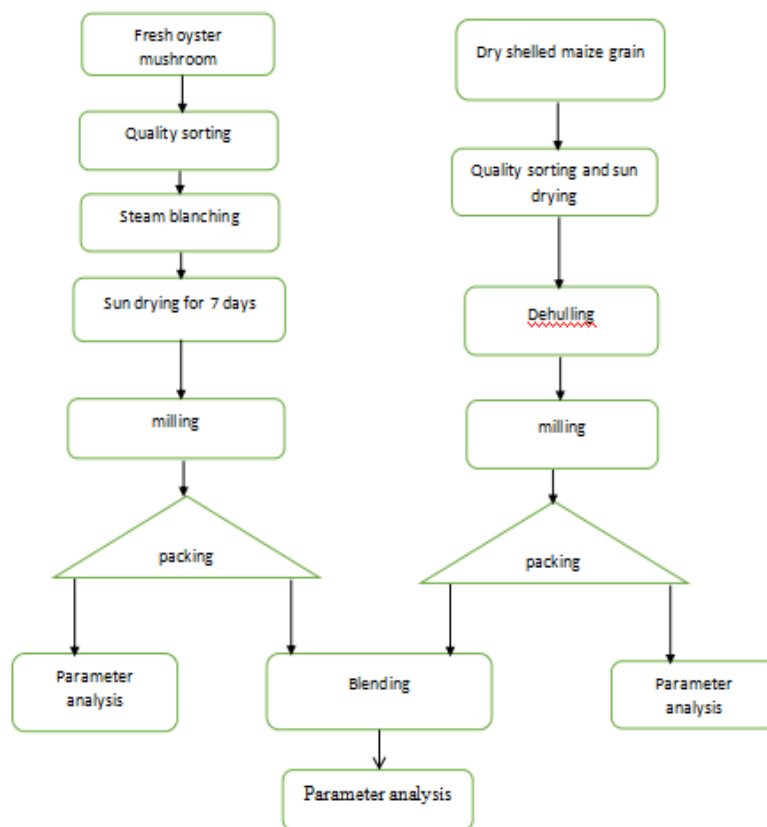


Figure 1. The process to formulate the composite from maize and mushroom and the

3.4 Preparation of maize-oyster mushroom composite

To obtain maize-oyster mushroom composite flour, maize and oyster mushroom flour were blended at the following ratios: 100:0; 90:10; 80:20; 70:30; 60:40 w/w, respectively. These ratios were calculated using the HarvestPlus Food Composition Matrix Table (2011), which took energy, zinc, and iron into account. The ingredient ratios were calculated basing on the minimum RDA (required daily allowance) of energy, which is 370kcal/100g, as well as 66.7 percent of the daily requirement for minerals such as iron 11mg/100g and zinc 3.0mg/100g for children aged 6 to 24 months (Desalegn et al., 2015; Haile and Getahun, 2018; Harika, Faber, Samuel, Kimiywe, et al., 2017). Following that, each composite was sealed in a polythene bag and stored at room temperature. After preparation, the control (maize flour) and the composites were analyzed to determine (i) proximate composition and mineral such as zinc, calcium, iron, and magnesium; (ii) microbial load in term of total microbial count, and moulds and yeast count; (iii) physico-chemical properties such as viscosity, water absorption capacity, oil absorption capacity, bulky density, compact density, compressibility index, and pasting properties; and (iv) consumer rating for the sensory properties between the composites and maize flour.

Table 2. Ratios of maize and oyster mushroom used in developing maize-mushroom composites

Ingredients			maize-mushroom Composite				
			1	2	3	4	5
white refined	Maize	100	90	80	70	60	
flour (%)							
Oyster mushroom (%)			0	10	20	30	40
formulation identity			K11	K22	K33	K44	K55

Source of information: Based on calculation using the HarvestPlus Food Composition Matrix 2011 Table

3.5 Determination of nutritional composition of maize-oyster mushroom composite

The nutritional composition was estimated in term of proximate composition and minerals content. For proximate composition, moisture content, crude protein, crude fat, ash, energy value, and crude fiber of the maize flour, and the composites flour was determined using procedures in the Association of Official Analytical Chemists (AOAC).

Moisture content (MC) of maize flour and the composite was determined using the oven drying method (AOAC, 2005; Method 930.15) in accordance with Bamidele and Fasogbon, (2020). Each sample (2g) was weighed and heated in a hot air oven (Model 202-0A, Huanghua Faithful Instrument Co.,Ltd) for 24 hours at 60⁰C using a pre-weighed dry clean crucible. After cooling in a desiccator for 20 minutes, each sample was weighed using an electronic analytical balance (FA2004). Every analysis was performed at least twice. The percentage (%age) of MC was calculated as presented in equation 2

$$\%age \text{ of MC} = (A_2 - A_3) \times (100/A_1) \dots \dots \dots \text{Equation 2}$$

Where A_1 = weight of the fresh sample, A_2 = weight of crucible + fresh sample, and A_3 = weight of crucible + dry sample

Crude protein content of maize and the composite was analyzed using Kjeldahl method (VELP SCIENTIFCA, A0000176, USA) according to Siyame et al. (2021). Each sample was weighed (1.5g) using an electronic analytical balance (FA2004) before being placed in a digestion tube. 13ml of concentrated Sulphuric acid was measured in a 50ml measuring cylinder, and 13ml of concentrated Sulphuric acid, two Kjeltec catalyst tablets, and one antifoaming agent tablet were added to each digestion tube. Each mixture was digested for 60 minutes at 400°C. After 60 minutes, 0.5M NaOH was added to 10 ml of digested samples to neutralize excess acid and liberate ammonia gas. The ammonia gas was collected using a delivery tube into a conical flask containing 20 ml of 4 percent boric acid; the reaction with boric acid produces ammonium ions and borate ions, which are then distilled. Following distillation, a drop of an indicator mixture of methyl red and bromocresol green was added and titrated against 0.1N Hydrochloric acid solution until the endpoint was obtained. Every analysis was performed at least twice. The percentage of nitrogen (N) in each sample was calculated as follows:

$$\% \text{age of N} = (V_1 - V_2) \times K \times f \times 0.014 \times (100/V) \times (100/S) \dots \dots \dots \text{Equation 3}$$

where V_1 = titer volume for the sample (ml); V_2 = titer volume for blank (ml); V = 10 ml of digested sample; K = normality of HCL solution; f = factor for standard HCL solution; 0.014 = milliequivalent mas of nitrogen; and S = mass of each sample taken. Every analysis was performed at least twice. Calculation of Crude Protein (CP) was done as presented in equation 4 by taking in to account a conversion factor of 6.25.

$$\% \text{age of CP} = \% \text{age of N} \times 6.25 \dots \dots \text{Equation 4}$$

Crude fat content of maize flour and the composite was determined using the Soxtec System HT2 fat extractor (FOSS, Hilleroed, Denmark) adopted from Adoko et al., (2021). Each dried sample (5g) was weighed in a preconditioned extraction cup of known mass. In the extraction chamber, the cup was immersed in 55ml of hexane (organic solvent). For six hours, the chamber was heated to 60°C. After six hours, the extraction cup was removed and dried for 30 minutes in a 70°C oven. The cup was then cooled, weighed, and recorded. Every analysis was performed at least twice. The percentage of CF was calculated as follows:

$$\% \text{age of CF} = [1 - (S_2/S_1)] \times 100 \dots \dots \dots \text{Equation 5}$$

Where S_1 = mass of each sample before extraction and S_2 = mass of each sample after extraction

Total ash content was determined using VULCAN furnace (AOAC 930.15, 2000) (Thermo SCIENTIFICA T16A, USA) in as described by Adoko et al., (2021). The weight of the dry empty crucible was measured and recorded using an electronic analytical balance (FA2004). The dry empty crucible was heated to 60°C for 24 hours before being cooled in a desiccator for 20 minutes. Each sample (2g) was weighed and placed in the crucible for six hours on a muffle furnace at 550°C. Following that, the burnt crucible and its contents were cooled in the furnace for two hours, then cooled in a desiccator for 20 minutes before being weighed and recorded. Every analysis was performed at least twice. The percentage of total ash was calculated as follows:

$$\% \text{age of total ash} = (K_3 - K_1) \times (100/K_2) \dots \dots \dots \text{Equation 6}$$

where K_1 =weight of empty crucible, K_2 = weight of each sample and K_3 = weight the crucible plus sample.

Amount of crude fiber in maize and the composite flour was determined using drying method after chemical digestion. Using a volumetric flask, a 1000ml solution containing 1.25 percent Sulphuric acid and 0.31N potassium hydroxide was prepared respectively. These two solutions were pre-boiled for one hour on a hot plate. Each sample (2g) was weighed, placed in a crucible of known weight, and then loaded into the fiber Raw extractor machine (FIWE6 6place 230V/60Hz, Velp Scientifica, USA). Each crucible was loaded with 150ml of Sulphuric acid solution, which was then boiled for 30 minutes. After 30 minutes, each sample was washed three times with 50ml distilled water. Following that, 150ml of potassium hydroxide solution was added to each crucible and boiled for 30 minutes. After 30 minutes, each sample was washed three times with 50ml of distilled water. Following that, each sample was washed twice with 25ml of acetone. The crucible for each sample obtained after washing was then dried overnight in the oven at 60°C. Following that, the weight of each dried crucible containing the sample was measured and recorded using an electronic beam balance. The crucibles were then heated in a muffle furnace for six hours at 550°C, cooled, weighed again, and the weight was recorded. The weight of crude fiber was calculated by subtracting the mass of dry empty crucibles from the total mass of crucibles and samples. Every analysis was performed at least twice. The crude fiber percentage was calculated as follows:

$$\% \text{age of crude fiber} = (K_1 - K_2) \times (100/K_3) \dots \dots \dots \text{Equation 7}$$

Where K_1 = weight of heated crucible + sample, K_2 =weight of crucible, and K_3 =weight of the fresh sample measured.

The percentage of total carbohydrate content was calculated using equation 8 according to Desalegn et al., (2015).

%age of total Carbohydrate = 100 - %age of (moisture + fat + protein + ash + fiber)...Equation 8

The energy value of each maize-oyster mushroom was determined through the summation of crude protein, carbohydrate, and crude fat values when multiplying with Atwater's conversion factors of 4, 4, and 9 calories respectively (Siyame et al., 2021). In addition, the contribution of maize-mushroom composites to recommended daily energy requirement was arrived at as a percentage ratio of observed energy in each composite to RDA (recommended Daily Allowance) for energy (Siyame et al., 2021) as presented in equation 9.

Contribution to RDA = (Energy content/RDA)*100.....Equation 9.

The mineral content of the maize and the composite flour that was considered are Zinc, iron, magnesium, and calcium. The minerals were analyzed using methods described in AOAC (2005). Zinc, iron, magnesium, and calcium concentration in maize flour, and the composite were analyzed using an atomic absorption spectrophotometer (AAS) (BUCK SCIENTIFICA, model 210 VGP, USA) in accordance with Paterne et al., (2019). Each sample (2g) was burned for 3 hours at 550°C in Vulcan furnace. Each sample's ash was dissolved in a 3% HNO₃ solution (10ml) and the filtered in 50ml volumetric flask using Whatman filter paper No. 41. Following that, the extract was diluted with deionized water up to the mark. The standard solutions for each mineral element were prepared according to AOAC (2005), with concentrations ranging from 0.5 to 5.0mg/L for Mg, 0.5 to 2.0mg/L for Zn, 0.5 to 5.0mg/L for Fe, and 0.5 to 5.0mg/L for Ca. The absorbance of calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn) was measured at 422.7, 285.2, 248.3, and 213.9

nm for both the samples and the standard solutions, respectively, using an air acetylene flame. Each diluted standard solution of Ca and Mg received 5ml of-Lanthanum nitrate solution (10% w/v) and HNO₃ (0.2%), and each sample for Ca and Mg received 5ml of 0.2% HNO₃ (3mol/L) and 5ml of Lanthanum nitrate solution (10%). To minimize interference, the Fe and Zn samples were run at lamp currents of 12 and 10mA, with band passes of 6.2 and 0.5nm, respectively. The absorbances of each mineral element ion against concentration to obtain a standard curve (ppm) (with R²=0.8 and 0.85 for Fe and Zn respectively, R² = 0.79 for Mg, and R²=0.7 for Ca). The content of mineral element ions in each ash sample was determined using the standard curve by extrapolating the absorbance of each sample. All minerals were expressed in milligrams per 100g of sample. Every analysis was performed at least twice. Also, the contribution of maize-mushroom composites to recommended daily allowance was arrived at as a percentage ratio of observed zinc and iron content in each composite to RDA (recommended Daily Allowance) for zinc and iron (Adoko et al., 2021) as presented in equation 10.

Contribution to RDA = (mineral content/RDA)*100.....Equation 10.

3.6. Analysis of microbial quality of maize-mushroom composite

Microbial analysis that was conducted include total microbial counts and moulds/yeast counts as described by Adoko et al., (2021). For total microbial count. To make a 500ml solution, 11.75 and 8.05g of total plate agar and buffered peptone water, respectively, were weighted and dissolved. These solutions were then autoclaved for 15 minutes. Each sample (2.5g) was weighed separately aseptically from each sampling point for each treatment into a sterile homogenizing container, followed by the addition of 22.5 ml of sterile buffered peptone water. The sample was homogenized for 2 minutes with a vortex machine before being serially diluted five times. To determine the load for total microbial count, 100l of inoculum will be pipetted from each dilution

and then plated on sterile plate count agar (BDH, Kampala, Uganda). The plates were incubated for two days at 37°C. Following that, counts were taken. To report the total microbial population, log CFU/g was used. Every analysis was performed at least twice.

For Yeast and Moulds count. To make a 500ml solution, 15.8, and 8.05g of rose Bengal agar and buffered peptone water, respectively, were weighted and dissolved. These solutions were then autoclaved for 15 minutes. Each sample (2.5g) was weighed separately aseptically from each sampling point for each treatment into a sterile homogenizing container, followed by the addition of 22.5 ml of sterile buffered peptone water. The sample was homogenized for 2 minutes with a vortex machine before being serially diluted five times. To determine the load for yeast and moulds, 100l of inoculum will be pipetted from each dilution and then plated on sterile Rose Bengal agar (BDH, Kampala, Uganda). The inoculated plates were incubated for 4 days at 24°C. To report the Yeast and Moulds population, log CFU/g was used. Every analysis was performed at least twice.

3.7. Analysis of physico-chemical properties and consumer sensory preference of maize-mushroom composites

Physico-chemical properties were laboratory analyzed and consumer sensory preference were evaluated using 5 points-hedonic scales.

3.7.1. Analysis of physico-chemical properties of maize-mushroom composites

Physico-chemical properties considered were viscosity, pasting properties, bulky density, compact density, compressibility index, water absorption capacity, and oil absorption capacity. The procedures described in AOAC (2005) were applied.

For viscosity, using a Viscometer (Model 3530LS+, AMETEK, USA) maize flour and maize-mushroom composite flour porridge was measured at 15% w/v of water. The porridge temperature was kept at heating temperature by placing them in a water bath and measuring their viscosity at 40°C. After cooking, gruel was poured into the Viscometer beaker, cooled to 40°C, viscosity (in centipoises, CP) was measured using spindle number 7 at a shear rate of 50rpm. The maximum and minimum viscosity readings were averaged within 5 minutes. Every analysis was performed at least twice.

For pasting properties, the Rapid Viscosity Analyzer (RVA) (RVA-3D, Newport Scientific, Narrabeen, Australia) was used to analyze the pasting properties of the maize flour and maize-mushroom composite flour. For each sample, 4.0 g flour was directly weighed into an RVA canister, followed by the addition of 25 g distilled water. The slurry was heated at a rate of 12°C/min from 50°C to 95°C, held at 95°C for 2.5 minutes, and then cooled to 50°C at the same rate. The paddle speed was set to 160 revolutions per minute. Peak viscosity (PV), trough viscosity (TV), breakdown (BD), final viscosity (FV), setback (SBV), peak time (PT), and pasting temperature were all measured (PTempt). Every analysis was performed at least twice.

For water absorption capacity, each sample (2g) was placed in a known-weight centrifuge tube, and 30 ml (initial volume) of hot water 70°C. The mixture was vortexed for 10 minutes and then set aside for 10 minutes to cool. At 25°C, the suspension was centrifuged at 4100Xg for 15 minutes. The supernatant was decanted into a 50ml measuring cylinder and the volume was recorded (final volume). Every analysis was performed at least twice.

%age WAC= (Initial volume-final volume) *100/ (sample weight)....Equation 9

For oil absorption capacity. The oil absorption capacity (OAC) of flours will be evaluated according to Eke and Akobundu method. Each sample (2g) was placed in a known-weight centrifuge. 10 ml (initial volume) refined olive oil was added and mixed. The mixture was vortexed for 2 minutes before being allowed to stand at 28⁰C for 30 minutes. After 30 minutes of standing, the suspension was centrifuged for 25 minutes at 300rpm. The volume of free oil decanted into a 50ml measuring cylinder was recorded (final volume). The amount of oil (in ml) absorbed by a gram of flour sample volume difference between initial and final volume is expressed as OAC. Every analysis was performed at least twice.

$$\% \text{age OAC} = (\text{initial volume} - \text{final volume}) * 100 / \text{sample weight} \dots \text{Equation 10}$$

For bulk density (BD), compact density (CD), and compressibility index (CBI). Each sample was weighed at 10 g using an electronic beam balance and transferred to a 50ml measuring cylinder. The initial volume was read and recorded. The cylinder was gently tapped on the laboratory bench until there was no change in volume. Every analysis was performed at least twice. The sample's final volume will be read and recorded. The percentages of BD, CD, and CBI were calculated as follow:

$$\text{For BD, } \% \text{age BD} = (\text{sample weight} / \text{initial volume}) * 100 \dots \dots \text{Equation 11}$$

$$\text{For CD, } \% \text{age CD} = (\text{sample weight} / \text{final volume}) * 100 \dots \dots \text{Equation 12}$$

$$\text{For CBI, } \% \text{age CBI} = (\text{initial volume} - \text{final volume}) * 100 / \text{initial volume} \dots \text{Equation 13}$$

3.7.2 Analysis of consumer sensory preference

Sensory evaluation was conducted with 67 individuals in Gulu District. The district was chosen due to the widespread use of polished maize flour, high oyster mushroom production from mushroom small enterprises, and widespread consumption among the residents. Caregivers of children aged 6-24 months who live in the district were invited to participate in the study because they cannot objectively evaluate the formulation's sensory qualities. The study did not include caregivers who had children whose ages fell outside of the range.

3.7.2.1 Sampling techniques

The selection process for caregivers who took part in the study involved multiple stages. Two sub-counties were chosen at random from Gulu district, and then two parishes were chosen. Two villages were randomly chosen from each parish, then purposively households with children aged 6-24 months were chosen. The district and local officials assisted in formally contacting the participants.

3.7.2.2 Sample size determination

Using a common formula, the sample size was determined as shown below.

$$(n) = (Z^2P(1-P)) \div e^2 \dots\dots\dots \text{Equation 1 adopted from Adoko et al., (2021)}$$

Where n=sample number; Z= 95% confident level (Zscore=1.96); p = prevalence of zinc (20 to 69%) and iron deficiency (53%) among children 6-24 months in Uganda, e=error margin of 5%. From equation 1, the required number of participants calculated is 67 after obtaining the average of n_1 at $p = 20\%$, n_2 at $p = 69\%$, and n_3 at $p = 53\%$. The total participant calculated fall in between the number of caregivers (50) who got involved in Alowo et al., (2018) in Acholi sub-region and number of caregivers (97) who participated in c study in Gulu and Omoro district.

3.7.2.3 sensory evaluation

A 5-point hedonic scale (from 1—Strongly dislike, 2—dislike, 3—neither like nor dislike, 4—like, and 5—like strongly) was used to evaluate the sensory qualities (aroma, color, taste, aftertaste, mouthfeel, and overall acceptability) (Alowo et al., 2018). Porridge samples were made from four maize-mushroom composites and a control by combining each flour with water at a 20% w/v ratio and cooked for 15 minutes. The evaluation was conducted on composites and maize flour porridge. Porridge of the composite, however, had a code: 501 for K11, 624 for K22, 830 for K33, 574 for K44, and 152 for K55. Caregivers were given instructions on each sensory characteristic's definition before the evaluate on exercise. Each sample of porridge served in single-use cup. Participants were also given clean water to rinse their mouth with before and after tasting each sample of porridge. This was done to prevent the sensory qualities from being over- or under-scaled. The participants were kept apart to prevent outside meddling.

3.8 Statistical Analysis

Statistical Package for the Social Sciences was used to examine the data (SPSS version 25). Additionally, ANOVA and descriptive statistics were used to compare the nutritional and microbiological qualities of maize flour (control) & maize-mushroom composite flour, and the rating level of each sensory characteristic of the composites and maize flour. furthermore, mean separation was done using studentized Post Hoc Tukey test. Additionally, Bar graph was showed the influence of physico-chemical determinants of caregivers' acceptability of maize-mushroom composites. Furthermore, bar graph was used to present sociodemographic characteristics of caregivers. A 95% confidence interval was used for the comparative analysis ($p \leq 0.05$). The results were shown as means $\pm$ standard deviation (SD).

3.9 Ethical considerations

The study was approved by Gulu University Research Ethics Committee (GUREC). The products were handled with extreme care during laboratory analysis. Permission from the CAO (Chief Administrative Officer) of the Gulu district was requested in order to conduct research in the community of Gulu district. Prior to taking part in the study, caregiver consent was requested and obtained in writing. In the study, there was no chance of injury or discomfort. This was because there was no allergic reaction. Allergy sufferers, however, were not permitted to take part in the evaluation. Additionally, because the evaluation was conducted in a hospital setting, medical personnel were on hand to assist the participant if they had any discomfort while tasting the various composite formulae.

3.10 Dissemination plan

The research will be published in peer reviewed journals and also disseminate to the community and other stakeholders through seminars.

3.11 Sustainability plan

For matter of sustainability, training of caregivers on mushrooms cultivation will be done so as they get skill on mushroom farming and will engage on mushroom farming. This will in turn break seasonal dependent on wild mushroom.

4.0 CHAPTER FOUR: RESULTS

4.1 Effect of blending maize flour with oyster mushroom on nutritional composition of maize-mushroom composite

Nutritional composition (proximate and mineral composition) of maize flour (control, (K11)) and different blends (K22, K33, K44, and K55) were assessed and the results are presented in the following sub-sections.

4.1.1. Proximate composition of maize-mushroom composite

The proximate composition of the different blends of maize-mushroom composite is presented in Table 3. Generally, blending maize flour with oyster mushroom altered its proximate composition. Notably, content of protein, ash, and fiber increased with increase in ratio of oyster mushroom flour. However, increase in ratio of oyster mushroom led to decrease in content of carbohydrate, crude fat, energy and moisture. For instance, the blend with 10%, 20%, 30%, and 40% oyster mushroom flour had a higher crude protein content by about 1.07%, 4.3%, 7.5%, and 11.2% respectively compared to the observed content in the control. Similarly, the content of crude fiber was significantly higher in the aforementioned blends compared to observed content in control by 0.22%, 0.67%, 1.6% and 3.04%, respectively. For ash content, addition of 20%, 30%, and 40% mushroom to maize flour significantly increased ash content ($p \leq 0.05$) by 0.82%, 1.62%, and 2.48% respectively. Dissimilarly, increase in the amount of oyster mushroom from 10%, through 20% to 40%, led to significant decrease ($p \leq 0.05$) of carbohydrate content by 1.19%, 3.95%, 8.13%, and 13.67% respectively compared to observed content in control. A similar trend was observed with moisture content. Similarly, contents of crude fat were significantly lower compared to that observed in the control with the highest decrease observed with 40% mushroom addition. The observed energy content of the blends significantly decreased ($p \leq 0.05$) with increase in mushroom ratios except for those with 10% and 20% oyster mushroom. The energy content of blends with

30% and 40% mushroom were 1.01 times and 1.036 times significantly lower ($p \leq 0.05$) than that of the control, respectively.

Table 3. Proximate composition of the maize flour and different blends of maize and mushroom flour

Parameter	Sample				
	K11	K22	K33	K44	K55
Crude Protein (%)	6.67±0.69 ^a	8.74±0.35 ^b	11.04±0.09 ^c	14.23±0.21 ^d	17.87±0.14 ^e
CHO (%)	84.44±0.5 ^a	83.25±0.54 ^b	80.49± 0.07 ^c	76.31±0.36 ^d	70.77±0.44 ^e
Crude fat (%)	1.65±0.22 ^a	1.45±0.35 ^b	1.37±0.08 ^c	1.32±0.08 ^c	1.29±0.01 ^d
Crude Fiber (%)	0.54±0.01 ^a	0.76±0.13 ^b	1.21± 0.11 ^c	2.14±0.14 ^d	3. 58±0.24 ^e
MC (%)	5.86±0.63 ^a	4.67±0.12 ^b	4.23±0.22 ^b	3.57±0.08 ^c	3.13±0.28 ^d
Ash Content (%)	0.83±0.02 ^a	1.14±0.07 ^{ab}	1.68±0.17 ^b	2.43±0.35 ^c	3.13± 0.28 ^d
Energy (Kcal/100g)	379.31±0.19 ^{ac}	380.96±0.61 ^{ab}	378.45±0.27 ^c	374.06±0.69 ^d	366.15±1.30 ^e

Mean ± Standard deviation (n=5) in the same row with different superscript are significantly different at $p \leq 0.05$. K11= maize flour (control, 100%), K22= blend of 90% maize and 10% mushroom flour, K33= blend of 80% maize and 20% mushroom flour, K44= blend of 70% maize and 30% mushroom flour, and K55= blend of 60% maize and 40% mushroom flour, CHO= Carbohydrate, MC = Moisture content.

4.1.2. Mineral composition of maize-mushroom composite flour

The mineral composition of different blends of the composite in comparison with the control is summarized in Figure 2. Generally, addition of oyster mushroom to maize flour significantly ($p \leq 0.05$) increased all the mineral content analyzed in the current study. Zinc content was 1.9, 3.24, 4.39, and 6 times higher than that observed in the control for 10%, 20%, 30% and 40% of mushroom addition, respectively. Similarly, iron content was 2.7, 5.9, 7.12, and 8.88 times higher than the observed content in the control for 10%, 20%, 30%, and 40% of mushroom addition, respectively. A similar trend was observed for calcium and magnesium. Furthermore, calcium recorded the highest increase (55.33%) with increase in mushroom ratio and zinc recorded the lowest increase (3.5%).

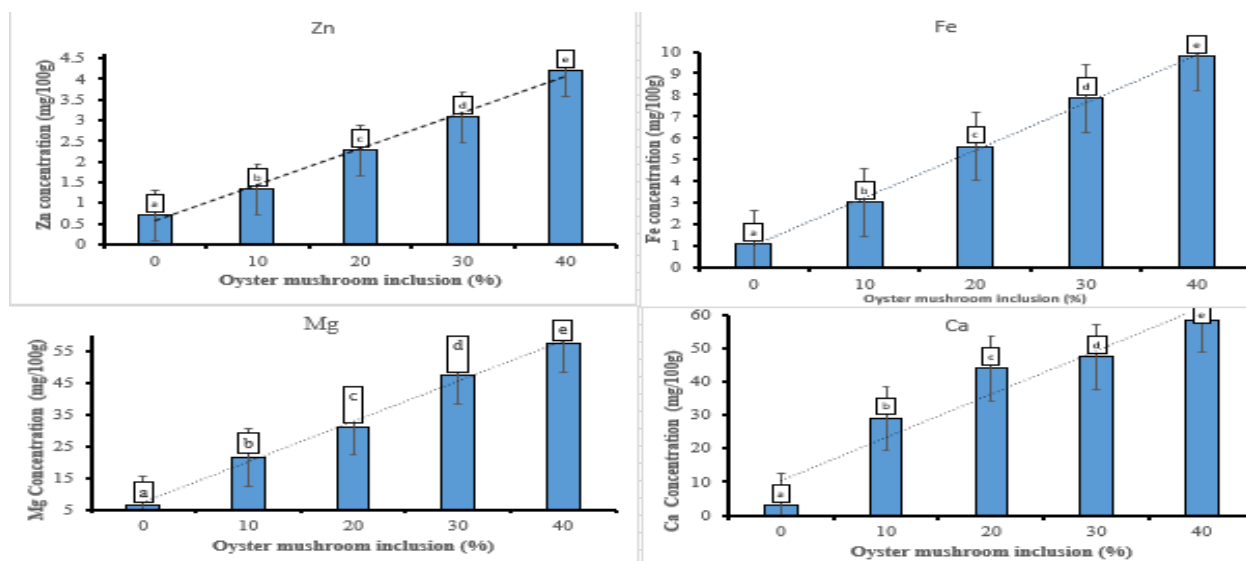


Figure 2. Effect of variation blending ratios of maize and oyster mushroom on concentration of selected minerals in maize flour.

Column bars with different letter superscript are significantly different at $p \leq 0.05$. Zn = Zinc, Fe = Iron, Ca = Calcium, Mg = Magnesium

4.1.3. Contribution of maize-mushroom composite to the recommended daily energy requirement for non-breastfed and breastfed children aged 6-24 months

Table 4 displays the contribution of various blends of maize-mushroom composite to the energy requirement for breastfed and non-breastfed children aged 6-24 months. For both non-breastfed and breastfed children, the contribution of the different blends reduced with an increase in mushroom blending ratio (Table 4). Despite the decline in energy contribution, the different blends contribute more than 53% and 100% of the energy gap for non-breastfed and breastfed children 6-11 months, respectively. However, the contribution of maize-mushroom composite to meet energy requirement for non-breastfed and breastfed children between the ages of 12 and 24 months is less than 43% and 77%, respectively.

Table 4. Contribution of maize-mushroom composite to energy requirement for non-breastfed and breastfed children 6-24 months

Sample	Energy (Kcal)	Contribution to meet energy requirement for non-breastfed infants (%)			Contribution to meet energy requirement for breastfed infants (%)		
		6-8 months	9-11 months	12-24 months	6-8 months	9-11 months	12-24 months
K11	379.31±0.19 ^{ac}	61.68	55.29	42.43	189.66	126.44	75.86
K22	380.96±0.61 ^{ab}	61.94	55.53	42.61	190.48	126.99	76.19
K33	378.45±0.27 ^c	61.54	55.17	42.33	189.23	126.15	75.69
K44	374.06±0.69 ^d	60.82	54.53	41.84	187.03	124.69	74.81
K55	366.15±1.3 ^e	59.53	53.37	40.95	183.08	122.05	73.23

Mean ± Standard deviation (n = 5) in the same column with different superscript are significantly different at $p \leq 0.05$. K11= 100% maize flour (control), K22= blend of 90% maize and 10% mushroom flour, K33= blend of 80% maize and 20% mushroom flour, K44= blend of 70% maize and 30% mushroom flour, and K55= blend of 60% maize and 40% mushroom flour. RDA (Recommended Daily Allowance) of energy for non-breastfed infants 6-8 months, 9-11 months, and 12-24 months is 615, 689, and 894kcal/day respectively and for breastfed infants 6-8months, 9-11months, and 12-24months is 200, 300, and 500kcal/day respectively (Abeshu, Lelisa, et al., 2016).

4.1.4 Contribution of maize-mushroom composite to Recommended Daily Allowance of zinc and iron for infants 6-24 months

The contribution of each blend of maize-mushroom composite to RDA for zinc and iron are presented in Table 5 and Table 6, respectively. As observed from Table 5, increasing mushroom ratio greatly increased the contribution towards meeting RDA for zinc. Additionally, blend K55 with a 40% mushroom recorded the highest contribution (42%) (Table 5). A similar trend was observed with the contribution to RDA for iron (Table 6). K55 contributed more than half (88.9% and 78.16%) to the RDA of iron for children aged 6-11 months and 12-24 months, respectively.

Table 5. Contribution of maize-mushroom composite to Recommended Daily Allowance of zinc for infants 6-24 months

Sample	Zn (mg/100g)	Contribution to RDA (%)
K11	0.7±0.001 ^a	23.33
K22	1.33±0.06 ^b	44.22
K33	2.27±0.23 ^c	74.89
K44	3.07±0.06 ^d	102.11
K55	4.20±1.29 ^e	139.0

Mean ± Standard deviation (n=5) in the same column with different superscript are significantly different at $p \leq 0.05$. K1= maize flour (control,100%), K22= blend of 90% maize and 10% mushroom flour, K33= blend of 80% maize and 20% mushroom flour, K44= blend of 70% maize and 30% mushroom flour, and K55= composite of 60% maize and 40% mushroom flour. RDA=Recommended Daily Allowance of zinc for infants 6-24 months = 3mg/day.

Table 6. Contribution of maize-mushroom composite to Recommended Daily Allowance of iron for infants 6-24 months

Sample	Fe (mg/100g)	Contribution toward RDA of Fe (%)	
		6-11 months	12-24 months
K11	1.1±0.005 ^a	10.0	8.8
K22	3.03±0.15 ^b	27.5	24.24
K33	5.6±0.38 ^c	50.9	44.8
K44	7.83±0.11 ^d	71.1	62.26
K55	9.77±0.06 ^e	88.9	78.16

Mean ± Standard deviation (n= 5) in the same column with different superscript are significantly different at $p \leq 0.05$. K1= maize flour (control,100%), K22= blend of 90% maize and 10% mushroom flour, K33= blend of 80% maize and 20% mushroom flour, K44= blend of 70% maize and 30% mushroom flour, and K55= blend of 60% maize and 40% mushroom flour. RDA (Recommended Daily Allowance) of iron for infants 6-11 months and 12-24 months is 11mg/day and 12.5mg/day respectively.

4.2. Effect of blending maize flour with oyster mushroom on microbial quality of maize-mushroom composite

4.2.1. Analysis of total microbial and mould/yeast count of maize-mushroom composite flour

The variation of total microbial, and moulds/yeast count in the control and different maize-mushroom composite blends are summarized in Table 7. The total microbial, and mould & yeast counts significantly ($p \leq 0.05$) increased with increasing blending ratio of mushroom, and

were higher in the different blend compared to the control. The microbial count ranged between 1.13-1.62 CFU/g and the mould and yeast count ranged between 0.95-1.56 CFU/g. Blend K55 with 40% mushroom had the greatest total microbiological count (43.4%) as well as the highest counts of molds and yeasts (64.2%).

Table 7. Total microbial, and Moulds and yeast colony forming unit per gram of maize-mushroom composites

Sample	Log ₁₀ Total microbial counts (CFU/g)	Log ₁₀ Moulds and yeast (CFU/g)
K11	1.13± 0.05 ^a	0.95± 0.05 ^a
K22	1.30± 0.02 ^b	1.02± 0.06 ^a
K33	1.45± 0.03 ^c	1.30± 0.03 ^b
K44	1.56± 0.02 ^d	1.46± 0.01 ^c
K55	1.62± 0.02 ^d	1.56± 0.02 ^c

Mean ± Standard deviation (n=5) in the same column with different superscript are significantly different at $p \leq 0.05$. K11= maize flour (control, 100%), K22= composite of 90% maize and 10% mushroom flour, K33=blend of 80% maize and 20% mushroom flour, K44= blend of 70% maize and 30% mushroom flour, and K55= blend of 60% maize and 40% mushroom flour.

4.2.2. Comparison of observed total microbial counts, and moulds /yeast count with their respective maximum acceptable limit in complementary feeding

The comparison of total bacterial and fungal count of the study samples to the maximum acceptable limit for complementary foods of 10^5 and 10^3 CFU/g by codex, respectively is presented in Figure 3. The total microbial counts, and mold/yeast counts were all significantly ($p \leq 0.5$) below the upper level that is permissible for complementary foods in flour form as seen in Figure 3.

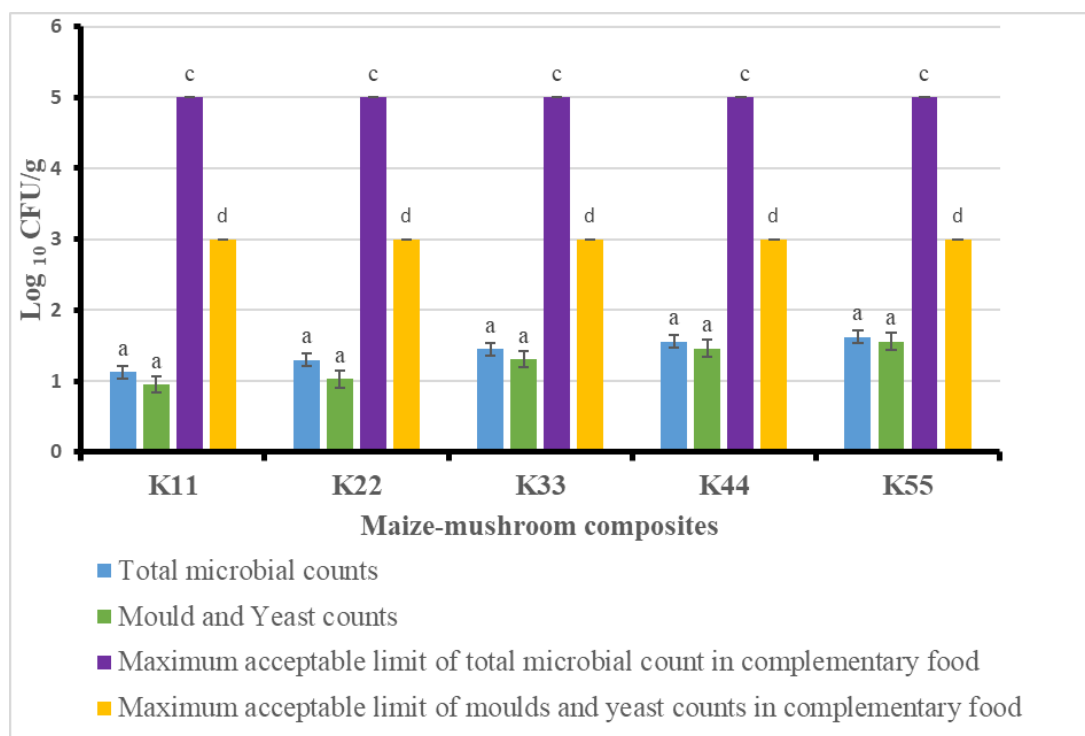


Figure 3. Comparison of total microbial and mould & yeast content of maize-mushroom composites with maximum acceptable limit of total microbial and mould & yeast load in complementary flour

CFU/g = Colony Forming Unit per gram, and bars with different letter superscript are significance at 5%. K11= maize flour (control,100%), K22= composite of 90% maize and 10% mushroom flour, K33=blend of 80% maize and 20% mushroom flour, K44= blend of 70% maize and 30% mushroom flour, and K55= blend of 60% maize and 40% mushroom flour, Codex Maximum acceptable limit of total microbial (105 CFU/g) and moulds & yeast counts (103 CFU/g) in complementary flour (Mekuria et al., 2021).

4.3 Effect of blending maize with mushroom on physico-chemical properties of maize-mushroom composites

4.3.1 Physico-chemical properties of maize-mushroom composite flour

The physico-chemical properties of maize-mushroom composites analyzed include viscosity, water absorption capacity, and oil absorption capacity presented in Table 8, compressibility index, compact density, and bulk density presented in Figure 4. Results indicate that addition of mushroom flour to maize flour had a significant ($p \leq 0.05$) effect on its physico-chemical characteristics. In general, when compared to the control, increasing mushroom ratio significantly reduced viscosity & water absorption capacity (Table 8) and bulk density (BD) & compact density

(CD) of the composite (Figure 4). For inclusion rate of 10, 20, 30, and 40% of mushroom, respectively, the observed viscosity was 970.51, 1707.83, 2198.88, and 2806.11cP lower than the viscosity observed in the control. Water absorption capacity (WAC) followed a similar trend, with the highest decrease (474.931%) observed in blend K55 (Table 8). On the other hand, increasing mushroom ratio in the blends by 10%, 20%, 30%, and 40% significantly increased OACs by 31.76%, 83.69%, 133.04%, and 157.4%, respectively.

Even though both CD and BD decrease with increasing mushroom ratio, CD was higher than BD, as seen in Figure 4. Contrarily, the CBI of maize-mushroom composites increased gradually (Figure 4), with a 40% inclusion of mushrooms producing a CBI that was significantly higher by 1.69% than the control.

Table 8. Variation in physico-chemical properties of maize-mushroom composites

Parameter	Sample				
	K11	K22	K33	K44	K55
Viscosity (cP)	4351.74±50.0 ^a	3381.23±45.13 ^b	2643.91±54.58 ^c	2152.86±47.63 ^d	1545.63±43.50 ^e
WAC (%)	572.201±27.14 ^a	440.11±14.12 ^b	291.09±14.08 ^c	149.72±0.14 ^d	91.27±14.38 ^e
OAC (%)	215.73±14.4 ^a	274.49±0.25 ^b	299.42±0.44 ^c	348.77±0.37 ^d	373.13±1.48 ^e

Mean ± Standard deviation (n=5) in the same row with different superscript are significantly different at $p \leq 0.05$. K11= maize flour (control, 100%), K22= composite of 90% maize and 10% mushroom flour, K33= composite of 80% maize and 20% mushroom flour, K44= composite of 70% maize and 30% mushroom flour, and K55= composite of 60% maize and 40% mushroom flour.

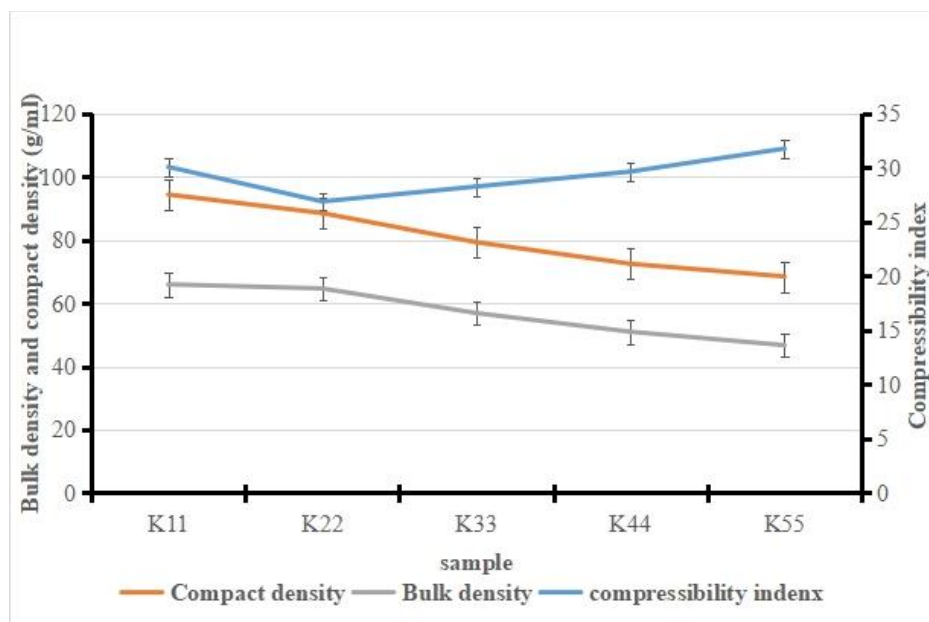


Figure 4. Variation in Bulk density, Compact density and Compressibility index of maize-mushroom composites

K11= maize flour (control,100%), K22= composite of 90% maize and 10% mushroom flour, K33= composite of 80% maize and 20% mushroom flour, K44= composite of 70% maize and 30% mushroom flour, and K55= composite of 60% maize and 40% mushroom flour.

4.3.2 Pasting properties of maize-mushroom porridge composite

Pasting characteristics results of the control and various maize-mushroom composite is presented in Table 9. Addition of mushroom to maize significantly ($p \leq 0.05$) reduced the peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV), and setback viscosity (SBV). For inclusion rates of 10%, 20%, 30%, and 40% of oyster mushroom, respectively, observed PV in the blends was 1.99, 2.35, 2.92, and 3.54 times lower than observed PV in the control. Similar trends were followed by TV, BV, FV, and SBV. In terms of peak time (PT), an increase in the mushroom ratio resulted in PTs that were 0.5, 1.13, 1.44, and 1.66 minutes higher than the control, respectively. Peak temperature (PTempt) followed a similar trend, with the highest elevation (7.93°C) recorded in blend K55 (Table 9).

Table 9. Variation in pasting properties of maize-mushroom porridge composite

Sample	Peak Viscosity (cP)	Trough Viscosity (cP)	Breakdown Viscosity (cP)	Final Viscosity (cP)	Setback Viscosity (cP)	Peak Time (Min)	Pasting Temperature (°C)
K11	3214.7±13 ^a	3050.4±56 ^a	477.0±8 ^a	4288.7±17 ^a	3221.9±3 ^a	5.14±0.10 ^a	80.37±0.55 ^a
K22	1615.7±56 ^b	1255.00±26 ^b	360.7±31 ^b	3248.7±41 ^b	1993.7±15 ^b	5.64±0.04 ^b	84.50±0.52 ^b
K33	1367.0±3 ^b	1143.7±49 ^{bcd}	223.3±16 ^c	2657.3±48 ^c	1513.7±53 ^c	6.27±0.07 ^c	85.87±0.51 ^c
K44	1102.3±5 ^b ^c	995.0±18 ^{bcd}	107.3±37 ^d	2044.7±96 ^d	1049.7±77 ^d	6.58±0.17 ^d	87.47±0.88 ^{cd}
K55	908.7±38 ^c ^d	875.7±26 ^{cd}	33.0±11 ^e	1533.3±70 ^e	657.7±45 ^e	6.80±0.20 ^e	88.30±0.48 ^{de}

Mean ± Standard deviation (n=5) in the same with different superscript are significantly different at $p \leq 0.05$. K1= maize flour (control,100%), K22= composite of 90% maize and 10% mushroom flour, K33= composite of 80% maize and 20% mushroom flour, K44= composite of 70% maize and 30% mushroom flour, and K55= composite of 60% maize and 40% mushroom flour, cP = Centipoise unit of viscosity

4.4 Effect of blending maize with mushroom on consumer sensory preference and acceptability of maize-mushroom composites

4.4.1 Socio-demographic statistics of caregivers that participated in sensory evaluation

The participant's sociodemographic data are compiled in Figure 5. The study included 67 female caregivers from Gulu District. Mature working age caregivers dominated the study, followed by prime- and early-working age caregivers (Figure 5). According to the Figure 5, more than three-quarters of the caregivers had attended school, with the majority completing their elementary education and less than 15.1% of the caregivers lacked formal education. Farmers and business person made up the majority of the participants' occupation (40% and 37%, respectively), whereas only 3% and 19% of the caregivers, worked as civil servants and casual workers respectively. However, the majority of caregivers (51%) fall below the poverty line, and 33% of them are in the lower middle-income class while just a small proportion belonged to the upper middle-income class.

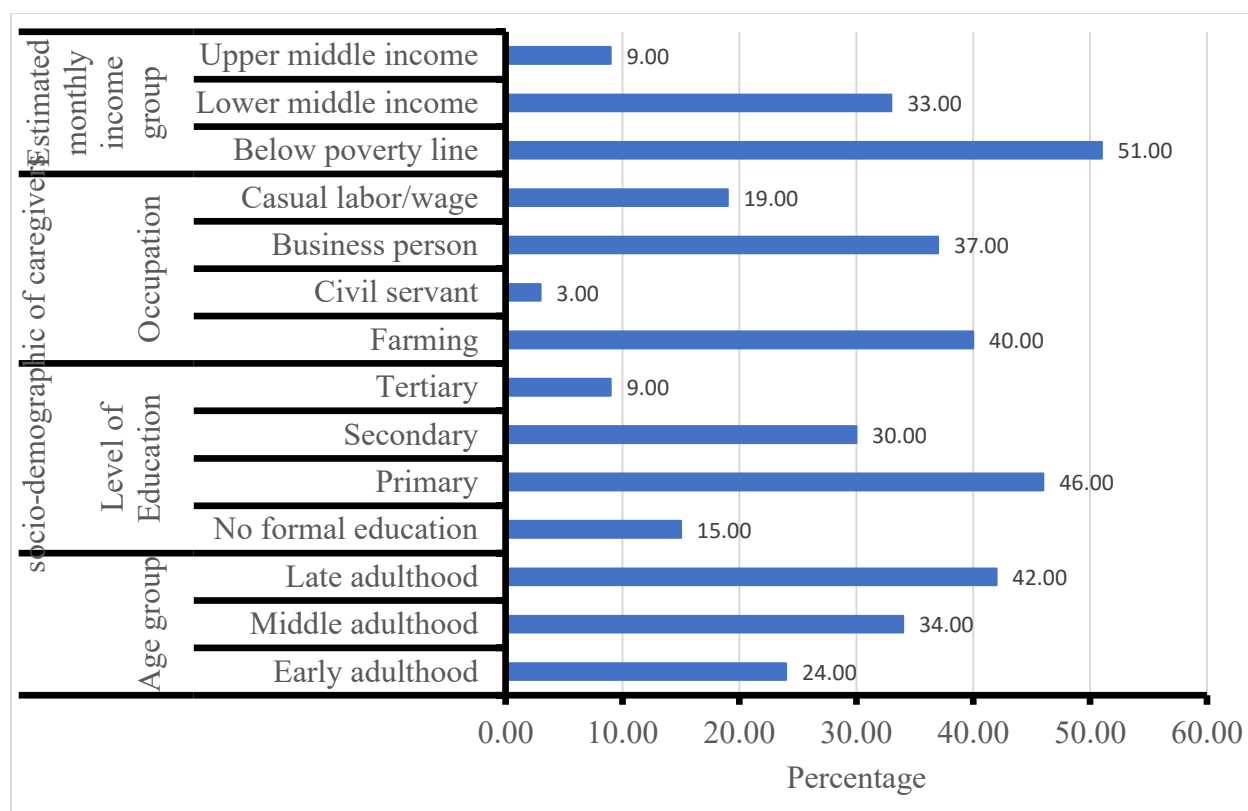


Figure 5. Socio-demographic statistics of female caregivers of Gulu district who participated in the study

Income groups were categorized according to Economic policy research center (EPRC) data on per capita daily consumption expenditure in purchasing power parity, Upper middle-class income (UG sh. 36,500-73,000), Lower middle income (UG Sh. 14,600-36,500), Below poverty line (< UG sh.7,300) (n = 67); Age group categorized based on Uganda Age Structure by Index mundi, Early Working age (15-24years), prime working age (25-54years), mature working age (> 54years).

4.4.2 Comparison of caregivers' sensory rating of different maize-mushroom composite porridge

Results of caregivers sensory rating of porridge made from maize-mushroom composites are presented in Table 10. The mean sensory rating for all sensory attributes decreased with increase in mushroom ratio. For instance, the mean sensory rating for aroma of the different blends was significantly ($p \leq 0.05$) lower by 0.54, 0.93, 1.78, and 2.39 for 10%, 20%, 30%, and 40% mushroom respectively compared to the control. However, for the composite with 10% mushroom, the mean sensory rating was not significantly ($p \leq 0.05$) different from that of the control. The mean sensory scores for color, mouthfeel, taste, and after-taste follow a similar trend as aroma (Table 10).

However, 10% mushroom ratio led to 0.28 points higher mean score for color compare to the control. For overall acceptability, for 10%, 20%, 30%, and 40% mushroom ratio in the composite led to significant ($p \leq 0.05$) 0.49, 1.29, 2.55, and 2.88 points lower mean score rating for overall acceptability compared to the control.

Table 10. Comparison of caregiver's sensory rating of caregivers of maize-mushroom composites

Sample	Mean sensory rating					
	Aroma	color	taste	Mouth feel	After-taste	Overall acceptability
K11	3.94±1.20 ^a	4.06±0.89 ^a	4.27±0.96 ^a	4.12±0.89 ^a	4.27±0.71 ^a	4.24±0.74 ^a
K22	3.40±1.21 ^{ab}	4.34±0.91 ^b	3.25±1.21 ^b	3.36±1.05 ^b	3.48±1.17 ^b	3.75±1.39 ^a
K33	3.01±1.27 ^b	3.18±1.03 ^b	2.84±1.59 ^b	3.39±1.24 ^b	2.90±1.44 ^c	2.95±1.45 ^c
K44	2.16±1.19 ^d	2.46±0.91 ^d	1.69±0.70 ^d	2.18±1.13 ^d	2.25±1.22 ^d	1.69±0.94 ^d
K55	1.55±1.02 ^d	3.13±0.81 ^d	1.46±0.91 ^c	1.28±0.67 ^c	1.39±0.74 ^c	1.36±0.73 ^c

Mean ± Standard deviation (n=67) in the same column with different superscript are significantly different at $p \leq 0.05$. K11 = maize flour (control, 100%), K22 = composite of 90% maize and 10% mushroom flour, K33 = composite of 80% maize and 20% mushroom flour, K44 = composite of 70% maize and 30% mushroom flour, and K55 = composite of 60% maize and 40% mushroom flour.

4.4.3 Influence of physico-chemical properties of maize-mushroom composites on caregivers' acceptability of the composites

Influence of physico-chemical properties on overall acceptability of maize-mushroom composite are presented in Figure 6. Physico-chemical properties, in general, heterogeneously associated with caregivers' acceptability of maize-mushroom composites. For instance, viscosity and water absorption capacity of maize-mushroom composites was associated with increase in caregivers' acceptability. Furthermore, viscosity and water absorption capacity greater than 3000cP and 400cP, respectively, associated with rapid increase in caregivers' acceptability for composite with 40% mushroom. In contrast, increase in oil absorption capacity of maize-mushroom was associated with a reduction in caregivers' acceptability of the composites. Furthermore, oil absorption capacity less than 275% was associated with rapid reduction in caregivers' acceptability for composite with 40%. Nevertheless, acceptability of composites without mushroom and with

40% mushroom had the highest and the lowest caregivers' acceptability, respectively at each point of each physico-chemical properties.

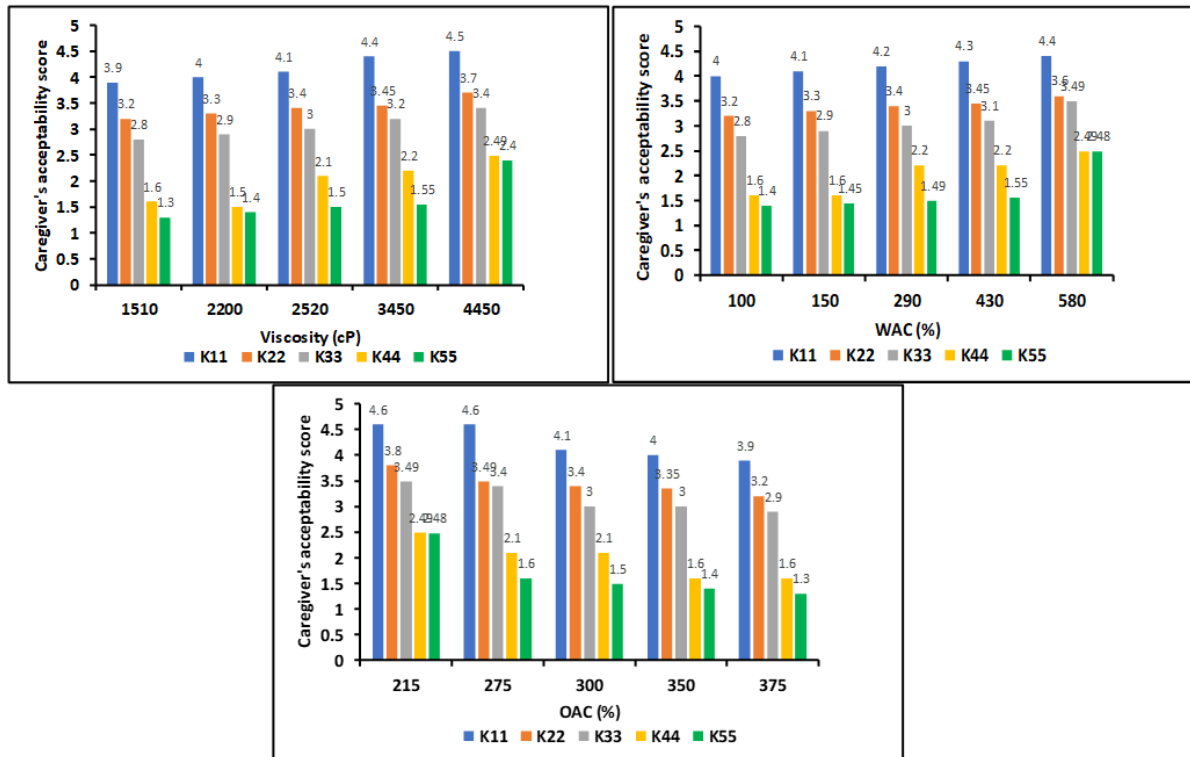


Figure 6. Variation in caregiver's acceptability of maize-mushroom composites with physico-chemical properties of the composites

K11 = maize flour (control, 100%), (n=5), K22 = composite of 90% maize and 10% mushroom flour, K33 = composite of 80% maize and 20% mushroom flour, K44 = composite of 70% maize and 30% mushroom flour, and K55 = composite of 60% maize and 40% mushroom flour. OAC = Oil absorption capacity, WAC = Water absorption capacity.

CHAPTER FIVE: DISCUSSION

5.1 Effect of blending maize flour with mushroom on nutritional composition of maize-mushroom composite

Most complementary foods are primarily plant-based in rural parts of Northern Uganda. Plant-based complementary foods are inadequate since they supply mainly starch. The need to enhance nutritional quality of plant-based complementary foods was called for, therefore, to demonstrate what is practically feasible in remote areas in order to enhance the nutritional quality of plant-based complementary foods. This study assessed the potential of mushroom addition in maize complementary flour in improving the nutritional quality of plant-based complementary food. The increment in crude protein levels after the inclusion of mushroom may be a result of high protein content in mushroom. Oyster mushroom is regarded to be a good protein source (22.61%) (Majesty et al., 2019) and its inclusion in the creation of recipes can boost the protein content of the complementary foods significantly as noted in the current study. This result concurred with results of previous studies of maize-mushroom composite (Bamidele and Fasogbon, 2020), maize-button mushroom composites (Ishara et al., 2018), and wheat-soy-oyster mushroom biscuit (Farzana and Mohajan, 2015) had high protein content. Nonetheless, the protein content (3.3-16.8%) observed in the current study is higher than protein content (2.3-6.9%) reported by Bamidele and Fasogbon, (2020) in maize-mushroom composites product. This difference could be attributed to geographical location factors like climate and soil (Hlongwane et al., 2022) which could explain the observed differences in nutritional composition of the substrate used for mushroom production leading to variation in nutritional composition of mushroom. The higher protein content reported in the current study demonstrate the efficacy of mushroom in enhancing protein content of complementary foods. This implies that consumption of maize-mushroom composites by children 6-24 months could enhance the intake of protein which is critical for protein deficiency alleviation.

Despite the increase in the amount of protein in maize-mushroom composites, it's crucial to recognize that the mushroom used in the current investigation could contain anti-nutritional elements like trypsin inhibitors (Majesty et al., 2019) that may reduce the bio-availability of proteins.

The decrease in fat content of the different maize-mushroom composites could be due displacement effect of mushrooms. This is because mushrooms have lower fat content (0.38%) compared to maize (4.16%) (Olusanya et al., 2020). Despite the lower fat content, mushrooms predominantly contain unsaturated fatty acid like oleic and linoleic acids (Siyame et al., 2021) which are associated with reduced incidences of non-communicable diseases (Tolera & Abera, 2017). The result of the current study contradicts with results obtained by Nguyen et al, (2020) in which the author reported a significant increase in lipid content of oyster mushroom-bean-barley-Dutch pea-potato formula could be attributed to high fat content in pea (26.3-36%) (Caprioli et al., 2016) compared to oyster mushroom (0.38%). Nevertheless, the percentage reduction (0.2%) in fat content caused by 10% mushroom blending ratio observed in the present study is higher than percentage reduction (0.01%) recorded in bread by Okafor et al., (2012). The difference in the percentage reduction recorded in maize-mushroom composite flour and wheat-mushroom bread could be due to variation in nutrition profiles of mushroom species used. Mushroom species (Pleurotus plumonarius) used to develop wheat-based composite contains 1.9% fat and mushroom species (Pleurotus ostreatus) used to develop maize-mushroom composite contains 0.38% fat content., Furthermore, difference could be attributed to variations in nutrition profiles of cereals used. Notably, the fat content of wheat (2.97-3.54%) (Narducci et al., 2019) is higher compared to that of maize (1.65%). Nevertheless, the fat content (1.29-1.45%) of the different maize-mushroom composites in this study is less than the recommended CODEX standard of 3.3%

maximum fat content in complementary foods. The relative low-fat content of maize-mushroom composites may demonstrate the potential of oyster mushroom offering better rancidity storage stability to the composites.

The increase in fiber content of the different blend of maize-mushroom composites is likely to the fact that mushroom has high fiber content, and hence a good fiber source (Bamidele and Fasogbon, 2020; Ogundele and Bamidele, 2020). Moreover, majority of the fiber in mushroom flour are insoluble (12-21g/100g) (Nile and Park, 2014). Insoluble fiber has numerous health benefits such as increasing the bulkiness, and thickness of stool resulting in reduced occurrences of functional gastro-intestinal disorders such as irritable bowel syndrome characterized by constipation (Lambeau & McRorie, 2017; Yang et al., 2012). This will probably have a favorable impact on the gut health of children taking into account the fact that sufficient amount of fiber in complementary foods has been observed as effective in alleviation of constipation in children as lactulose (Adoko et al., 2021). Furthermore, sufficient amount of fiber plays a crucial role in establishment of healthy micro-flora population during first year of growth and development (Cronin et al., 2021; Holscher, 2017). In comparison, for 30 and 40% mushroom inclusion rate in the Siyame et al, (2021) study, the percentage increment (7.67-9.27%) in fiber content reported is higher than the percentage increment (1.6-3.04%) in the present study. The different could be attributed geographical location effect on mushroom nutrient quality and typed of maize flour used. Though, the increase in fiber content in the present study is similar to result obtained by Gemedede (2020), the fiber content (0.76-3.58%) of different maize-mushroom composites is lower than CODEX threshold (5%) in complementary foods. The low fiber implies that the disruption efficacy of absorption of various nutrients by fiber could be minimal in the different blend of

maize-mushroom composite. Therefore, consumption of the composites by children 6-24 months could lead to adequate absorption of nutrients is vital in alleviation nutrients deficiencies.

The decrease in carbohydrate in the maize-mushroom composites can be attributed to the limited quantity ($67.32\mu\text{g}/100\text{g}$) of carbohydrates found in mushroom compared to maize (Sawangwan et al., 2018). The carbohydrate content (ranged from 83.25-70.77%) observed in the current study is higher than the carbohydrate content (64.25%) recorded in NUTREND developed by Nwadi and Uvere (2019). The different in carbohydrate content in maize-mushroom composites in the current study and NUTREND could be due to effect of germination on the carbohydrate composition of maize grains used to develop NUTREND. In fact, during germination, the catalytic activity of alpha amylase increase, hence more starch is hydrolyzed to simpler and absorbable monomers which is utilized by seedling at an early start of germination (El-safy et al., 2013). Additionally, the carbohydrate contents observed in the different blends of maize and mushroom in the present study are above the CODEX standard (range 64-75%) for complementary flour. The implication of this is that the different blends are adequate to meet the energy requirement of children 6-24 months. The reduction of carbohydrates notably, led to decrease in energy content of maize-mushroom composites. Despite this limitation of energy decrease, theoretically the composites meet more than 40% and 70% of the energy gap for non-breastfed and breastfed children 6-24 months respectively. This result demonstrates the potential of different blends of maize-mushroom composites in meeting the physiological energy need of children 6-24 months.

The current study revealed a decrease in moisture content of maize flour when compounded with mushroom. The decrease in moisture content could be due to decrease in starch levels in the composites as result of mushroom substitution effect on maize fraction in the formulae. In fact, refined maize flour contains 73% starch, and has a strong capacity to bind water as a result of

highly available water binding sites of starch granules (Remya and Jyothi, 2015). The moisture content observed in this study was lower compared to moisture content reported by Fikiru et al. (2016) in maize-roasted pea-malted barley composite. This could be attributed to the difference in starch granules composition between the product developed in the current study and the aforementioned product. It is noteworthy that the lower starch granules composition the lower moisture content of any products. For instance, Enriched Weanimix had lower moisture content compared to maize-bean composites. This could be ascribed to the lower starch granules composition (46.72%) in Enriched Weanimix (Kweku Amagloh et al., 2013) compared to maize-bean composites with 52% starch granules composition (Ndagire et al., 2015). The low moisture content implies that consumption of maize-mushroom composite may provide children with substantial nutrient that are usually deficient in the diet at home. According to Fikiru et al. (2017), cereal-based foods prepared from cereals with 3–8% moisture content have higher quality standards.

Increasing mushroom ratio increased ash content of the composites, and is most likely as a result of the higher mineral content (8.8%) of mushroom (Majesty et al., 2019; Tolera & Abera, 2017). This result corroborates with results obtained in previous studies. For instance, Farzana and Mohajan (2015) investigation reported that incorporation of mushroom and soy in wheat had a favorable impact on the total ash content of the composite. This is majorly due to the high mineral content of mushroom and soy bean. Nonetheless, the increase (37.3-277.1%) recorded in this study is higher than the increase (11.0-17%) observed in maize-pea-anchote composite reported by Gemede (2020). This could be attributed to difference in total mineral composition of oyster mushroom used in this study and pea & anchote used in the aforementioned product. In fact, oyster mushroom has higher ash content (6.37%) (Thinley Gyletshen, 2019) compared to pea and anchor

which has 2.56% and 3.87% ash content, respectively. The high ash content implies that mushroom could be more suitable for enhancing total mineral contents of maize than legumes. The increase in the level of total minerals in the different maize-mushroom composites indicate that these composites would contribute to the children's overall micronutrient intake. For instance, calcium, zinc, iron, and Magnesium levels were found to be greater in foods containing mushroom (Bamidele and Fasogbon, 2020). This is plausible due to high concentration of calcium, magnesium, iron, phosphorus, and zinc in mushroom (Majesty et al., 2019). However, the amount of these nutrients was determined in the flour not in the porridge, therefore, the impact of food preparation on the content of these nutrients was not examined. Therefore, there is need for future focus on the impact of food preparation on nutrients content of the composites.

Complementary foods should supply 30 to 97% of the overall daily requirement of micronutrients (Abeshu, Lelisa, et al., 2016). Mushroom flour is a good source of zinc, and in this study, it has been adduced that ingestion of 100g of blends containing 10% and 20% mushroom inclusion would account for 44.22% and 74.89% of RDA of zinc respectively. This means that consumption of maize-mushroom composite may alleviate zinc deficiency among children and ought to be encouraged in areas where maize is consumed. The increased level of the zinc concentration in the maize-based composites after being compounded with oyster mushroom supports the conclusions of earlier research. Common examples are studies that showed a strong positive correlation between increase in zinc concentration and the percentage of substitution effect of mushroom in the various blends (Farzana and Mohajan, 2015; FO et al., 2019; Irakiza et al., 2021; Ishara et al., 2018). The RDA of zinc for children aged 6-24 months reported in the current study is lower than that reported by Bamidele and Fasogbon (2020). This could be attributed to substrate effect on the zinc composition of the mushroom. In fact, Hoa et al. (2015) noted that zinc content of mushroom

grown on sawdust is lower than zinc content of mushroom grown on sugarcane bagasse. On the other hand, 10% and 20% mushroom inclusion account for less than 51% RDA of iron for children 6-24 months. Nonetheless, contribution of 30% mushroom inclusion in the present study towards RDA of iron is higher than the contribution of maize-bambara ground nut malt composite and maize-cowpea malt composite as recorded by Nwadi and Uvere (2019). This could be attributed to the higher iron content in mushroom compared to cowpea and bambara ground nut malt. This implies that mushroom is more effective in enhancing the iron content of maize-based complementary foods than bambara ground nut and cowpea. The amount of anti-nutritional factor, however, that might have an impact on the bioavailability of iron, zinc, and other nutrients in the blends were not examined. Therefore, future should examine the effect of antinutritional factors on the bioavailability of nutrients of the composites. In general term, the current study has demonstrated clearly that when mushroom flour is added to maize flour, it is not only the content of micro-nutrients like iron and zinc that increase but also other crucial nutrients such as protein necessary for healthy children's growth. Extrapolating from this, it might be hypothesized that the promotion of consumption of maize flour blended with mushroom may increase intake of key micro-nutrients such as iron and zinc by children 6-24 months.

5.2 Effect of blending maize flour with mushroom on microbiological quality of maize-mushroom composite

The microbial load of any food material is a helpful indicator of microbiological quality as well as providing information about the products' potential safety for human consumption. The higher total microbial load found in maize-mushroom composites at each sampling point is most likely a result of the higher nutrient contents caused by the addition of oyster mushroom, as microbial load is influenced by nutrient content (Adeyeye et al., 2019). It is plausible to suggest that the higher

level of microbial load detected in the different maize-mushroom composite was mostly caused by mushroom since it is the main source of protein. Mefferd et al. (2020) reported that proteinous diets encourage growth of spoilage microbes than non-proteinous foods. The composites had higher contribution of yeast and mold to total microbial counts, which makes maize-mushroom composite more susceptible to fungal attack (Irakiza et al., 2021b). In comparison, the total microbial count in the current study is higher than the total microbial count noted by Adeoye et al. (2019). This could be attributed to variation in genetic structure of mushroom (Owaid et al., 2017) which makes it prone to microbial attack. Complementary composites with microbial loads of 4 to 5 log CFU/g or less are microbiological safe for consumption, whereas those with microbial loads of 6 log CFU/g or more are undesirable (Lohia and Udipi, 2015). The current study demonstrates that, the microbial counts of blends were within the acceptable limit. This suggests that, from a microbiological perspective, the combination of maize and oyster mushroom are microbiologically safe for consumption for children.

5.3 Effect of blending maize flour with mushroom on physico-chemical quality of maize-mushroom composite

The viscosity, water and oil absorption capacities, bulk density, compressibility index, compact density, and pasting properties of the composite flours were examined because they have effect on consumer sensory preference and acceptability (Ekpa et al., 2019). Food viscosity has drawn a lot of attention as one of the crucial sensory qualities. When compared to the viscosity of the maize-mushroom composites, the control flour had a higher viscosity. The gelatinization that occurs as a result of structural changes in starch granules causes the viscosity to increase (Chakraborty et al., 2022), which is likely the cause of the increased viscosity observed in blend K11 without mushroom. These alterations include water absorption, irreversible starch granule enlargement,

crystallite melting, and amylose leaking out which led to higher viscosity. However, retrogradation happens at roughly 40 °C when cooling porridge, as result of molecular reassociation. This usually causes viscosity of porridge to increase. However, the viscosity of maize-mushroom composites decreased as the oyster mushroom ratio increased. Therefore, the likelihood of retrogradation occurring is expected to reduce. The viscosity observed for the maize-oyster mushroom composites in the present study is lower than that reported by Ishara et al., (2018) for maize-button mushroom composites. The difference in viscosity could be attributed to interspecies starch composition difference in mushrooms. Oyster mushroom has lower amylose content (49.3g/100g of starch) (Alobi et al., 2017) compared to button mushroom which is largely responsible for increased viscosity during gelatinization. Furthermore, the variation in viscosity between the two products could be due to substrate effect on starch composition of the mushroom species. Nevertheless, excessive viscosity, low energy density, and poor nutrient quality are the three main characteristics of traditional complementary porridges from Africa (Oladiran and Emmambux, 2020). These characteristics have frequently been linked to the development of micronutrient deficiencies (Abeshu, Lelisa, et al., 2016). According to Oladiran and Emmambux (2020) children can consume porridges with a viscosity of 1-3 Pa.s. For instance, porridge served to Congolese children 4-11 months had a viscosity of 0.5 to 2.8 Pa.s (Ishara et al., 2018). However, the viscosities of composites were the within the recommended level (approximately 1-3 Pa.s.) for the complementary porridges intended for young children by adding mushrooms. Therefore, this implies the porridge prepared from the composites is easily consumed by children aged 6-24 months.

The ability of food material to absorb water during processing is indicated by its water absorption capacity (WAC), and this ability depends on the amount of protein and carbohydrate polymer

present in food products (Godswill et al., 2019). The decrease in WAC of maize-mushroom in the current study with increase in mushroom ratio, could be because the polymeric protein and carbohydrate molecules in oyster mushrooms have molecular structures that prevent water absorption. The WAC of acha-mushroom composites reported by Ayo et al. (2018) contradicts with the WAC observed in maize-mushroom composites in the current study. This could be attributed to variation in protein structural components of different genetic structural of mushroom used. In comparison to previous studies, 10% mushroom incorporation produced a composite with a higher WAC. The higher WAC of K22 indicates that 10% mushroom can be utilized to make other food products such as sausage, dough, processed cheese, and bakery goods with high binding property, desired finished product consistency and characteristics could be obtained.

On the other hand, flours' oil absorption capacity (OAC) has been recognized as being crucial to food formulation and sensory qualities. OAC of the flour refers to the protein's capacity to physically bind fat through capillary attraction (Godswill et al., 2019). This property is crucial because fat is widely known for retaining flavor and enhancing the mouthfeel of foods (DiPatrizio, 2014). The fact that maize-mushroom composites had greater OAC values may be due to high degree of amino acids non-polar side chain of the protein composition of the composite, which may bind the hydrocarbon side chain of the oil in the flours. Protein, which is made up of both hydrophilic and hydrophobic components, is the main chemical factor affecting OAC (Séré et al., 2021). Non-polar amino acid side chains have the potential to bind hydrophobically with lipid hydrocarbon chains (Godswill et al., 2019). The result recorded in the current study is also comparable with previous studies of (Arise et al., 2019; Ukeyima et al. 2019) on ABARI composite and maize-garden pea composite respectively. However, Ukeyima et al. (2019) observed a lower OAC in maize-garden pea composites compared to the current study. A plausible explanation for

this contrast could be due to variation in protein structure of the individual ingredients used. The higher OAC demonstrates the potential of mushroom to be helpful in food structure interactions, specifically in flavor retention and palatability improvement of foods which could increase consumption of nutrient dense maize-mushroom composites.

The lower bulk density (BD) of the flour indicates that relative amount of maize-mushroom composite in a package would not degrade significantly during storage, since it depends on solid particles heaviness present (Alawode et al., 2017). In food sector, it aids in determining the various packaging needs, material handling, and applications (Alamri et al., 2021). The lower BD content may be due to reduction in solid particle heaviness of the different blends of maize and oyster mushroom in the present study. The most prevalent polysaccharide in mushrooms, beta-glucan, has also been linked to a decrease in bulk density when compared to maize flour, which comprises of starch (amylose and amylopectin) as a polysaccharide (Jantaramanant et al., 2014).

Compact density (CD) of a powder is an accurate representation of its dense packing. CD of Maize-mushroom flour was found to be roughly 21% greater than its bulk density. This is because of the size, solid density, geometry, and surface characteristics of the flour (Iwe et al., 2016). The non-cohesive qualities of the material are indicated by the low compact density of the maize-mushroom composite. A higher CD compared to BD is appropriate for the packaging, and more material is packed in a given quantity of space (Verma et al., 2022).

Flowability of a flour is gauged by the compressibility index. The CBI reveals the capacity of flour to lose volume. The results of CBI of maize-mushroom composite flour were more than 15% for good flowability. This could be a result of stability and strength of the flour bridges (Barretto et al., 2021) as result of stronger magnitude of Van Der Waal forces of attraction, and hence the composites flour exhibits poor flowability. Poor flowability is represented by a CBI (%) score of

23 to 31 (Falade et al., 2019), coupled with decrease in water absorption capacity as exhibited in this current study could negatively impact consumers acceptability.

The different results of peak viscosity (PV) of maize-mushroom composites in the current study show variations in starch gelatinization levels and amylose content of the composites. According to Raigar & Mishra (2017) high PV is closely related to high starch degradation, which in turn increases viscosity. The results of peak viscosity observed in the present study is lower than that recorded in maize-plantain composite and maize-cooking banana composite by Agbaje et al., (2022). This could be attributed to differences in the rate of water absorption and starch granules swelling during heating. These results imply that, in comparison to the other maize-mushroom composites in the present study, the composite containing 10% mushrooms will have a higher gel strength and a propensity to create gels during cooking or reconstitution. This suggests that the composite with 10% mushroom would be suitable for making complementary food gruel. The lower breakdown viscosity (BV) of maize-mushroom composites suggests that the composites will be more able to withstand heat and shear stress during cooking (Adams et al., 2019). This indicate that swollen granules of maize-mushroom composites will have good stability against mechanical shearing due to resistance to swelling disintegration. The difference between the final viscosity (FV) and the trough viscosity is known as the setback viscosity (SBV). In addition to reflecting the interaction between leached amylose chains during the cooling cycle and the presence of intact and/or fragmented granules embedded in the amylose network, Yan et al. (2021) showed that SBV monitors recrystallization of gelatinized starch during the cooling phase. Retrogradation of starch molecules are associated with this phase, which is also referred to as the setback area. The SBV is typically thought of as an indicator of the likelihood for the paste made from a starchy food to retrograde (Yan et al., 2021) and the tendency to retrograde increases with increasing value of

SBV. Mushroom addition, however, decreased the SBV of maize-mushroom composites. This implies that there will be significant granule disturbance during the heating cycle and minimal resistance to the stirring of paddles action during the cooling cycle. Reduced SBV therefore indicates a decreased tendency for retrogradation and syneresis together with increased digestibility (Denchai et al., 2019) of the starch in maize-mushroom composites. An essential factor in determining the final textural quality of food in terms of its hardness and elasticity is the final viscosity (FV). The reduction in FV after addition of mushroom may be because of reduction in the amount of the starch molecules particularly amylose molecules (Amagloh & Coad, 2015; Yadav et al., 2014). The conclusion that can be drawn from these observations is that the composites that demonstrated relatively low values of FV, may be the most suitable for developing weaning foods attributed to the porridge smoothness and softness for easy swallowing by infants. Therefore, complementary foods can be produced using maize-mushroom composites. On the other hand, due to the granule size of the composites, the high pasting temperature of maize-mushroom composite suggests that the blends exhibited a strong resilience to swelling and breakage (Chakraborty et al., 2022).. The pasting temperature gives an estimate of energy cost and minimal temperature needed to cook the sample. The simplicity of product of preparation is indicated by the low peak time (Godswill et al., 2019). Hence maize-mushroom is easy to cook as demonstrated by the pasting time of the present study.

5.4 Effect of blending maize flour with mushroom on sensory attributes of maize-mushroom composite

Consumer acceptability is a crucial variable that has been linked to the success of food-based nutritional innovations in society (Guiné et al., 2020). The degree of consumer preference for the

specific sensory qualities of food product is a major determinant of acceptability. The nature of the various ingredients utilized in the formulation, the blending ratios, and the processing techniques (Otolowo et al., 2018) used all have a significant impact on the sensory qualities (Fikiru et al., 2017). Therefore, the negative sensory appeal brought about by the inclusion of mushroom could be the reason why nutritionally enhanced maize-mushroom composites were not accepted. The results on color contradicts with the results reported by Farzana and Mohajan (2015), where consumers liked the color of soy-mushroom biscuits. The difference in the findings could be due to the masking effect of soybean used in that study on mushroom. Thus, porridge of maize-mushroom composites had a poor color compared to the control which could negatively affect its consumption among children 6-24 months. Aroma is sensory quality that can sometimes have a significant impact on how well-liked foods are, and it is influenced by the ingredient used in the formulation (Kiefl and Schieberle, 2013). The aroma that comes from mushroom is less liked by people (Ng et al., 2022). Therefore, the presence of mushroom may be the reason why the nutritionally enriched maize-mushroom scored lower for aroma. The unpleasant smell and odor could be due to Lanthionine and sulphur containing-S-alkyl cysteine in mushroom (Berger and Zorn, 2004; Deepalakshmi and Mirunalini, 2008; Moliszewska, 2014). These compounds might have negatively altered the aroma of the composites. Additionally, the lower sensory rating for aroma of the nutritionally enhanced maize-mushroom may have been influenced by little fat content observed in the current study. Fundamentally, high fat content of food improves texture, flavor, and aroma. This claim is supported by the fact that high fat content increases food palatability and hence enhancing its acceptability (Johnson and Wardle, 2014). The taste of any product is the main determinant of its acceptability and has the greatest influence on its likelihood of commercial success. According to Teferra et al. (2015), the sensation of taste is a chemical

process that involves stimulation of taste receptors by taste stimuli. Therefore, the higher starch content of maize flour (Mlotha et al., 2016) and the associated chemical processes as a result of heat treatment could be the cause of higher scores for taste observed in the control compared to the composites. Additionally, the lower sensory score for taste could be explained by the intense aroma of mushroom. The lower rating of after-taste of the maize-mushroom composites could be due to slight bitter after-taste of mushroom as evidenced in the present study on the bitter after-taste of the blends with mushroom. The result of mouthfeel found in the current study could be due to intense mushroom flavor, and thus, the composites had a poor mouthfeel. Additionally, the lower rating for mouthfeel could plausibly be explained by low fat content of maize-mushroom composites as found in this study. Okafor et al. (2012) reported that mushroom inclusion in wheat flour resulted in lower rankings for the overall acceptability of wheat-mushroom bread. This is consistent with the result of the present study, which shows a significantly lower score in terms of overall acceptability for maize-mushroom composites. Indeed, this offers more proof of how mushroom contributes to the unpleasant sensory appeal of the composites. However, this study contradicts with findings of Adeoye et al. (2019) in that 10% mushroom inclusion did not affect overall acceptability of the composite. The present study further recorded that addition of $\leq 20\%$ mushroom had the best sensory rating compared to composites with $> 20\%$ mushroom inclusion. In comparison to the present findings, Okafor et al. (2012) found that adding more mushroom powder to an 85:15 composite of wheat and mushroom flour resulted in lower ratings for the bread's aroma, flavor, texture, and overall acceptability. As a result, compounding maize flour with the smallest amount of mushroom flour ($\leq 20\%$) may produce complementary flour blends with better sensory qualities for children aged 6-24 months.

Nevertheless, it is not unique to this study that the blending ratio have a detrimental impact on consumer sensory appeal. For instance, Ishara et al. (2018) also reported that compounding maize with different mushroom species negatively impacted on the sensory appeal among panelists. Unfortunately, there hasn't been much focus on enhancing the sensory quality of composites. The observed negative sensory effect is a potential factor that should be carefully taken into account in future attempts to improve the efficacy of the mushroom for enhancing nutrient contents from plant-based foods, given that acceptability of food is largely driven by sensory appeal as opposed to nutritional quality (Imtiyaz et al., 2021).

5.4.1 Influence of Physico-chemical properties of maize-mushroom composite on caregivers' acceptability of maize-mushroom composite

The Physico-chemical properties such as viscosity, water and oil absorption capacities of composites flour have been the main focus in the effort to improve quality of complementary foods. This is primarily due to their influences on consumer's acceptability of complementary foods (Ekpa et al., 2019).

Food viscosity has drawn a lot of attention as one of the crucial sensory qualities and hence become crucial during complementary feeding. Fundamentally, viscosity of complementary foods affects acceptability. As noted in the current study, the negative effect of decrease in viscosity on acceptability of maize-mushroom composite could be due to thinness of the porridge of the composite compared to the control. This was evidenced by caregiver's complain about the thinness of the porridge of the composites as they are more familiar with viscous porridge. This finding is consistent with Konyole et al. (2012) study in that the author reported higher preference for thicker porridge by caregivers primarily due to its being heavy. The effect of viscosity on caregivers' acceptability of the composite's porridge is higher in the present study compared to the effect of

viscosity on maize-winged termites composites by Ezeocha et al., (2020) and maize-soya-whole millet composites by Akinjayeju et al., (2019). This could be attributed to differences in viscosity of maize-mushroom composites and the aforementioned products. The unfavorable impact of viscosity may have an impact on infants' acceptability of the composites due to caregiver's unfavorable response. However, from a nutritional perspective, this provides an opportunity to increase the viscosity of the porridge to the recommended viscosity of porridge for children by WHO (Abeshu, Lelisa, et al., 2016) by adding more flour. By implication therefore, the porridge will have a higher nutrient density per meal and hence increasing nutrient intake among children aged 6-24 months.

Water absorption capacity of flour determine desired consistency and swelling power of the porridge, which in turn affect the overall acceptability. The positive impact of water absorption capacity (WAC) of the composites could be due to desired viscosity, softness, and smoothness of porridge produced. The result of the present study is comparable with previous study (Ezeocha et al., 2020). For instance, 10% mushroom had a lower percentage reduction (10%) of caregivers' acceptability than the percentage reduction (12.2%) recorded by Ezeocha et al., (2020) when 10% winged termite is included in maize flour, could be due to variation in viscosity of the two products as result of difference in water absorption capacity of the aforementioned products. In fact, composites with 10% mushroom in the present study had higher WAC (274.49%) than WAC (30%) recorded in maize-winged termite composites (Ezeocha et al., 2020) as result the viscosity of maize-mushroom composites was higher than maize-winged termites composites.

OAC of flour increases palatability of foods; and hence it improves sensorial appeal especially mouthfeel and flavor which in turn enhances food acceptability (DiPatrizio, 2014). However, the negative impact of OAC of the composites on caregivers' acceptability could plausibly be due to

effect of mushroom on mouthfeel and aroma rendering the composites with poor mouthfeel and aroma.

6.0 CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1. Conclusion

The current study investigated how mushrooms affected the qualities of maize-mushroom composites and how their physico-chemical characteristics affected how well the composites were generally accepted by consumers. These results allow for the following inferences to be made. This study has shown that mushrooms have the potential to be used in complementary foods to address the micronutrient deficiencies of children 6-24 months. The developed maize-mushroom composites offer the optimal macro- and micronutrient contents pertinent for children between the ages of 6-24 months. Additionally, the current study shows that the composites are microbiologically acceptable and that blends of maize and oyster mushrooms are safer for consumption by children 6-24 months. Furthermore, the current investigation showed that the inclusion of mushroom maize enhanced the composite's physico-chemical properties, specifically viscosity relevance for children 6-24 months. The sensory qualities of the composites, specifically their color, scent, taste, aftertaste, and mouthfeel, were greatly reduced, but this did not lower overall acceptability of the composite which was comparable to maize porridge commonly consumed by the participants studied. Therefore, the inclusion of mushrooms should not make up more than 20% of the composite in order to produce an acceptable maize-mushroom composites that is nutritionally enriched. Despite these benefits, maize-mushroom composites' enhanced physico-chemical characteristics had a detrimental impact on consumers' overall acceptability and put them at a lower acceptance and use in society. This indicates that promoting the nutritionally improved maize-mushroom composites will probably have problems with acceptability.

6.2. Recommendation

6.2.1 Recommendation for practice

To effectively benefit from maize-mushroom composites, households and caregivers should blend maize with 10-20% mushrooms for breastfed infants.

To ensure that caregivers have thorough understanding of mushrooms, community-wide training is necessary. Such training should concentrate on educating caregivers about (i) mushroom cultivation to eliminate seasonality dependence and (ii) the nutritional advantages of using mushrooms.

To encourage widespread adoption and usage of the formulae produced in this study, long exposure is needed. This can be achieved by carrying out community children feeding trials for long period of time.

6.2.2. Recommendation for future studies

The observed negative sensory effect is a potential factor that should be carefully taken into account, and hence future research should be tailored more toward improving sensory quality of the maize-mushroom composite.

Microbial stability during storage was not examined, therefore, future research could focus on ascertaining the effect of blending maize flour with mushroom on storage stability of maize-mushroom composite.

The amount of nutrients was determined in maize-mushroom flour not in the porridge; therefore, future studies should focus on optimizing the effect of traditional food preparation on nutritional composition.

The amount of anti-nutritional factor in the composite, that might have an impact on the bio-availability of iron, zinc, and other nutrients in maize-mushroom composite was not assessed.

Future research should focus in this area.

6.2.3. Recommendation for policy

The study recommends that, the government should tailor public investment toward the use of local resources and technologies in alleviating micronutrient deficiency.

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APPENDICES

Appendix I: Laboratory data collection tool for parameters being analyzed

The maize and oyster mushroom blends, together with the control (maize flour), are used for laboratory analysis, primarily for microbiological, physico-chemical, and nutritional content purposes. The results on the quality of the maize-mushroom composite for children between the ages of 6 and 24 months are given in the table below.

Parameter	Sample (results indicate means $\pm$ SD of samples analyzed in triplicates)				
Proximate analysis	K11	K22	K33	K44	K55
Moisture content					
Crude protein					
Crude fat					
Ash content					
Crude fiber					
Crude carbohydrate					
Energy level					
Mineral analysis					
Calcium					
Zinc					
Magnesium					
Iron					
Microbial analysis					

Total microbial load					
Mould and yeast load					
Physico-chemical analysis					
Viscosity					
Oil Absorption Capacity					
Water Absorption Capacity					
Pasting properties					
Compressibility Index					
Bulk density					
Compact Density					

Appendix II: Sensory Evaluation Form

I'm **KILAMA GODFREY**, a student of Gulu University conducting research on the topic: Effect of blending ratio of maize and mushroom on nutritional quality maize and mushroom porridge.

You will be given four different formulations of maize-mushroom composite porridge and maize flour to sample as part of the study. After tasting each one, please rate them according to your level of preference using the scale below.

You are given fresh water to rinse your mouth with before and after tasting each sample, and you are asked to rate how much you liked each one.

ADMINISTRATIVE DETAILS

Date	Household number
Questionnaire identity Number	Team leader
Team number	Questionnaire check by:
Village and sub-county name	Socio-demographics

SCALE

1. Very dislike, 2. Dislike, 3. Neither like nor dislike, 4. Like, 5. Like strongly

Sample code	Aroma	Color	Taste	Mouthfeel	After-taste	Overall acceptability
624						
830						
501						
574						
152						

Thank you for your participation

Appendix III: Informed consent form

Title of the study: Effect of blending ratio of maize and mushroom on quality of maize-mushroom porridge.

Investigator(s): Kilama Godfrey

Institution(s): Gulu university

Introduction

Good morning/afternoon! My name is (Interviewer mentions his/her name)

I'm a student of Gulu University and I'm conducting research on the topic: **Effect of blending ratio of maize and mushroom on quality of the maize-mushroom porridge**

The Gulu University Research Ethics Committee has approved the study (GUREC).

You will be given five different types of composite porridge to try during the study. You will be asked to rate each one according to how much you liked it using the aforementioned scale. The study included 66 caregivers in all.

You will be given clean water to rinse your mouth with before and after tasting each sample, and you will be asked to rate how much you liked each one.

Please feel free to ask any questions you may have at any time regarding the study. You will be asked to sign a consent form, which you will be given a copy of to keep, once the study has been described, any questions you may have been answered, and you have agreed to participate in the study.

1.1.1 Purpose:

The goal of the study is to increase the micronutrient consumption of supplemental foods made from maize by children aged 6-24 months. The results of this study are anticipated to support national initiatives focused at reducing micronutrient deficiencies, notably iron and zinc insufficiency among children in Uganda between the ages of 6 and 24 months.

1.1.2 Procedures

You will be required to prepare the porridge for each sample as part of your participation in this study. You will be given the made porridge in a plastic cup and asked to sample it and rate how much you like the individual ingredients for the various formulations.

Because you are a caregiver of children aged 6-24 months, you have been selected to take part in this study.

1.1.3 Risk/discomforts

You cannot reasonably expect any harm or pain as a result of taking part in this study. This is because there is less chance that an allergic reaction may result from the composites' constituents. Allergy sufferers, however, would not be permitted to take part in the trial. Additionally, because the evaluation will be conducted in a hospital setting, medical experts will be on hand to assist the participant if they experience any discomfort while tasting the various composite formulae. In addition, participants are able to leave the study whenever they want.

1.1.4 Benefits

Therefore, we are asking for your permission to take part in the study. Your participation at every stage of the research will teach you how to combine and cook the various ingredients to create wholesome complementing foods. Additionally, this will aid in lowering the likelihood of childhood malnutrition in your household and the larger community. Additionally, your participation in the study has no financial or other personal benefits associated with it.

1.1.5 Confidentiality

No one will know your identity because we'll use codes to identify participants. Only the research team will have access to the data collected. Hard copy files will be maintained behind closed doors, and password protection will be used to protect soft copies of the data. Only the lead investigator will have access to confidential data.

1.1.6 Alternative

You do not have to participate in this study if you are not interested

1.1.7 Cost

If you agree to take part in the study, there will be some additional expenditures involved, such as time and any equipment you need to prepare porridge.

1.1.8 Questions

You can reach the lead researcher, Kilama Godfrey, by phone at 0788258718 or by email at kilamagodfrey632@gmail.com if you have any questions about the study or your rights as a research participant.

1.1.9 Statement of voluntariness

You can choose whether or not to participate in the research study at your own free will. You are free to leave the study at any time without incurring any fees.

Please get in touch with the chair of the Gulu University Research Ethics Committee, Dr. Gerald Obai, at his telephone number of 0772305621, via email at lekobai@yahoo.com or lekobai@gmail.com, or at the Uganda National Council for Science and Technology, located at plot 6 Kimera Road, Ntinda, Kampala, on the number 0414705500.

1.1.10 Statement consent

.....have explained to me what would be done, the dangers, the advantages, and my rights as a participant in this study. I am aware that my choice to take part in this study will not have any impact on me. My identify will remain hidden while this data is used. I am conscious of my right to withdraw at any time. I am aware that signing this form just signifies that I have been told about the research project in which I voluntarily accept to participate. I do not, therefore, surrender any of my legal rights in doing so. I will receive a copy of this form.

Name of child.....

Name of care giver/parent/Gaudian.....

Signature/Thumbprint.....Date.....

Name of witness.....signature.....Date.....

Name of interviewer..... Signature.....Date.....

Appendix IV: informed consent form in the local language (Luo)

Kara tac mi yee. Gin mi akwana: adwogi pa size mi irubu kede obwol ki anyogi ikom mocam, kwidi and pec pa moko pa obwol ki anyogi ma ki rubu.

Lakweda: Kilama Godfrey

Gang Kweda: Gulu University

Nyutt,

Apwoyo coo/apwoyo rii Nyinga (Lakweda wacco nyinge)

An latin kwan mi Gulu University atye ka timo kweda na adwogi pa size mi irubu kede obwol ki anyogi ikom mocam, kwidi and pec pa moko pa obwol ki anyogi ma ki rubu.

Dul ma neno ki kweda I gang pwonye me Gulu University oyee, dul mi Research Ethics Committees (GUREC).

Dong wabi miyo abic calo lanyut pa nyuka ki penyo in pi rwom ma imaro gi kwede, dong tii ki gin matye piny eni we ocung pi mitti ni. Kikweda man dong obi mitto lugwok latini 66 (omi kony). Dong ki bi miyo it wu pii ci uwuk dog wu maleng mapudi piya imato ki dong inge mato nyuka ka dong owacco mitti wu.

Bedagonya me penya ikom gin ma watye ka kwano ne cawa kiken. Inge tyeko pwony, lapeny mongo kiken mi apenya nongo ki gamo kidong, dok iyee I ribbe I pwony man. Dong wabi legi mi yee ketto cingi ikara tac man ma bene ki bi bot wu mogo.

1.1.1 Mitti

Kwan man neno kit kwayi diro mi adwogi pa size mi irubu kede obwol ki anyogi ikom mocam, kwidi and pec pa moko pa obwol ki anyogi ma ki rubu.

1.1.2 Yoo me tic

Lukwed in gin me kwan, gi obi oyubu moko nyuka abic. Lanyut pa nyuka ki bi miyo botti mi abilla wek iwac rwom ma imaro kwede ki diro mapat pat I yoo mapat pat bene. Wa yeri pien in imiyo kony bot lution oyabe kine dwe 6-24 I mwaka.

1.1.3 Lworo

Pe tye gin marac onyo neno mongo marac ikwan man. Kade kabledni, tye lworo mogo ibilo nyuka ni, jo ma gi pe agonya gi twero aa cawa moo kiken labongo dic.

1.1.4 Mugoba

Watye mitto yee wu I kwan man, ribbe ni ikwan man obi wekko I pwonyo kit mi yubu nyuka matye ki moo cam. Mi metto ne, eni bene obi dwokko piny nok pa moc cam ikin lutino ikin pacci madwong. Kony mi lim onyo gin mongo pe pi pwonye man.

1.1.5 Mung/Lakidi ikut

Lanyut pa ngat acel pe obinen ka mongo kiken pien watye ka tic ki namba. Ngec mongo kiken, dul mi ki kweda aye gubi neno kiken. Ki be wabi gwokko jami ki pungu ki kopulu I nyonyo computer. Ladit ma kit me kwedo piny kiken aye obiedo ki kara mi neno ki kweda man.

1.1.6 Tunu tunu

Ka gwok ipe ki mitti ci pe I dony

1.1.7 Acara

Ibi nongo acara ma nok tutwal calo cawa ki jami tic ma ki bi penyi ni eyub nyuka kwade ikwan man.

1.1.8 Lapeny

Ka gwok itye ki lapeny moo kiken ma kwakko kwan eni, obedo twero ni calo ngat ma tye ka kwan man mi penyo ladit maneno ki kweda Kilama Godfrey I cim namba 0788258718 onyo I mel: kilamagodfrey632@gmail.com.

1.1.9 Lok midyere

Dano ducu ma I kwan man tye pi mitti gi labongo dic dok bene nono. Itye ki twero mi aa labongo dic moo dok labongo kit pwod moo bene. Ka itye ki gin moo ma kwakko twero ni ikwan man, ribbe ki won won kom I Gulu University Research Ethics Committee, Doktor Gerald Obai I namba 0772305621 onyo I mel: lekobai@yahoo.com/lekobai@gmail.com; onyo Uganda National council for Science and Technology I pulora namba 6 kimera road Ntinda, Kampala in namba: 0414705500

1.1.10 Yee

.....owacca gin ma mito atima, acara, mugoba ki twero na ikwan man. Atye ki ngec maber ni kwan man pe twero kelo kit acara moo ikom. Inge kwan man, inge tic ki lok man ki bi ngolo ne weng dok atye aromo aa ki I kwan man cawa moo kiken. Angeyo be ni inge ketto cinga I kara tac man, pe dong nongo ni abolo cik ento lanyut ni abedo ikwan mi ki kweda ma aye bedo iye. Kara tac ki kweda man ki bi miyo ki bi miyo mogo botta.

Nying latin.....

Nying lami kony/lanyodo/lagwok.....Cing.....Nino
dwe.....

Nyin latim kweda.....Cinge.....Nino dve.....

Appendix V: Ethical clearance from Gulu University Research Ethical Committee



GULU UNIVERSITY

P.O. Box 166, Gulu (U)
Website: www.gulu.ac.ug
Email: gures@gulu.ac.ug

Tel: +256 471 432 096
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Mob: +256 772 355 621
+256 776 812 147

RESEARCH ETHICS COMMITTEE

14/06/2022

To: Kilama Godfrey

0788258718

Type: Initial Review

Re: GUREC-2022-207: Effect of blending ratio of maize and mushroom on nutritional, microbial and physico-chemical quality of maize-mushroom composite, ,

I am pleased to inform you that at the 81st convened meeting on 17/02/2022, the Gulu University REC, committee meeting, etc voted to approve the above referenced application.

Approval of the research is for the period of 14/06/2022 to 14/06/2023.

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval **prior** to the activation of the changes.
3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC.
4. Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
5. Continuing review application must be submitted to the REC **eight weeks** prior to the expiration date of **14/06/2023** in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study.
6. The REC application number assigned to the research should be cited in any correspondence with the REC of record.
7. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following is the list of all documents approved in this application by Gulu University REC:



No.	Document Title	Language	Version Number	Version Date
1	revised informed consent form	Acholi (luo)	2.0	2022-06-14
2	Revised data collection 2	English	2.0	2022-06-14
3	Approved revised protocol	English	2.0	2022-06-14
4	covid 19 risk management plan	English	2.0	2022-04-30
5	revise informed consent form	English	2.0	2022-04-30

Yours Sincerely,


GULU UNIVERSITY
INSTITUTIONAL REVIEW COMMITTEE
APPROVED UNTIL
 ★ 14 JUN 2023 ★
FACULTY OF MEDICINE
 Dr. Gerald QBAI P. O. Box 166, Gulu
 For: Gulu University REC