NUTRITIONAL COMPOSITION, MICROBIOLOGICAL ASSESSMENT, SHELF-LIFE AND SENSORY PROPERTIES OF WHEAT MUFFINS ENRICHED WITH AFRICAN EMPEROR MOTH (*Gonimbrasia zambesina*, W) CATERPILLAR(S) FLOUR

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A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Award of Master Degree in Food Security and Sustainable Agriculture of Jaramogi Oginga Odinga University of Science and Technology

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DECLARATION AND RECOMMENDATION

Declaration

This research thesis is my original work and has never been submitted in part or whole for the award of degree or diploma in any other university or institution.

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Recommendation

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DEDICATION

To my beloved mother, Mary Atieno and my father, Jeremiah Ouma.

ABSTRACT

Protein malnutrition is a challenge in sub-Saharan Africa. However, a cheap source of protein (*Gonimbrasia zambesina* caterpillars) is currently underutilized in the coastal region of Kenya. The study aimed to promote the utilization of *G. zambesina* caterpillar(s) by enriching wheat muffins with its flour. The specific objectives were: to evaluate the nutritional composition of wheat muffins enriched with *G. zambesina* caterpillar(s) flour; to evaluate the microbial properties of wheat muffins enriched with *G. zambesina* caterpillar(s) flour; to determine the shelf life of wheat muffins enriched with *G. zambesina* caterpillar(s) flour and to evaluate the sensory properties of wheat muffins enriched with *G. zambesina* caterpillar(s) flour. Substitution of wheat flour with *G. zambesina* caterpillar(s) flour was done at five levels with 0% as the control while 5%, 10%, 15% and 20% as the experimental variance. Nutritional composition, microbiological assessment and peroxide values (PVs) for predicting shelf life were determined by standardized analytical methods (AOAC, 2000). The sensory properties of the enriched wheat muffins were evaluated on a 5-point hedonic scale based on five parameters: colour, aroma, taste, texture and overall acceptability. PROC GLM was used to carry out an analysis of variance (ANOVA) to test the hypotheses of the study at 95% confidence level. Enriching wheat muffins with *G. zambesina* caterpillar(s) flour led to an increase in protein content by up to 73.6% for wheat muffins enriched at 20% substitution level. There was a significant difference ($p<0.05$) in the *in vitro* protein digestibility of wheat muffins enriched with *G. zambesina* caterpillar(s) flour. The mineral and tocopherol contents increased with an increase in substitution level. The total viable count (TVC) was <30 colony forming units (CFU)/g, total coliform count (TCC) was <30 cfu/g, *Salmonella* spp and *Staphylococcus aureus* were not detected while yeast and moulds were <30 cfu/g over the 21 days evaluation period. The PVs of enriched wheat muffins increased with a corresponding increase in substitution level, storage time and temperature. The predicted shelf-life of *G. zambesina* caterpillar(s) flour-enriched wheat muffins was significantly different at ($p<0.05$). The sensory parameters evaluated except taste were significantly different ($p<0.05$). Sensory scores for colour, texture, aroma and overall acceptability decreased with corresponding increasing substitution levels. The results of this study implied that enriching wheat muffins with *G. zambesina* caterpillar(s) flour at 10% substitution level has the potential to improve the nutritional profile and health benefits of wheat muffins without compromising acceptability, microbiological quality and shelf life.

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LIST OF ABBREVIATIONS AND ACRONYMS

^{o}C:	Degrees Celsius
AAS:	Atomic Absorption Spectrophotometry
ANOVA:	Analysis of Variance
AOAC:	Association of Analytical Chemist
BMI:	Body Mass Index
CRD:	Completely Randomized Design
EFSA:	European Food Safety Authority
FAO:	Food and Agricultural Organization of the United Nations
FSV:	Fat soluble vitamin
GHP:	Good Handling Practices
GMP:	Good Manufacturing Practices
HPLC:	High Performance Liquid Chromatography
HSD:	Honest significance difference
ICP-OES:	Inductively Coupled Plasma Optical Emission Spectroscopy
ILRI:	International Livestock Research Institute
IVPD:	*In vitro* Protein Digestibility
JOOUST:	Jaramogi Oginga Odinga University of Science & Technology
KDHS:	Kenya Demographic and Health Survey
KEBS:	Kenya Bureau of Standards
MA:	MacConkey Agar

MMUST Masinde Muliro University of Science & Technology

MUFA: Mono-unsaturated Fatty Acids

NAMAMWG: North America Miller's Association Microbiology Working Group

PCA: Plate Count Agar

PDA: Potato Dextrose Agar

PEM: Protein Energy Malnutrition

PUFA: Poly-unsaturated Fatty Acids

RDA: Recommended Daily Intake

SMA: Salt Mannitol Agar

UN: United Nations

w/w: Weight per weight

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

About 25.9 per cent of the world's population, is experiencing hunger or did not have regular access to nutritious and sufficient food as of 2019 with Africa and Asia bearing the heaviest burden (FAO, 2020). An additional 112 million more than in 2019 did not have access to healthy diets in 2020, reflecting the inflation in consumer food prices stemming from the economic impacts of the COVID-19 pandemic and the measures put in place to contain it (FAO, 2022). Despite hopes that the world would emerge from the COVID-19 pandemic in 2021 and food security would begin to improve, world hunger rose further in 2021. The increase in global hunger in 2021 reflects exacerbated inequalities across and within countries due to an unequal pattern of economic recovery among countries and unrecovered income losses among those most affected by the COVID-19 pandemic (FAO, 2022). Therefore, there is a higher demand for highly nutritious food for the most affected; children, women, and the elderly in society (FAO, 2019). Most people in rural Sub-Saharan Africa continue to suffer from protein-energy malnutrition (PEM) since they cannot afford a diet that meets the required levels of essential nutrients with some not able to afford the cheapest healthy diets (FAO, 2020; Mlcek *et al*., 2014; Nantanga & Amakali, 2020). Lack of regular access to sufficient and good-quality protein is associated with stunting and wasting (FAO, 2019). Both acute and chronic forms of PEM exist among these vulnerable groups (Reinhardt & Fanzo, 2014).

Approximately, 7.3% of children under the age of five years globally are currently affected by wasting. This is far above the target figure of less than 5% and 3% of childhood wasting by the years 2025 and 2030, respectively. Globally, the prevalence of stunting has been on a 10.1% decline among children under the age of five years from 2012 to 2018 with 21.9% affected by 2018. However, this falls short of the percentage decline targeted over the same period to reduce the number of stunted children by one-half by 2030 (FAO, 2019). About 36.5% of Kenyans are food insecure with 2.6 million severely food insecure (FAO, 2018). Thus, their access to good-quality protein is limited. Some 9% of women aged 15-49 years are reported to be thin (BMI<18.5 Kg/m2). The prevalence of stunting stands at 26% while wasting is at 4% among children under five years in Kenya (KDHS, 2014; Momanyi *et al*., 2019). Protein malnutrition prevalence is quite

high in Kwale County with some 29.2% of the children being stunted while 13.4% are severely stunted (Ndemwa *et al*., 2017).

The best estimated average requirement for protein and amino acid requirement in human nutrition is 105 mg nitrogen/kg body weight per day or 0.66 g protein/ kg per day (Schönfeldt & Hall, 2012). However, these standards are difficult to attain in sub-Saharan Africa due to food insecurity among vulnerable households. The impact of the COVID-19 pandemic has even made it more difficult to achieve the protein standards since the steps that had been made towards achieving these standards have been reversed (FAO, 2020, 2021, 2022). To tackle food insecurity and protein malnutrition among vulnerable groups, both multi-sectorial and multi-level approaches present the best opportunity since malnutrition is an outcome of a combination of determinants (Gillespie & Bold, 2017). Both the nutrition-specific programs and interventions such as agriculture, food security and, social safety nets and nutrition-sensitive programs and interventions such as micronutrient fortification, dietary diversification and, dietary supplementation should be of serious consideration (Gillespie & Bold, 2017; Ruel & Alderman, 2013).

Entomophagy in all its forms at the household, community, national and global levels can help bridge the existing protein and energy deficit in human food (Dauda *et al*., 2014; Van Huis *et al*., 2013). Edible insects are available and are a sustainable source of food as they can be used alone or along with other sources of food. Thus, insects play a very important role in human diet and nutrition (Ramos-Elorduy, 1997). In Africa, Asia, and Latin America, entomophagy forms part of the majority of the community's culture and heritage (Raheem *et al*., 2019). More than 2000 species of insects are edible around the world with most being an important source of protein (Roos & van Huis, 2017). In Africa alone, over 470 species of insects are being consumed with the central part of the African continent leading in the utilization of edible insects as food. Some of the commonly consumed insects are of the order Lepidoptera, Orthoptera, and Coleoptera (Kelemu *et al*., 2015). In East Africa, entomophagy is much more embraced in Uganda as it is known for the production of *Ruspolia* spp which has provided a good background for entomophagy.

In Kenya, insect consumption is practiced among certain communities. Some of the commonly consumed insects in western parts of Kenya are edible winged termites (*Macrotermes subhylanus*), black ants (*Carebara vidua*), and longhorn grasshoppers (*Ruspolia differens*). Around the Kenyan

coast, palm weevil larvae (*Rhynchophorus phoenicis*) are consumed by most members of the communities. Most of these insects are important sources of protein and other nutrients to members of these particular communities (Ayieko *et al*., 2012; Kinyuru *et al*., 2009; Kinyuru *et al*., 2013). Insects in the order lepidoptera, class Saturniidae are diverse in Kenya even though their consumption is not widespread (Subramanian *et al*., 2017). Saturniids are a rich source of proteins that are adequate in both quantity and quality (Dauda *et al*., 2014; Subramanian *et al*., 2017; Van Huis *et al*., 2013). From the literature reviewed, it has been proved that consumption of 100g/ day of caterpillars can provide up to about 76% of the recommended daily protein requirements in adults (Ayensu *et al*., 2019). They are a rich source of minerals and other micronutrients in comparison to edible grasshoppers and palm weevils. Their fat contents are also fairly reasonable in comparison to most insects e.g. termites and palm weevil larvae (Dauda *et al*., 2014; Van Huis *et al*., 2013).

Consumption of Saturniid caterpillars presents one of the most exciting opportunities to improve the PEM situation in sub-Saharan Africa. Though initially not widespread in Kenya, recent studies have shown that the caterpillars are diverse (Subramanian *et al*., 2017). Some of the Saturniid caterpillars that have been earmarked in the coastal region, semi-humid lake zone, eastern lowland, and central highlands of Kenya include: African emperor moth (*Gonimbrasia zambesina* Walker), cabbage tree emperor moth (*Bunaea alcinoe* Stoll), pine tree emperor moth (*Nudaurelia krucki* Hering), mopane worm (*Gonimbrasia belina*), pallid emperor moth (*Cirina forda*) and speckled emperor moth (*Gynansia maja*) (Subramanian *et al*., 2017). In the coastal region, the African emperor moth (*G. zambesina*) is the dominant species (Subramanian *et al*., 2017). Coastal people locally refer to this caterpillar as "maungu ya mwembe", a Swahili word that loosely translates to mango tree caterpillar. *Gonimbrasia zambesina* caterpillars are associated with the mango tree as their host plant. The caterpillars are harvestable at the 5^{th} and 6^{th} larval instars stages of development. At these instar stages, the caterpillars have significantly higher protein contents (Mbata *et al*., 2002; Van Huis *et al*., 2013). When conditions are optimal, full larval development occurs by late December. The caterpillars are harvested by handpicking. They are then degutted and can either be boiled, dried or roasted (Mbata *et al*., 2002).

Gonimbrasia zambesina caterpillars are seasonal but form part of the human diet when available, especially in central and southern African countries where their consumption is well embraced

(Mbata *et al*., 2002). *Gonimbrasia zambesina* caterpillars can be utilized as an alternative potential source of food to bridge protein and energy deficits in human food as an enrichment ingredient (Clover, 2003; FAO, 2018, 2019; Ramos-Elorduy, 1997). The use of edible insect's flour in fortifying or enriching foods is not a new phenomenon in the bakery industry (Ayensu *et al*., 2019; Dauda *et al*., 2014; de Oliveira *et al*., 2017; Osimani *et al*., 2018). Enrichment/ fortification is performed to improve the nutritional value of food, enhancing health-promoting properties or correcting nutrient deficiencies (Zielińska *et al*., 2021). In food products supplementation, for a food to qualify to be supplemented then it must be consumed in adequate quantities and widely; bakery products made from refined wheat flour are a staple food in many countries (Agrahar-Murugkar, 2020; Awobusuyi *et al*., 2020; Zielińska *et al*., 2021). Enrichment by direct addition of an ingredient during processing not only improves the nutritional value of the bakery product but also leads to changes in sensory properties, microbiological quality and shelf-life of the enriched bakery products (Cappelli *et al*., 2019; Zielińska *et al*., 2021). Thus, the purpose of this study was to develop wheat muffins enriched with *G .zambesina* caterpillar(s) flour at different substitution levels of wheat flour with *G. zambesina* caterpillar(s) flour and to determine the nutritional composition, microbiological quality, shelf life and sensory properties of the enriched wheat muffins.

1.2 Statement of the problem

Protein malnutrition is a challenge of no less magnitude in most rural parts of Kenya due to existing protein-energy deficits in the diet (Momanyi *et al*., 2019). Kwashiorkor, marasmus, and marasmic-kwashiorkor are some of the clinical conditions associated with PEM (FAO, 2022; Oluchina, 2017). It affects mostly children under the age of five years, the elderly, and women who are the most vulnerable (Diane *et al*., 2008; Oluchina, 2017). Poor cognitive, mental and physical development are some of the severe consequences of PEM in children. Among women, it causes anxiety, depression, and poor physical health (Momanyi *et al*., 2019). Among the elderly, protein malnutrition has often resulted in poor physical and mental health, polypharmacy, depression, and eating disorders (Diane *et al*., 2008).

Strategies to bridge protein gaps as an alternative to unsustainable conventional meat production lie in promoting the consumption of *G. zambesina* caterpillars. The caterpillars are currently

underutilized mainly due to their disgusting nature, unappealing sensory attributes and neophobia amid the growing influence of the western culture (Barton *et al*., 2020; Mishyna *et al*., 2020; Subramanian *et al*., 2017). Hence, the need to transform the caterpillar(s) into an unrecognizable state to improve its acceptability (Mlcek *et al*., 2014). To effectively promote its entomophagy, knowledge is required of the potential of *G. zambesina* caterpillar(s) flour as an enrichment ingredient for wheat flour with a low protein content (Purnoma *et al*., 2012). Information on the effect of enriching wheat flour with *G. zambesina* caterpillar(s) flour on the end bakery products is currently limited. Physicochemical characterization, microbiological assessment, shelf life determination and sensory evaluation of *G. zambesina* caterpillar(s) flour-enriched wheat products might reveal the effects of *G. zambesina* caterpillar(s) flour on the enriched products.

Inadequate attention to the improvement of the protein contents of important staple foods such as refined wheat flour is a cause of protein deficiency due to the high consumption levels of refined wheat products (Agrahar-Murugkar, 2020). *Gonimbrasia zambesina* caterpillar(s) provides a desirable option for improving the protein profile of refined wheat flour since its protein content constitutes more than 50% of its dry mass weight (Rumpold & Schlüter, 2013b; Subramanian *et al*., 2017). The caterpillar(s) flour also has minerals, retinol, β-carotene and satisfactory levels of fat unsaturated fats and levels of energy (Subramanian *et al*., 2017; Van Huis, 2013). Therefore, this study seeks to promote large-scale utilization of *G. zambesina* caterpillar(s) by using its flour as an enrichment ingredient in wheat muffins.

1.3 Objectives of the study

1.3.1 General objective

To promote utilization of *G. zambesina* caterpillars by developing and characterizing wheat muffins enriched with *G. zambesina* caterpillar(s) flour and determine its suitability in the baking industry.

1.3.2 Specific objectives

i. To evaluate the nutritional composition of wheat muffins enriched with *G. zambesina* caterpillar(s) flour.

ii. To assess the microbiological properties of wheat muffins enriched with *G. zambesina* caterpillar(s) flour.
iii. To determine the shelf life of wheat muffins enriched with *G. zambesina* caterpillar(s) flour.
iv. To evaluate the sensory properties of wheat muffins enriched with *G. zambesina* caterpillar(s) flour.

1.3.3 Hypotheses

i. Enriching wheat muffins with *G. zambesina* caterpillar(s) flour has no significant effect on the nutritional composition
ii. Enriching wheat muffins with *G. zambesina* caterpillar(s) flour has no significant effect on the microbiological quality
iii. Enriching wheat muffins with *G. zambesina* caterpillar(s) flour has no significant effect on the shelf life
iv. Enriching wheat muffins with *G. zambesina* caterpillar(s) flour has no significant effect on the sensory properties

1.4 Significance of the study

Promoting utilization of *G. zambesina* caterpillar(s) is visualized to help fight protein malnutrition and alleviate food insecurity among vulnerable groups in line with Sustainable Development Goal 2 on zero hunger by 2030 and the Kenyan Big 4 Agenda initiative on food security. This research aimed to create new interest in the role *of G. zambesina* caterpillar(s) flour as an enrichment medium/ ingredient in the bakery industry and subsequently stimulate its commercialization as a bakery food product ingredient. The research will provide important lessons for the food industry for further promotion of entomophagy. It will also generate information and education on the effect of using *G. zambesina* caterpillar(s) flour as an ingredient in wheat muffins formulation on the nutritional properties, microbiological quality, shelf-life and sensory properties of the enriched wheat muffins.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

Insects have been in existence for very many years (Laar *et al*., 2017; Van Huis *et al*., 2013). Some are important sources of food to certain communities whereas some act as pollinators. Other insects also have medicinal value (Kelemu *et al*., 2015; Van Huis *et al*., 2013). This review seeks to draw on a range of scientific research on how the baking industry can develop wheat muffins enriched with *G. zambesina* caterpillar(s) flour as a non-wheat and sustainable source of protein and energy to improve dietary protein diversification to bridge the protein-energy deficit in human food. This will help in alleviating the PEM situation in sub-Saharan Africa.

2.2 Entomophagy

Entomophagy refers to the consumption of edible insects by human beings to achieve their daily dietary needs (Rumpold & Schlüter, 2013b; Van Huis *et al*., 2013). The edible insects are an important resource globally as they play a key role in human nutrition and food security. Individuals of all ages and races and those of low social status practice entomophagy (Gahukar, 2011; Imathiu, 2019; Ramos-Elorduy, 1997; Van Huis, 2013, 2015). Insect consumption has been practiced among various communities and societies of the world as part of their culture and heritage (Gahukar, 2011; Ramos-Elorduy, 1997). More than 2000 species of edible insects are consumed by roughly 3071 ethnic groups (Imathiu, 2020; Ng'ang'a *et al*., 2019; Ramos-Elorduy, 2009; Roos & van Huis, 2017). Usually, the insects are consumed at different development stages, though there is a preference for the immature stage when the exoskeleton is still soft (Ramos-Elorduy, 1997) Some of the edible insects consumed include those of the orders: Lepidoptera, Coleoptera, Orthoptera, Isoptera, Hymenoptera and Homoptera (Bukkens, 1997; Van Huis *et al*., 2013)

Entomophagy is practiced all over the world. However, it is most prominently practiced in the African and the American continents (Gahukar, 2011; Ramos-Elorduy, 2009; Ramos-Elorduy, 1997; Van Huis, 2015). The consumption of insect depends on the abundance and its distribution which is influenced by the various climatic and geographical conditions of the world (Ramos-Elorduy, 2009). Approximately 209 species of insects are eaten daily as either a component of the diet or as delicacies (Akullo *et al*., 2017).

In Europe and North America, cases of insect consumption have been reported despite the negative influence of the media in western countries (Shockley & Dossey, 2013). Dishes such as tortillas obtained from the addition of mealworm larvae (*Tenebrio molitor*) powder in maize flour are an indicator of entomophagy in Europe (Mlcek *et al*., 2014). In a composition by Pliny the Elder, the larvae of the great Capricorn beetle (*Cerembyx cerdo*) were popular among the ancient Romans (Govorushko, 2019). In seasons of abundance, terrestrial and aquatic bugs, butterflies' larvae and sphenarians grasshopper are collected and eaten in Mexico. In the United States of America, *Coloradia pandora* larvae are eaten. Certain species of grasshoppers are packed and exported to the United States for consumption (Ramos-Elorduy, 2009).

In southern Asia, 150-200 species of insects are consumed. Red palm weevils (*Rhynchophorus ferruginous*) are some of the delicacies in most regions of the Asian continent. In Thailand, Brazil and Colombia, different species of adult ants of the order Hymenoptera are eaten just before the rainy seasons. Crispy fried locusts are also a delicacy in Thailand (Melo *et al*., 2011; Van Huis *et al*., 2013). In Africa and more specifically sub-Saharan Africa, entomophagy is more common because most countries are still developing. Insects are found in abundance throughout the African continent and thus an important source of food. Africa alone consumes approximately 500 species of different insects (Kinyuru, 2020; Niassy & Ekesi, 2016; Van Huis *et al*., 2013). Most of the edible insects in sub-Saharan Africa are collected from the wild and are either cooked fresh, boiled or roasted before either storage or consumption (Mbata *et al*., 2002; Mutungi *et al*., 2019). In the western part of Kenya, some of the insects commonly consumed are: longhorn grasshopper (*Ruspolia differens*), winged termite (*Macrotermes subhylanus*), black ants (*Carebara vidua*), lake flies (*Chaoborus edilus*) and crickets (*Acheta domesticus*) (Ayieko *et al*., 2010; Ayieko *et al*., 2012, 2016; Kinyuru *et al*., 2009; Kinyuru *et al*., 2010; Kinyuru *et al*., 2013).

2.2.1 Consumption of Saturniids

According to literature, consumption of caterpillars in Africa is most common in the southern and the central parts of Africa where about 95% of forest dwellers depend on insects as a source of protein (Kelemu *et al*., 2015; Mbata *et al*., 2002). In northern Africa, the literature points to no history of caterpillars' consumption (Kelemu *et al*., 2015). Caterpillars such as Mopane worms (*Gonimbrasia belina*), pine tree emperor moth (*Nudaurelia krucki* Hering), cabbage tree emperor moth (*Bunaea alcinoe*), speckled emperor moth (*Gynansia maja*), shea butter caterpillar (*Cirina*

forda), *Urota Sinope* and *Eumeta cervina* Druce are among the diverse edible caterpillars that are unpopular in Eastern Africa (Kelemu *et al*., 2015; Mutungi *et al*., 2019; Subramanian *et al*., 2017). In Kenya, Saturniids diversity has been reported (Subramanian *et al*., 2017). Apart from being a source of protein, energy, vitamins and minerals, they also contribute to human livelihoods (Dauda *et al*., 2014; Mbata *et al*., 2002; Van Huis *et al*., 2013).

2.2.2 African emperor moth (*Gonimbrasia zambesina*)

This is a moth of the family Saturniidae, genus *Gonimbrasia* and the species *zambesina*. The adult moth has orange wings. The moth oviposit its single clusters of white eggs on the host plant leaves. Usually, mature caterpillars of this moth are large and black in colour with white spots. Their bodies have black spines which they use as a defence tool against predators. The head and the anus of the caterpillars are always black. On the anterior sides of the caterpillar's body is orange spots (Mbata *et al*., 2002).

These caterpillars are abundant in the coastal region though their consumption is not widespread (Subramanian *et al*., 2017). With the increasing demand for more alternative protein sources to bridge the existing protein gap in human food, *G. zambesina* caterpillars can be consumed as the only source of protein or along with other sources of protein to account for a given percentage of daily protein intake (Gahukar, 2011; Kelemu *et al*., 2015). The adult male and female *G. zambesina* moths and their caterpillars are shown in Figures 1 and 2, respectively.

Figure 1: Image of male and female adult African emperor moth (*G. zambesina*) (https://www.shutterstock.com/search/african+emperor+moth)

Figure 2: Image of *Gonimbrasia zambesina* caterpillars feeding on mango leaves

2.2.3 Life cycle of *Gonimbrasia zambesina* caterpillar

The life cycle of the African Emperor moth is presented in Figure 3. Caterpillars are the larval stage of the African emperor moths' life cycle. After mating, the female moths oviposit separately clusters of about 50-300 white eggs on the leaves of the host plant around mid-September (Sekonya *et al*., 2020). The eggs then hatch into larvae after about 10-14 days from the day of oviposition. The larvae has a series of developmental stages from 1st instar to 6th instar which lasts for a period of 3 to 6 weeks to attain full maturity (Mbata *et al*., 2002). Between the 1st and the 3rd instars, the caterpillars are gregarious i.e. they feed and migrate from one leaf to the next in a group of about 30-200 caterpillars (Klok & Chown, 1999). This enables them maintain their body temperature and defend themselves against possible predators (Klok & Chown, 1999; Sekonya *et al*., 2020). During the day, the caterpillars often hide under the leaf to protect themselves from direct sunlight (Frears *et al*., 1999). However, at the 4th and 5th instars, the caterpillars tend to forage in solitary as they prepare to pupate by burying into the sand (Klok & Chown, 1999; Sekonya *et al*., 2020). Before pupation, the caterpillars stop feeding (Sekonya *et al*., 2020). The pupation stage is the longest in the life cycle of the *G. zambesina* caterpillar. It begins in mid-December to mid-September which is approximately 9 months. The pupae metamorphose into adults. The emperor moths live for 3 days during which they mate and do not feed (Klok & Chown, 1999; Mbata *et al*., 2002; Sekonya *et al*., 2020).

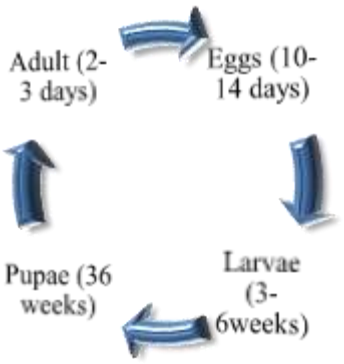


Figure 3: Life cycle of *Gonimbrasia zambesina*

2.3 Insects based bakery products

Among those who eat insects, opinions differ on what the food from insects should look like. Some prefer to eat the insect whole or see insect body parts in food. However, other people who have no strong culture of entomophagy prefer consuming insects in a way that they are not visible (Dobermann *et al*., 2017; Mlcek *et al*., 2014). Save for ones who are allergic, some people can eat insects only if they are eating them in an unrecognizable state (Mlcek *et al*., 2014). Dried insects can be ground into powder/flour which can either be mixed with refined wheat flour, finely ground meat or even mashed potatoes and baked to produced insects based bakery products (Ayieko *et al*., 2010; Dobermann *et al*., 2017; Kinyuru *et al*., 2009; Mlcek *et al*., 2014).

Some of the insect-based food products that have been developed include: bread enriched with cockroach powder, cookies enriched with cricket powder, and wheat buns enriched with edibles termites flour, muffins enriched with cricket (*Gryllodes sigillatus*) and mealworm (*Tenebrio molitor*) flours and pork pâté enriched with cricket powder among other others (de Oliveira *et al*., 2017; Kinyuru *et al*., 2009; Osimani *et al*., 2018; Smarzyński *et al*., 2019; Zielińska *et al*., 2021). In their study, Kinyuru *et al*. (2009) focused on the development of wheat buns enriched with winged termites (*Macrotermes subhylanus*) flour and reported that at 5% substitution level, the enriched wheat buns exhibited an increase in the nutritional content and were acceptable to consumers. A study by Ayieko *et al*., (2010) also focused on the development of meatloaf, crackers, sausages and muffins enriched with either mayfly or termite flour. They found out that the enriched products were generally acceptable and preferred by consumers to conventional insects in the market. Additionally, biscuits fortified with palm weevil larvae (*Rhynchophorus*

phoenicis) and orange fleshed potato have been developed by Ayensu *et al*., (2019) and were also generally acceptable by pregnant women.

2.4 Nutritional value of edible insects and insect based bakery products

The nutritional content of edible insects varies considerably depending on many factors such as the diet of the insect, their habitats, stage of development and the processing methods (Bukkens, 1997; Mbata *et al*., 2002; Mutungi *et al*., 2019; Van Huis *et al*., 2013). Proteins and fats are the major nutrients in edible insects (Belluco *et al*., 2013; Van Huis *et al*., 2013). Generally, the amounts of energy and protein provided by edible insects are capable of satisfying body needs (Subramanian *et al*., 2017). Insects are also high in the desirable unsaturated fatty acids i.e., monounsaturated fatty acid (MUFA) or polyunsaturated fatty acids (PUFA). The amino acid contents in the edible insects are adequate to meet the human requirements of the same. Edible insects are also a source of manganese, copper, zinc, phosphorous, iron, selenium, zinc, phosphorus, pantothenic acid, biotin, riboflavin, cobalamin and folic acid in certain cases (Belluco *et al*., 2013; Bukkens, 1997; Mishyna *et al*., 2020; Roos & van Huis, 2017; Rumpold & Schlüter, 2013a; Van Huis *et al*., 2013)

In the literature reviewed, insects including lepidopterous larvae are high in protein content. The protein is both good quality and quantity e.g *G. zambesina* larvae protein content accounts for more than 50% of its total dry body weight (Belluco *et al*., 2013; Cavallo & Materia, 2018; Roos & Van Huis, 2017; Subramanian *et al*., 2017; Van Huis *et al*., 2013). Processing methods has got an effect on the nutritional composition of edible insects. Thus depending on the processing method adopted, a variation in the nutritional content is likely as was the case in processed mopane caterpillars (*G. belina*) (Mutungi *et al*., 2019). Though literature on nutritional composition of *G. zambesina* caterpillars is limited, the available literature is in agreement that members of the genus *Gonimbrasia* are nutritionally rich (Rumpold & Schlüter, 2013a; Van Huis *et al*., 2013).

The incorporation of insect powder into bakery products has shown some remarkable improvement in their nutritional profile from conventional bakery products. A study by Kinyuru *et al*. (2009) on enriching wheat buns with winged termites (*Macrotermes subhylanus*) at 5% substitution levels of the flours showed that the enriched wheat buns had a superior nutritional advantage to the control wheat buns (100% wheat flour). A similar finding by (Ayensu *et al*., 2019) showed that at 70% substitution level of wheat flour with *Rhynchophorus phoenicis* larvae powder, biscuits recorded

higher nutritional content than the control biscuits except for the carbohydrates and crude fibre contents. The fibre content is determined by the nature of the exoskeleton of the insect. Soft skinned insects like *R. phoenicis* are low in fibre while those with hard exoskeleton are high in crude fibre (Mishyna *et al*., 2020).

2.5 Microbiological quality of insects and insect-based bakery products

Despite the high nutritional quality associated with edible insects, certain consumers and various players in the public health sector have had certain concerns about the lack of sanitary and quality control since obtaining microbiologically free insects is hard to achieve (Melgar-Lalanne *et al*., 2019; Nyangena *et al*., 2020). During harvesting, processing, storage and marketing, insects are often exposed to unhygienic conditions thus making them highly susceptible to contamination by microorganisms (Braide & Nwaoguikpe, 2011). There is fear and uncertainty among some quarters of the consumers over the introduction of these insects as an enrichment media in food products that it might compromise the quality in terms of microbial hazards (Belluco *et al*., 2013; Melgar-Lalanne *et al*., 2019; UN, 2015). All the edible insects, like any other food products, are susceptible to either or both the pathogenic and spoilage microorganisms. This can be a result of their water activity and high nutritional contents which acts as a substrate when the condition for the growth of microorganisms are favourable (Imathiu, 2020; Kelemu *et al*., 2015; Klunder *et al*., 2012; Musundire *et al*., 2016; Nyangena *et al*., 2020). Contamination of insect and insect-based products by micro-organisms often leads to deterioration and ultimate loss in quality of the product (Meyer-Rochow *et al*., 2021)

Previous literature has accounts the presence of either pathogenic or spoilage microorganisms in fresh, boiled or dried edible insects (Ayensu *et al*., 2019; Belluco *et al*., 2013; Fasolato *et al*., 2018; Kelemu *et al*., 2015; Musundire *et al*., 2016; Mutungi *et al*., 2019). The microbiological risk of edible insects depends on several factors including the origin of the insect, feed of the insect, method of processing as well as hygiene of insect handlers (Braide & Nwaoguikpe, 2011; Imathiu, 2020; Klunder *et al*., 2012; Mpuchane *et al*., 2000; Mutungi *et al*., 2019; Ng'ang'a *et al*., 2019). Some of the fungal species of microorganisms that have been detected in insects and insect-based products include *Penicillium verrucosum, Aspergillus flavus, Fusarium poae* and *Clostridium* spp. It has also been found that edible insects are potential carriers of pathogens with health implications such as *Salmonella* spp, *Shigella* spp and *Campylobacter* spp (Ayensu *et al*., 2019;

Imathiu, 2020; Meyer-Rochow *et al*., 2021; Mutungi *et al*., 2019). *Klebsiella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus* spp, *Micrococcus* spp, *Pseudomonas*, *Escherichia coli*, and *Fusarium* have also been detected in edible insects and insect-based bakery products (Ayensu *et al*., 2019; Imathiu, 2020; Mutungi *et al*., 2019).

In the determination of the microbial aspect of processing and storage of edible insects, Klunder *et al*. (2012) observed that Enterobacteriaceae and the bacterial spores were detected on mealworm larvae (*Tenebrio molitor*) and house crickets (*Acheta domesticus*). A study by Belluco *et al*. (2013) on the total microbial load of house crickets (*Acheta domesticus*) showed a high number of gram-positive bacteria (10^5 to 10^6 cfu/g) mostly *Micrococcus* spp, *Lactobacillus* spp and *Staphylococcus* spp. Similar findings were observed by Braide & Nwaoguikpe (2011) in their study on the microbiological quality of processed *R. phoenicis* in which they found out that both fungi and bacteria were present in the processed larvae.

Literature review on quality deterioration of edible caterpillar of the emperor moth, *Gonimbrasia belina* by Mpuchane *et al*. (2000) pointed out that most isolates from the samples were spore formers though isolates of gram-positive and gram-negative bacteria were also present in significant numbers. *Aspergillus* spp, *Fusarium* spp, *Penicillium* spp, *Cladosporium* spp and *Phycomycetes* spp were the most observed fungal isolates in their study. According to Kachapulula *et al*. (2018), an investigation on aflatoxin contamination in dried *Gynanisa maja*, *Gonimbrasia zambesina* and *Macrotermes falciger* in Botswana was reportedly present above safe levels. Aflatoxin is an indicator of fungus *Aspergillus* contamination. In the analysis of mycotoxins in edible stink bugs, Musundire *et al*. (2016) identified carcinogen mycotoxin (aflatoxin B1) at low levels in those that were stored in granny bags and dung smeared wooden baskets. These studies also resonate well with literature on the presence of moulds (fungus) in proliferated mopane worms (*Imbrasia belina*) (Mutungi *et al*., 2019). Pathogenic microorganisms on the gut and skin of *Bunaea alcinoe* caterpillar larva that cause foodborne illnesses have also been documented (Kelemu *et al*., 2015; Mutungi *et al*., 2019).

However, good handling practices (GHPs), good manufacturing practices (GMPs) and storage practices have played a significant role in the reduction of the microbial load and decontamination of most insect-based food products to within the acceptable limits (Kelemu *et al*., 2015; Musundire *et al*., 2016; Mutungi *et al*., 2019; Nyangena *et al*., 2020). For instance, in the production of biscuits

fortified with palm weevil larvae (*R. phoenicis*), it was observed that total aerobic counts (TAC) were within the acceptable limits. The cfu/g for the thermo-tolerant Coliform Counts (TCC) was zero for all the biscuits. *Staphylococcus aureus* and *Salmonella* cells were not detected as well as fungi which were zero for all the biscuits (Ayensu *et al*., 2019). This is despite other literature on the microbial quality of *R. phoenicis* indicating that it hosts a range of food microorganisms. Hence confirming the assertions on the GHPs and GMPs (Kelemu *et al*., 2015; Mutungi *et al*., 2019). Despite this observation, it is imperative to ascertain the microbial quality of each product enriched with the novel insect flour to assure the consumers that the product they are due to consuming would not pose health risks to them.

2.6 Accelerated shelf life test of insect-based baked food products

Shelf-life is defined as a period in which a food product retains most of its nutritional and sensory qualities (Gichau *et al*., 2019; Prchalová *et al*., 2016). Shelf life is one of the most important parameters in highly nutritious food production in the wake of a necessity to transform our food systems to achieve food security across the globe (Lartey *et al*., 2018; Prchalová *et al*., 2016). Even though achieving this transformation is a tall order, much effort is still being put in place to explore alternative sources of food with rich nutritional profiles. This entails promoting large scale adoption of entomophagy by enriching other food products with edible insect powder (Lartey *et al*., 2018; van Huis *et al*., 2013). However, edible insects are rich in unsaturated fatty acids whose main components are a series of omega 9, omega 6 and omega 3 (Ahmed *et al*., 2016). Substituting wheat flour with insect flour introduces these unsaturated fatty acids into the final bakery product making them more susceptible to chemical changes

The most universally accepted method for monitoring oxidative stability is sensory evaluation. However, its effectiveness is limited since the panelists need to be well trained, it's costly and requires sensory booths hence difficult to conduct on regular basis (Farhoosh, 2007). As a result, chemical tests such as peroxide value are used to monitor deterioration in the quality of lipid-containing food products. Accelerated shelf-life test by increasing the temperature has been adopted to reduce the time needed to generate data on shelf life since the rate of autoxidation is slow at ambient temperature (Farhoosh, 2007; Manzocco *et al*., 2012). The choice of temperature to accelerate lipid oxidation is because it exponentially increases the rate of reaction (Farhoosh & Hoseini-Yazdi, 2013).

2.6.1 Lipids oxidation

Oxidation of lipids occurs when lipids are exposed to atmospheric oxygen, a process known as autoxidation (Figure 4) (Kontio, 2017). It causes a loss in the nutritional value of food products and also leads to the formation of off flavours and rancidity compounds (Farhoosh & Hoseini-Yazdi, 2013). Lipid oxidation is a process that involves three main steps: initiation phase, propagation phase and termination phase (Ahmed *et al*., 2016). In the initiation step, a free alkyl radical is formed by the removal of hydrogen from a molecule of olefinic acid. In the propagation phase, the alkyl radicals formed during the initiation step react with an oxygen molecule to form peroxyl radicals. The peroxyl radicals then react with the other surrounding molecules of fatty acids to form hydro peroxides and free radicals. These free radicals react with another molecular oxygen in the chain. In the termination stage, alkyl radicals are removed by combining with peroxyl radicals leading to the formation of secondary oxidation compounds such as aldehydes and ketones. These products are non-radical and can be detected by the organoleptic senses (Kiokias *et al*., 2010; Kontio, 2017; Kubow, 1992).

Exposure of bakery products with unsaturated lipids contents to either oxygen, high temperature and light results in their deterioration due to lipid's susceptibility to oxidation (Ahmed *et al*., 2016; Flick *et al*., 1992; Kubow, 1992). This does not only result into loss of nutritional benefits but also reduces the shelf-life of the product (Barden *et al*., 2015). Rancidity resulting from unsaturated fats oxidation is characterized by loss in colour, the development of off-flavour and the production of compounds that are toxigenic, carcinogenic and cytotoxic. This poses serious health concern among consumers and the public health sector (Ahmed *et al*., 2016)

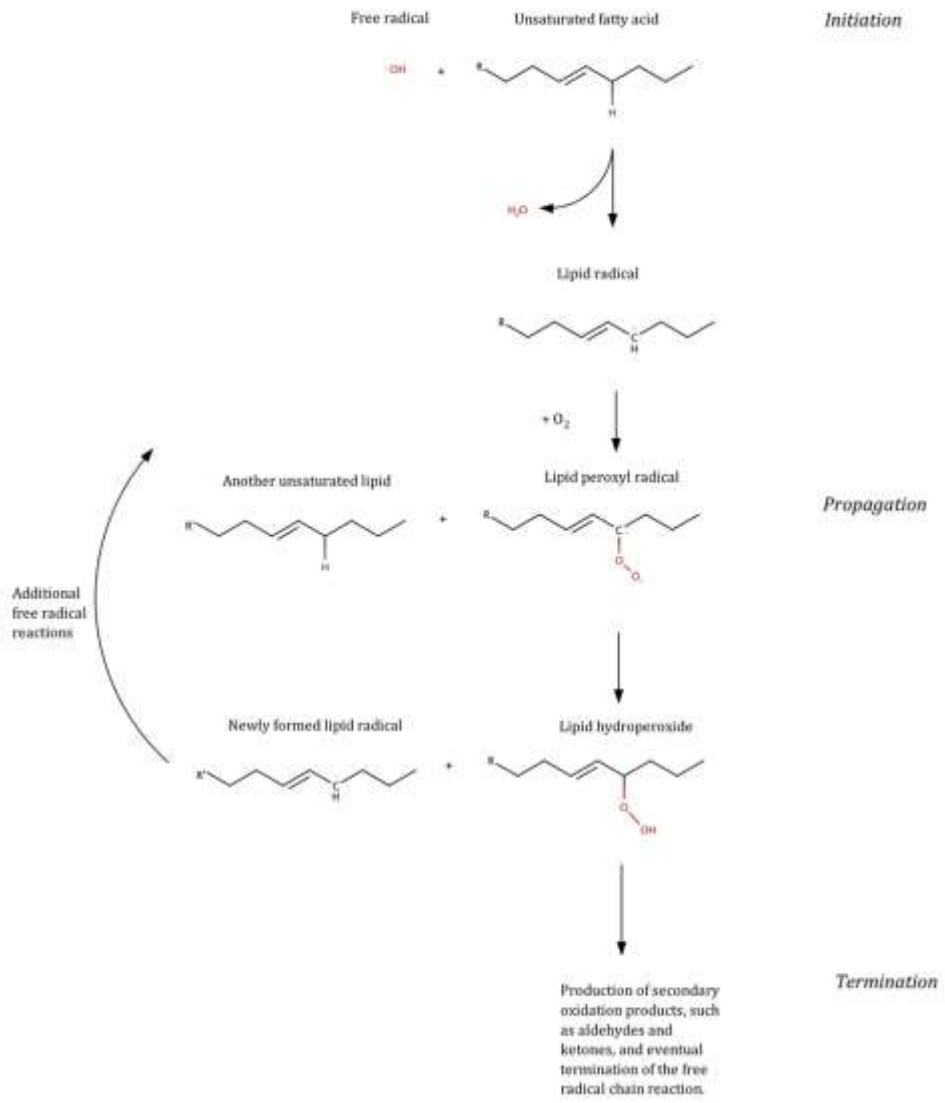


Figure 4: Stages of lipid oxidation (Kontio, 2017)

2.7 Consumer acceptability of insect-based food products

When edible insects and food products containing insect parts are presented to people mostly with no clear background of entomophagy, the emotion of disgust is triggered which is a major hurdle in adopting entomophagy at large scale levels (Ardoin & Prinyawiwatkul, 2020; Dobermann *et al*., 2017; Mlcek *et al.*, 2014). Disgust is an emotionally triggered response of revulsion as a result of ingestion of a food product considered by the consumer as offensive, dangerous or dirty (Castro & Chambers, 2019; Rozin & Fallon, 1987). The low consumption and unwillingness to consume insects and insect-base food products are also attributed to neophobia (hesitancy to try a novel food product which is unfamiliar), unappealing sensory properties and the seasonal availability of

these food products in the market (Barton *et al*., 2020; Mishyna *et al*., 2020). Limited access to information on the benefits of entomophagy is also a contributing factor to low consumption levels (Ardoin & Prinyawiwatkul, 2020). The perception that insects are pests, dirty, disease carriers, disease transmitters, and primitive has also contributed to the emotion of disgust among consumers (Castro & Chambers, 2019; Mishyna *et al*., 2019).

Changing the perception of consumers on entomophagy is one of the major key aspects of ensuring that it is widely adopted. Several academic institutions are engaged in research on the sustainable use of insects as food whereas food industries are involved in the production of insect-based food products (Castro & Chambers, 2019). These are aimed at providing adequate information on the benefits of entomophagy and improving the acceptability of insects as food and their products. It is essential to transform insects into powder or other ingredients and incorporate them into familiar food products for consumers to exploit their high protein and energy contents (Castro Delgado *et al*., 2020). Whenever edible insects are processed into a fine powder and incorporated into other food products such as cookies, buns and biscuits with no visible insect parts, consumers' willingness try to eat the insect based-food is provoked (Castro & Chambers, 2019).

According to research findings by Ayieko *et al*. (2010), it was observed that consumers were much more receptive to wheat muffins enriched with lake flies (*Chaoborus edilus*) powder and *M. subhylanus* flour. They also preferred consuming these enriched muffins to consuming the insects in their original form. Biscuits fortified with palm weevil larvae (*Rhynchophorus phoenicis*) powder at 70% substitution level were also generally acceptable among pregnant women in Ghana (Ayensu *et al*., 2019). Furthermore, to improve consumer acceptability of *B. alcinoe* larva so as to maximize on the caterpillar's high nutritional contents among children, *B. alcinoe* larvae was made into flour and used to make pulp (Dauda *et al*., 2014).

2.8 Research gap identified from the literature review

Gonimbrasia zambesina caterpillar(s) and other lepidopterous larvae have rich nutritional profiles and can be utilized by the baking industry as an ingredient to improve the nutritional profile of refined wheat flour (Subramanian *et al*., 2017; Van Huis, 2013). Refined wheat flour has got low protein content yet it is one of the major staple food in most developing countries hence the need to improve its protein content using conventional protein sources (Purnoma *et al*., 2012; Zielińska *et al*., 2021). Insects are host to food microorganisms and consumers' concerns about the

microbiological safety of insect-based bakery products call for the microbiological assessment of these products (Melgar-Lalanne *et al*., 2019; Musundire *et al*., 2016; Nyangena *et al*., 2020). The presence of unsaturated fatty acids in lepidopterous larvae makes them susceptible to lipid oxidation which affects the shelf life of food products (Lehtovaara *et al*., 2017). Hence, shelf life tests for bakery products enriched/ fortified with *G. zambesina* caterpillar(s) flour would help in the determination of the effects of its addition to the shelf life of the final products. Furthermore, consumption of *G. zambesina* caterpillar(s) is not widespread in Kenya despite its abundance in the coastal region and this has been attributed to their poor sensory appeal (Subramanian *et al*., 2017). To improve consumer acceptability of *G. zambesina* caterpillar(s), there is a need to transform it to a form that would be more appealing to the senses of the consumers and used as an enrichment ingredient in familiar bakery products (Mlcek *et al*., 2014).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Description of the study area

Gonimbrasia zambesina caterpillar(s) were collected from Arabuko Sokoke forest reserves and its surrounding villages. Some of the caterpillars were also reared in a butterfly-rearing unit at Kipepeo Butterfly Centre, Gede, Kenya between September to December 2020 and others were purchased from the caterpillar farmers attached to Kipepeo Butterfly Centre. Arabuko Sokoke forest is located in Kilifi County in the coastal part of Kenya. It is between Gede (south of river Galana) and to the north of Kilifi creek, between 03°10″ and 03°30″S and 39°40″ and 39°50″E as presented in Figure 5 (Oyugi *et al*., 2007).

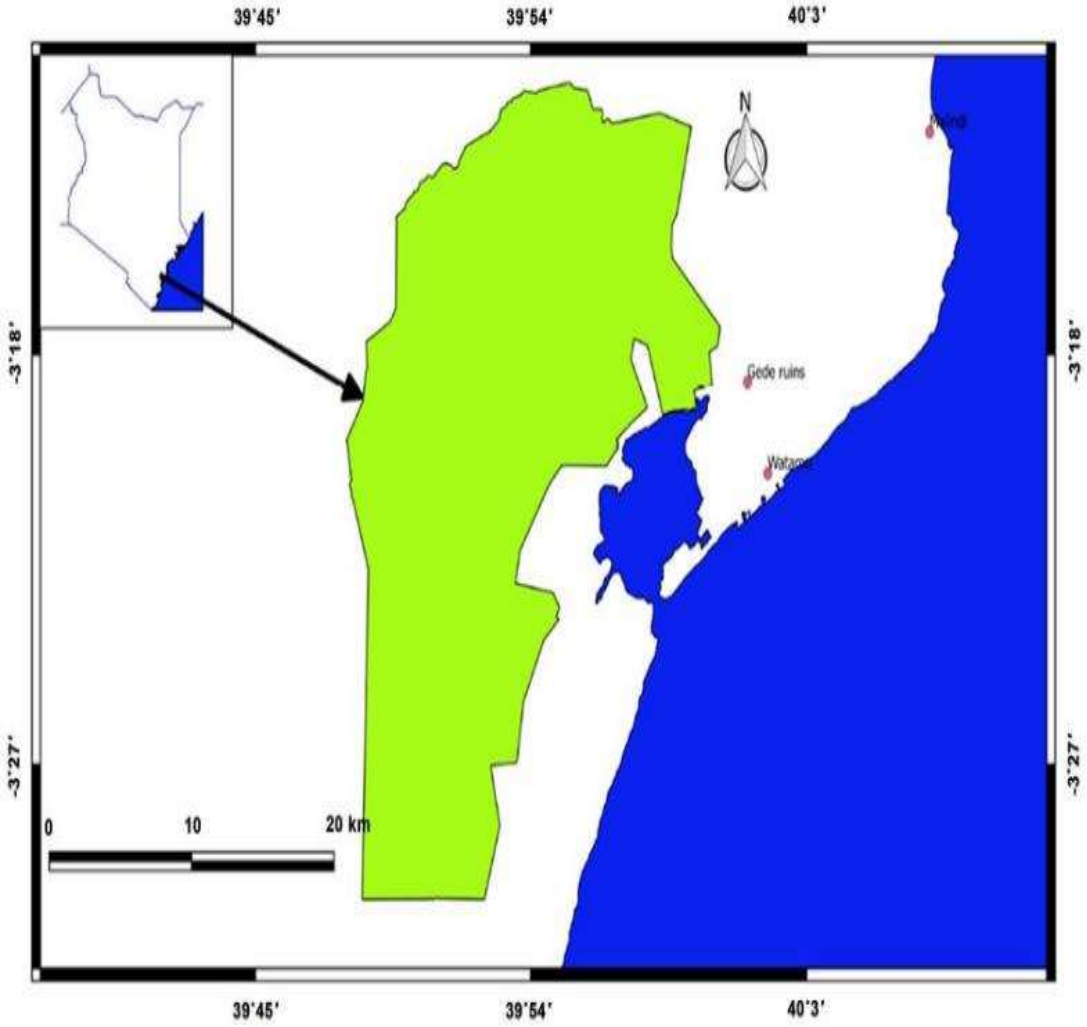


Figure 5: Map of Arabuko Sokoke Forest Reserve (http://geoportal.rcmrd.org/)

Enrichment of wheat flour with *G. zambesina* caterpillar(s) flour, product development, baking, sensory evaluation, and analysis of the microbiological quality of wheat muffins was done at JOOUST, Bondo, Kenya. Proximate composition and *in vitro* protein digestibility analysis was done in the Food Chemistry laboratory and Animal Science laboratory of Egerton University, Njoro, Kenya. Peroxide value was determined at the chemistry laboratory of MMUST. Fat soluble vitamins and mineral analysis was done at ILRI, Nairobi, Kenya.

3.2 Experimental design

A completely Randomized Design (CRD) was adopted for all the objectives. Substitution of wheat flour with *G. zambesina* caterpillar(s) flour was done at five levels with 0% as the control while 5%, 10%, 15% and 20% as the experimental variance. The baking process for every sub-level was repeated thrice. From the replicated batches of baked wheat muffins, muffins were randomly picked for nutritional analysis, microbiological assessment, shelf-life determination and sensory evaluation. Each experiment was replicated three times for every sub-level resulting into 15 experimental units.

Statistical model; $Y_{ij} = \mu + T_i + \varepsilon_{ij}$ (1)

where; Y_{ij} is the response from the jth unit receiving the ith treatment, μ is the overall mean, T_i is the fixed effect of the ith treatment, ε_{ij} is the random effect error

3.3 Rearing of *G. zambesina* caterpillars

Gonimbrasia zambesina moth lay their eggs on the leaves of mango trees (*Mangifera indica*) and *Euclea natalensis* (family Ebenaceae). Leaves on which the eggs of *G. zambesina* moth were deposited were gently plucked, placed in open baskets, and transported to rearing cages at Kipepeo Butterfly Centre, Gede, Kenya. The cages were rectangular in shape made of steel and wire mesh and covered with a net. The height of each cage was 0.5 m, width 1.0 m, and length 1.2 m. The cages were fitted with four stands, every 0.6 m as shown in Figure 6. Each stand stood in an ice cream box filled with water. This was to create a barrier for predators such as rats and snakes which would otherwise find their way into the cages. The rearing cages were stationed inside one major cage to prevent monkeys from eating the reared caterpillars. Leaves with *G. zambesina* caterpillar eggs were placed inside the two cages. They were then stacked with fresh mango leaves

and the entrance sealed. The leaves were to shelter the eggs from strong rains, direct sunlight and also acted as a starter feed for newly hatched larvae. The eggs were monitoring for hatching at 8:00 am, 12:30 pm and 4:30 pm daily. The eggs of *G. zambesina* moth took about 2-5 days to hatch. However, this was not uniform since, during their collection, the exact time and day of deposition on the leaves was not known. The hatched caterpillars were continuously fed on fresh *M. indica* leaves. The stalks of consumed mango leaves were removed every morning and evening during routine checking and replaced with fresh ones. Molting of *G. zambesina* larvae from the 1st instar to the 6th instar was at an interval of 4-6 days for 3-4 weeks. This was dependent on the date on which a larvae was hatched.

Figure 6: *Gonimbrasia zambesina* caterpillars rearing cages

3.4 Sample collection

Sample collection was done between September and December 2020. Approximately 10 kg (wet weight) of *G. zambesina* caterpillars were randomly collected by plucking from the foliage either by bending the mango trees or climbing the mango trees to pluck. Mature *G. zambesina* caterpillars that fell off the mango trees to pupate into the soil were randomly picked from the ground. Another five kg (wet weight) of reared *G. zambesina* caterpillars were randomly picked from the rearing cages. The caterpillars were put in ice cream boxes, sealed with lids, and inactivated at -25^{o}C for 30 min in a freezer for further processing. During the harvesting *G. zambesina* caterpillars from

the cages, about 10% of the total caterpillars was not harvested for them to complete their life cycle. This was to avoid overexploitation which would lead to extinction.

3.5 Processing of *G. zambesina* caterpillars

3.5.1 Evisceration

The processing of *G. zambesina* caterpillars from degutting to dripping is presented in Figure 7. *Gonimbrasia zambesina* caterpillars were degutted using sterile surgical blades. After which, they were washed in running tap water and rinsed twice to remove all the soil particles, and gut content and to ensure they remain fresh. The caterpillars were then spread on drying racks for 30 min for water to drip. The caterpillars were then put in tightly sealed ice cream boxes and frozen-stored at -25^{o}C.

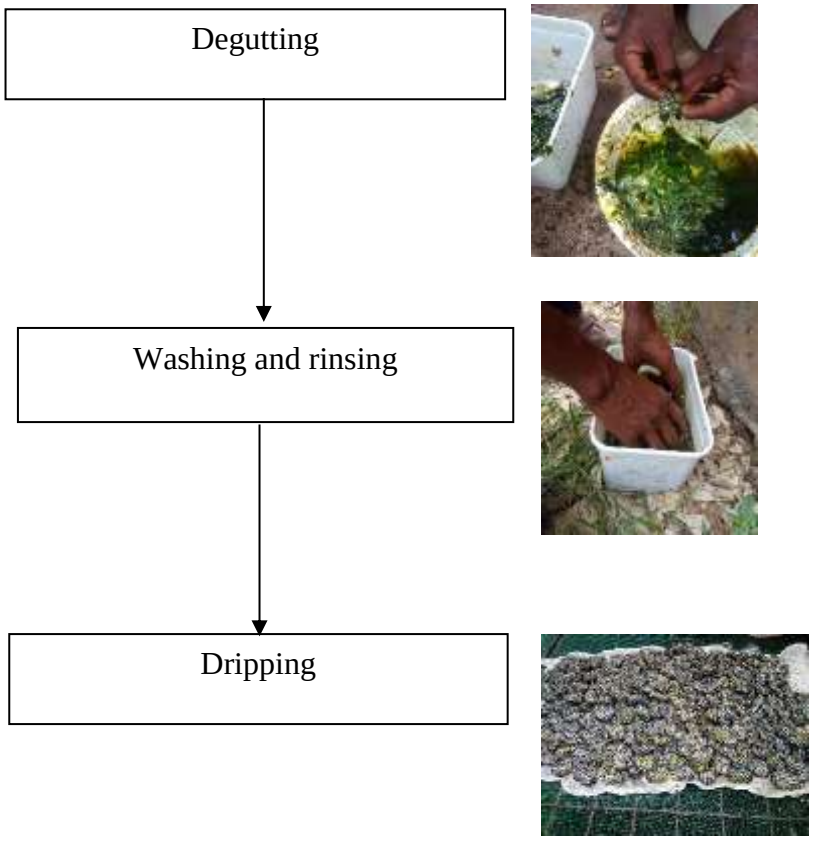


Figure 7: Flow diagram of *G. zambesina* caterpillars processing from degutting to dripping

3.5.2 Drying

Drying was done in a locally made wooden electric dyer (Figure 8). The dryer was 0.46 m high, 0.615 m wide and 0.62 m long resting on two stands of 0.03 m high from the ground. The lid was

made of wood and within the dyer was fitted two 100 W bulbs. On one opposite end of the dryer were 12 closely spaced 1 cm diameter holes for air circulation in and out of the dryer. The temperature within the dryer before the introduction of *G. zambesina* caterpillars was 55^{o}C. This was determined by a mercury bulb thermometer which showed constant temperature (55^{o}C) for 3 h. *Gonimbrasia zambesina* caterpillars were thawed at room temperature for 15 min, placed in the electric dryer, and left to dry to a constant weight at 55^{o}C for 3 days. Dry *G. zambesina* caterpillars were removed from the drying chamber and left to cool at room temperature for 20 min. The caterpillars were packaged in 1kg grey packaging bags, placed in polyethylene zip-lock bags (Vinayak Udyog, New Delhi, India), and refrigerated at 4^{o}C for 24 h.

Figure 8: Wooden electric dyer

3.5.3 Milling

Figure 9 show the milling process of *G. zambesina* caterpillars into flour. *Gonimbrasia zambesina* caterpillars were milled using a coffee bean grinder (DeLonghi KG-79, Wuhan, China) and sieved

through a 250 µm mesh sieve to obtain fine flour. The caterpillar flour was packaged in tightly sealed polyethylene zip-lock bags (Vinayak Udyog, New Delhi, India) and frozen-stored at -25^{o}C for 6 months over which baking and raw material characterization was done.

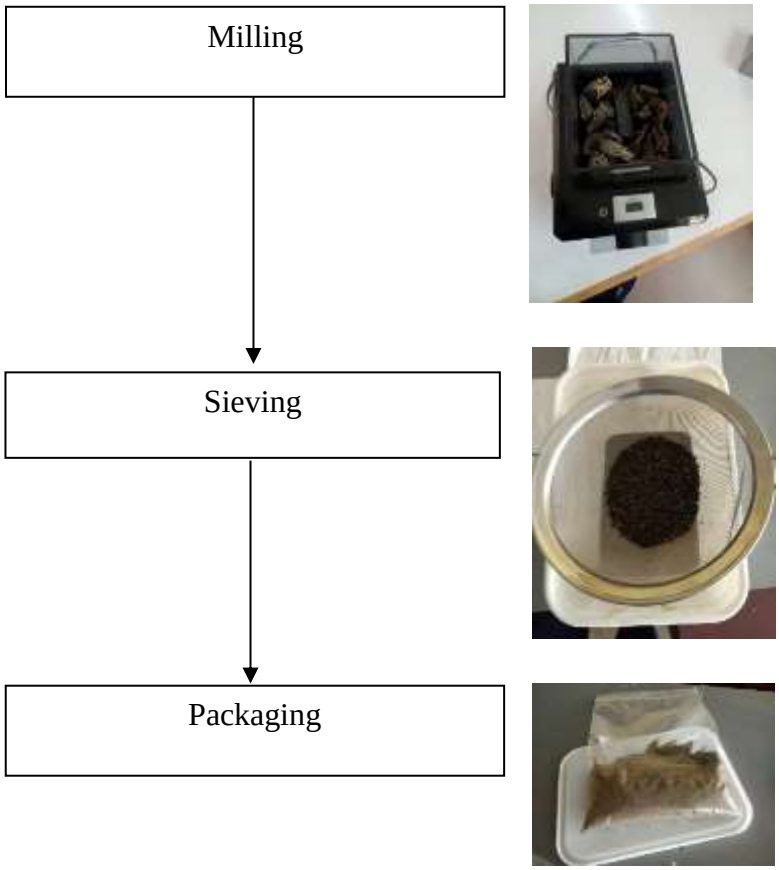


Figure 9: Flow diagram for *G. zambesina* caterpillar(s) flour processing

3.6 Materials

Refined wheat flour, baking powder, sugar, margarine, water and eggs were sourced locally. *Gonimbrasia zambesina* caterpillar(s) flour was processed in the Food Processing Laboratory of JOOUST, Bondo, Kenya. Analytical food-grade chemicals were used for analyses.

3.7 Formulation of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

Wheat flour was substituted with *G. zambesina* caterpillar(s) flour at 0%, 5%, 10%, 15% and 20% substitution levels. The formulation and baking of wheat muffins enriched with *G. zambesina* caterpillar flour was according to basic muffins formulas by Purnoma *et al*. (2012) with slight modifications shown in Table 1.

Table 1: Basic muffin formulas

Ingredients	Amount (g)
Wheat flour	525
Eggs	300
Margarine	345
Salt	3
Water	165
Refined sugar	320
Baking powder	7.5

The choice of the substitution levels of wheat flour with *G. zambesina* caterpillar(s) flour was informed by the Kenyan standards, (KEBS, 2020) which require that while enriching bakery products with insects flour, the least substitution level limit for substituting the main flour (wheat flour) with insect flour should be 5%. The choice was also further informed by previous research by Kinyuru *et al*. (2009) in which they reported that at a 5% substitution level of wheat flour with *M. subhylanus* flour, wheat buns were more acceptable to consumers. The highest allowable limit for substituting wheat flour in baked products is 20% (Kinyuru *et al*., 2009). Hence, the choice of 20% substitution level of wheat flour with *G. zambesina* caterpillar(s) flour as the maximum limit of substitution. The modified quantities of raw materials adopted for wheat muffins enriched with *G. zambesina* caterpillar(s) flour at five substitution levels are shown in Table 2.

Table 2: Optimized muffin formulation in grams (g)

Ingredients	Treatment levels				
	0%(control)	5%	10%	15%	20%
Wheat flour	150.00	142.50	135.00	127.50	120.00
G. zambesina flour	0.00	7.50	15.00	22.50	30.00
Margarine	98.57	98.57	98.57	98.57	98.57
Refined sugar	91.43	91.43	91.43	91.43	91.43
Beaten eggs	85.71	85.71	85.71	85.71	85.71
Baking powder	2.14	2.14	2.14	2.14	2.14
Water	47.14	47.14	47.14	47.14	47.14

3.8 Baking of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

The procedure for baking wheat muffins enriched with *G. zambesina* caterpillar(s) flour at 0%, 5%, 10%, 15%, and 20% substitution levels is presented in Figure 10. Baking was according to the procedures by Purnomo *et al*. (2012) with slight modifications. The quantities of ingredients used for the control wheat muffins (100% wheat flour) and other formulations (5%, 10%, 15%, and 20% *G. zambesina* caterpillar(s) flour are shown in Table 2. Wheat flour and *G. zambesina* caterpillar(s) flour were sieved into a mixer bowl. A hand mixer (Model No: RM/382, Guangdong, China) was set at high speed (as written in the equipment), and the flours were mixed for 1 min. Melted margarine, water, and whisked egg were added to the mixer bowl at 30 sec intervals and further mixed for 2 min at the same speed. The hand mixer speed was adjusted to moderate speed (as written on the equipment) after which baking powder and refined sugar were added respectively into the mixer bowl and mixed for 5 min to form the batter. The batter was poured in muffin liners into standard 6-well muffin pans and filled to the top. The muffins were placed in preheated oven (Electrolux AR 85, Italy) (180^{o}C) and baked at 180^{o}C for 20 min. (NB: A preliminary run was conducted to ascertain the most suitable baking temperature and time to be applied). The muffins pans were removed from the oven (Electrolux AR 85, Italy), and baked wheat muffins were removed from the wells, and cooled on trays for 20 min at room temperature. Wheat muffins samples for chemical analysis were frozen-stored in polyethylene zip lock bags (Vinayak Udyog, New Delhi, India) by deep freezing at -25^{o}C.

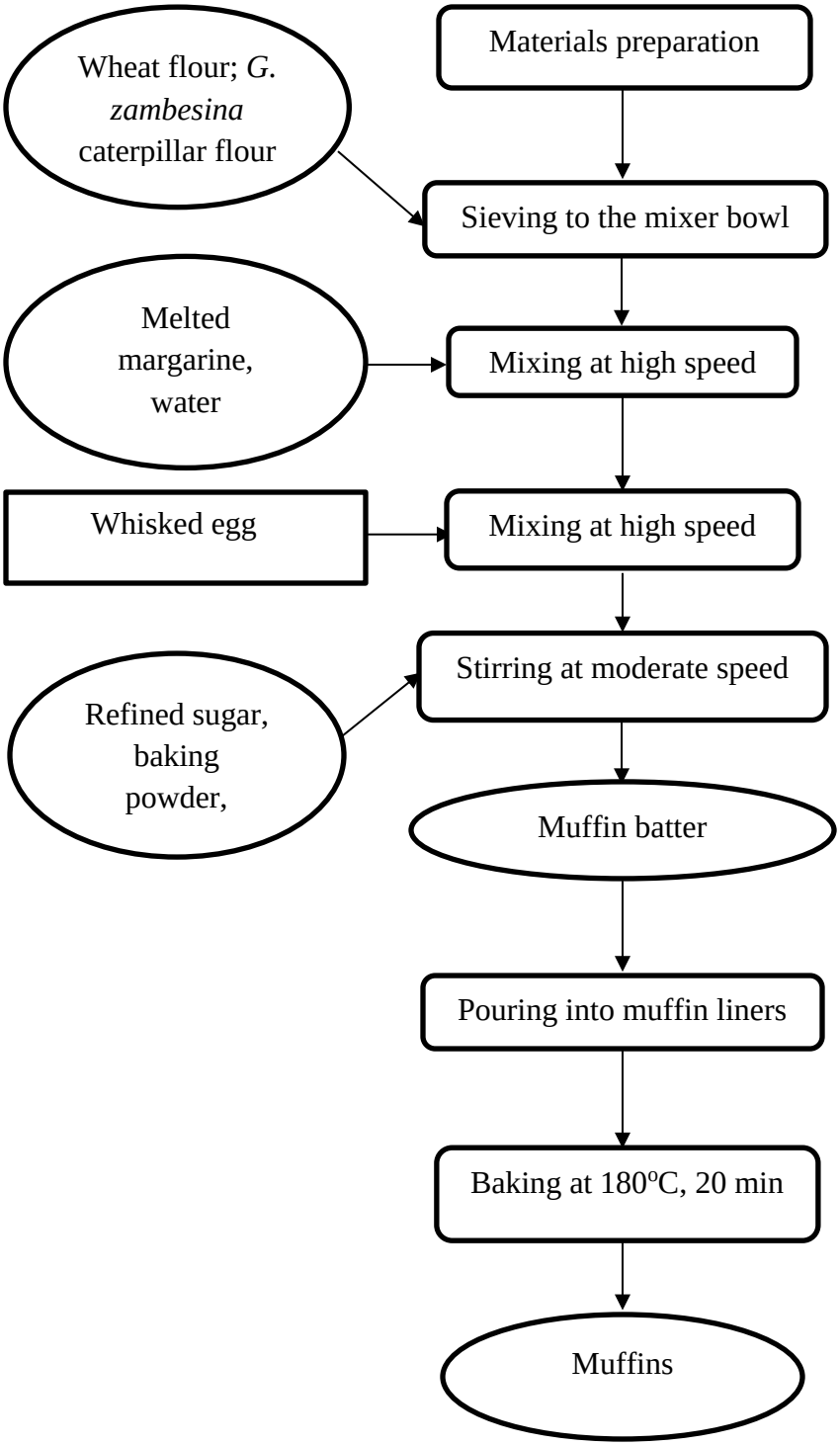


Figure 10: Flow diagram of muffin production

The quantity of ingredients for Figure 9 are shown in Table 2.

3.9 Proximate analysis

3.9.1 Determination of dry matter content

Dry matter content of *G. zambesina* caterpillar(s) flour, wheat flour, and enriched wheat muffins was determined as moisture loss on drying in an oven (Memmert, Schwabach, Germany) according to AOAC (2000) Method 930.15. Sample (2 g) was weighed into dry pre-weighed moisture dishes. The sample in the dishes was dried in an oven (Memmert, Schwabach, Germany) at 130^{o}C for 1 h 15 min. The moisture dishes were cooled in a desiccator to room temperature and the weights were taken. The percentage moisture content was expressed as average weight loss after drying the sample as per the formula below.

$$\%Moisture\ content = \frac{W2-W3}{W2-W1} \times 100 \qquad 2$$

where: $W1$ is the weight of the dish; $W2$ is the weight of the dish and wet sample; and $W3$ is the weight of the dish and dried sample.

$$Dry\ matter = 100\% - \%\ moisture\ content \qquad 3$$

3.9.2 Determination of Crude fibre content

The crude fibre content of *G. zambesina* caterpillar(s) flour, wheat flour, and enriched wheat muffins was determined as a loss on ignition following acid-base sample digestion according to AOAC (2000) Method 962.09 with slight modification. Defatted sample (2 g) was weighed into a 600 ml graduated glass beaker. Approximately 100 ml of hot water was added to the sample in the beaker followed by 25 ml 1.01M H_2SO_4 and topped to 200 ml mark with hot water. The solution was boiled for 30 min on a hot plate. The content was filtered by use of filter stick packed with glass wool and washed with boiling water three times until all the acid was removed. Approximately, 100 ml of hot water was added to the residue in the 600 ml glass beaker followed by 25 ml 1.78M KOH. The solution was topped to 200 ml mark with hot water and boiled on hot plate for 30 min. The residue was filtered and washed three times with boiling water. The glass wool with the residue was transferred into sintered crucible, weighed, dried in an oven overnight at 105°C, cooled in a desiccator and reweighed. The residue was ashed at 550°C for 3 h in a muffle furnace. The sintered crucible was cooled in a desiccator, and reweighed. Percentage crude fibre content was expressed as the difference in the weight of crucible and its content before weighing and after ashing.

$$\%\ Crude\ fibre = \frac{(X-Y)}{W} \times 100 \quad (3)$$

where: X is the weight of crucible and dried sample before ashing, Y is the weight of the crucible and sample after ashing, W is the weight of the sample used.

3.9.3 Determination of Crude fat content

The crude fat content of *G. zambesina* caterpillar(s) flour, wheat flour, and wheat muffins was determined by Soxhlet extraction according to AOAC (2000) Method 920.39. Dry samples (2 g) were transferred into extraction thimbles and covered with balls of cotton wool. The sample-filled thimbles were connected to Soxhlet extractor and connected to dry-weighed 250 ml round-bottomed flasks with 150 ml diethyl ether. The apparatus was connected to a quick fix condenser and refluxed for 3 h on an electro thermal extraction unit. After fat extraction, the flasks were removed and diethyl ether evaporated on the electro thermal extraction unit. The flasks with fat were then cooled in a desiccator and weighed. The crude fat content was calculated as per the formula below:

$$\%Crudefat = \frac{(weight\ of\ the\ flask + fat) - weight\ of\ flask}{weight\ of\ the\ sample} \times 100 \quad (4)$$

3.9.4 Determination of Crude protein content

The crude protein content of *G. zambesina* caterpillar(s) flour, wheat flour, and enriched wheat muffins was determined in the Kjeldahl apparatus (Gerhardt, Königswinter, Germany) according to AOAC (2000), Method 955.04 with slight modifications. Homogenized samples (0.2 g) were weighed and transferred into digestion tubes and 0.5 g of selenium tablets were added to each sample. Approximately, 10 ml of concentrated H_2SO_4 was added into each tube and the tubes were agitated to wet the samples. The samples were then digested at 410°C for 1 h 30 min until clear solutions were obtained. The solution in the digestion flask was made alkaline by the addition of 30% NaOH. Ammonia gas liberated from the solutions were subjected to excess boric acid to form ammonium borate. The samples were cooled to room temperature and distilled. About 3 drops of a mixed indicator of methylene blue and methylene red indicator were added to the distillate. The distillate was titrated against 0.1 M HCl solution. The acid was added until the colour changed to light pink. The volume of acid used for titrating each sample and the blank was recorded. Samples were analysed in triplicate. The percentage of nitrogen was estimated using the formula:

$$\%\ Total\ nitrogen = \frac{100\times(VA-VB)\times NA\times 14}{W\times 1000} \quad 5$$

where: VA is the volume in ml of standard acid used in titration, VB is the volume in ml of standard acid used in the blank, NA is the normality of acid (HCl), and W is the weight in grams of the sample.

Protein content was calculated using 6.25 as the conversion factor for % Nitrogen (Kinyuru, 2020).

$$\%\ Crude\ protein = \%\ Nitrogen \times 6.25 \quad 6$$

3.9.5 Determination of Ash content

The crude ash content of *G. zambesina* caterpillar(s) flour, wheat flour, and enriched wheat muffins was determined according to AOAC (2000), Method 923.03 with slight modifications. Sample (2 g) was ashed in a muffle furnace at 550°C for 6 h. The samples were cooled in a desiccator and weighed. Ash content was expressed as the percentage of residual weight. The percentage of ash was calculated according to the formula below:

$$\%\ Ash\ content = \frac{C-A}{B-A}\times 100 \quad 7$$

where: A is the weight of the crucible; B is the weight of crucible and raw sample, and C is the weight of crucible and dried sample.

3.10 Determination of carbohydrate content

The carbohydrate content of *G. zambesina* caterpillar(s) flour, wheat flour, and enriched wheat muffins was calculated by difference method according to Dauda *et al*. (2014).

$$\%\ CHO = 100\% - (moisture + crude\ fat + ash + crudefibre + crude\ protein)\% \quad 8$$

3.11 Determination of *in vitro* protein digestibility (*IVPD*)

In vitro protein digestibility of *G. zambesina* caterpillar(s) flour and wheat muffins enriched with *G. zambesina* caterpillar(s) flour were determined according to method by Wang *et al*. (2010) with slight modifications. In the digestion tube, 0.5 g protein-containing sample was suspended in 9.5 ml 0.1M HCl and mixed with 5mg pepsin (1:3000; activities 0.8 Anson units/mg, Mumbai, India) in 0.5 ml 0.1M HCl and covered. The mixture was gently shaken in a water bath at 37°C for 2 h. The solution was neutralized by adding 10 ml 1.0M phosphate buffer (pH 8.0) followed by the

addition of trypsin (Pankreasprotease; activity min 200 FIPU/g, Merck Darmstadt, Germany) (100:1 ratio of substrate/enzyme ratio, w/w). The digestion tubes were covered and incubated again in the water bath at 37°C for 2 h. Enzyme activity was terminated by addition of equal volumes (10 ml) of trichloroacetic acid (10% w/v). Samples were immediately transferred to a freezer (-25°C) for 20 min after which, samples were vortexed (Henry Troemner, LLC, USA) for 15min (2500 g, 20°C) and left for 1 h to settle. The supernatant was discarded and the residue was transferred into a moisture dish and dried at 105°C for 3 h. About 0.2 g residue was weighed and the nitrogen content of the residue was determined by Kjeldahl nitrogen analysis. The % nitrogen of the residue was determined as follows

$$\%Nitrogen = \frac{1.4007(VBL-VB)\times 100}{W\times 1000} \quad 9$$

where: VBL is the volume of the blank titre, W is the weight of the sample and VB is the volume of the base.

Protein digestibility (*in vitro*) was calculated by getting the difference between the total amount of protein and the residual amount of protein after pepsin and trypsin digestion divided by the total amount of protein and expressed as a percentage.

$$\%IVPD = \frac{TAP-RAP}{TAP} \times 100 \quad 10$$

where: TAP is the total amount of protein and RAP is the residual amount of protein

3.12 Mineral analysis

The mineral element concentrations in *G. zambesina* caterpillar(s) flour and enriched wheat muffins samples were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) quantitation according to Series, (1992) and Phan-thien *et al*. (2012) with modifications. Briefly, samples (0.5 g) were weighed into the digestion tubes and homogenized. Approximately, 8.0 ml of concentrated HNO_3 and 1 ml of 30% H_2O_2 were added to the digestion tubes. The digestion tubes were put into Multiwave Go Plus equipment (Anton Par, Graz, Austria) and the samples were digested for 10 min ramp to 100°C, 10 min hold followed by second digestion for 10 min ramp to 180°C, 10 min hold. The digestion tubes were then removed from the digester and the digest quantitatively transferred to 50 ml falcon tubes. The digest was diluted with 2% Nitric acid to the mark and the solution was taken for quantification of minerals in the

ICP-OES equipment (Model: Optima 2100DV PerkinElmer, Massachusetts, USA). The operating conditions for ICP-OES (Optima2100 DV Perkin Elmer, Massachusetts, USA) is presented in Table 3. For quantification of mineral elements, ICP-OES mixed standard CatNo.43843 (Sigma Aldrich, USA) was used. Serial dilution of the standard was performed using 2% nitric acid to obtain calibration standards of 400, 800, 2000, and 4000 µg/L and an external standard calibration method was applied. Calibration was performed using Perkin Elmer Winlab 32 software. The obtained data were used to calculate the final elemental concentration for each element in mg/100g using the formula below

$$Element(mg/100g) = \frac{C \times V \times D \times 100}{W \times 1000} \qquad 11$$

where: C= concentration of the sample in µg/L, V= total dilution volume in L, D= Dilution factor, W= weight sample, g, 1000= conversion of µg to mg, 100 = Conversion factor to report results in mg/100

Table 3: Operating conditions of ICP-OES Perkin Elmer (optima2100 DV)

RF power (W)	**1450**
Plasma gas flow rate (L min−1)	15
Auxiliary gas flow rate (L min−1)	0.2
Nebulizer gas flow rate (L min−1)	0.8
Sample flow rate (L min−1)	1.5
View mode	Axial
Read	Peak area
Source equilibration time (s)	10
Read delay (s)	10
Replicates	1
Background correction	2-point (manual point correction)
Spray chamber	Scott type spray chamber
Nebulizer Cross-	Flow GemTip Nebulizer (HF resistant)
Detector CCD	CCD
Purge gas	Nitrogen
Shear gas	Air
Plasma gas	Argon
Wavelength (nm)	Mg-285.213, Fe-259.939, Mn-257.61, Ca-317.933, P-213.617, Mo-202.031, K-766.49, Al-396.153, Co- 228.616, Zn- 213.857

3.13 Vitamin analysis

3.13.1 Determination of retinol, and α, and γ-tocopherol

Retinol, α, and γ-tocopherol concentrations of *G. zambesina* caterpillar(s) flour and enriched wheat muffin samples were analysed by HPLC according to methods described by Bhatnagar-panwar & Bhatnagar-mathur (2013) and Hosotani & Kitagawa (2003). Briefly, 0.5 g powdered product sample was weighed in a 25 ml tube in triplicate. Accurately, 6 ml ethanol with 0.1% (BHT) was added, homogenized for 1 min and then 120 µl of potassium hydroxide 80 % (w/v) was added and the sample mixed by vortexing. The samples were incubated for 5 min at 85 °C in a water bath after which were removed and immediately placed on ice to cool. Deionized water (5 ml) was added to each tube and mixed by vortexing. About 5 ml of hexane was added, mixed by vortexing, and the samples centrifuged (Eppendorf model: centrifuge 5810, Hamburg, Germany) at 1791 *g* for 5 min. The upper phase (hexane) was transferred to a centrifuge tube using Pasteur pipette. Extraction was done thrice with 4×3×3 ml hexane pooling the extract into the 25 ml tube. About 5 ml of deionized water was added to the extract, vortexed for 1 min, and centrifuged (Eppendorf model: centrifuge 5810, Hamburg, Germany) at 1791 *g* for 5 min. The hexane layer was recovered into a clean test tube and evaporated under nitrogen in an N-Evap (Organomotion, Massachusetts, USA) to complete dryness. Samples were reconstituted into 1 ml of methanol: tetrahydrofuran (85:15 v/v) vortexed and sonicated for 30 sec. Some 0.8 ml of the reconstituted residue was transferred to vials of the HPLC system (Shimadzu Nexera UPLC, Kyoto, Japan) with a rephrased phased column, YCM C30, carotenoid column (3 µm, 150×3.0 mm, YMC Wilmington, NC) and an injection volume of 10 µl. The HPLC has mobile phase A: methanol/tert-butyl methyl ether/water (85:12:3, v/v/v, with 1.5% ammonium acetate in the water) and mobile phase B: methanol/tert-butyl methyl ether/water (8:90:2, v/v/v, with 1% ammonium acetate in the water) with a flow rate of 0.4 ml/min. Retinol, α-tocopherol, and γ-tocopherol were monitored at 290 nm wavelength on an SPD -M2A detector. Standards were compared to the extracts for vitamins A and E concentrations determination. The liquid chromatogram elution program flow method is shown in Table 4.

Table 4: Liquid chromatogram elution flow method

	Time	Flow	% A	% B
1		0.40	100.0	0.0
2	1.00	0.40	100.0	0.0
3	10.00	0.40	90.0	10.0
4	22.00	0.40	45.0	55.0
5	33.00	0.40	5.0	95.0
6	37.00	0.40	5.0	95.0
7	39.00	0.40	100.0	0.0
8	40.00	0.40	100.0	0.0
9	61.00	0.40	0.0	0.0

3.14 Microbiological analysis of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

The microbiological assessment of wheat muffins enriched with *G. zambesina* caterpillar(s) flour was determined 48 h after baking except for yeast and moulds which was enumerated on day 1, 7, 14, and 21 of baking, respectively.

3.14.1 Total viable count (TVC)

The TVC was determined according to AOAC (2000) Method 966.23. Sample (5 g) was diluted with 45 ml of sterilized peptone water to make a stock dilution. The stock dilution was vortexed to homogenization at 3000 rpm for 5 min and serial dilutions made up to 10^{-3}. A 1 ml aliquots from each serial dilution was inoculated in triplicate in Petri dishes with Plate Count Agar (PCA) (CM0152). The inoculum was gently swirled and left to sit for 15 min. The plates were inverted and incubated at 35ºC for 48 h. The plates were observed for growth and results expressed as cfu/g of the sample.

3.14.2 Total coliform count (TCC)

The TCC was determined according to AOAC (2000) Method 966.24. Sample (5 g) was diluted into 45 ml of sterilized peptone water to make a stock dilution. The stock dilution was vortexed to homogenization at 3000 rpm for 5 min and serially diluted up to 10^{-3}. Aliquots (1 ml) from each serial dilution were inoculated in triplicate Petri dishes with MacConkey Agar (MA) (Himedia Ref M043) and swirled gently and left to sit for 15 min. The plates were inverted and incubated at 35ºC for 48 h. The plates were observed for any colonies and results expressed as cfu/g of the sample.

3.14.3 Enumeration of *Staphylococcus aureus*

Enumeration of *S. aureus* was done according to method by Nyangena *et al*. (2020) with slight modifications. Accurately, 5 g of the sample was diluted into 45 ml sterilized peptone water to make a stock dilution. The stock dilution was vortexed to homogenization at 3000 rpm for 5 min and serially diluted up to 10^{-3}. Aliquots (1 ml) from each serial dilution were inoculated in triplicate in Petri dishes with Baird-Parker media (Himedia Ref M043) enriched with egg-yolk tellurite emulsion (Himedia, India). The plates were retained in an upright position until inoculum was absorbed by the medium and then inverted and incubated at 35ºC for 48 h. The petri dishes were observed for growth of colonies of typical *S. aureus* appearance (grey-black to jet black, circular, smooth, convex, and moist and 2-3mm in diameter).

3.14.4 Detection of *Salmonella*

Detection of *Salmonella* was done according to method by Nyangena *et al*. (2020) with slight modifications. Sample (25 g) was diluted into 225 ml nutrient broth (Himedia Ref M002) (1.5 g yeast extract, 1.5 g beef extract, 5 g sodium chloride, 5 g peptone per 1000 mL water, pH 7.4) in a closable container. The container was gently shaken and incubated at 35ºC for 24h. The enriched homogenate (25 ml) was transferred to 225 ml tetrathionate broth (Himedia, India) and incubated at 37ºC for 24 h. A 3 mm loopful of incubated culture was streaked on *Salmonella-Shigella* Agar (SSA) (Himedia, India) in triplicate plates. The plates were incubated at 37ºC for 24 h and observed for the growth of typical *Salmonella* colonies (colourless colonies with black centers).

3.14.5 Yeast and moulds determination

Yeast and molds were determined according to Nyangena *et al*. (2020) with slight modifications. Test sample (5 g) was diluted into 45 ml sterilized peptone water to make a stock dilution. The stock dilution was homogenized by vortexing at 3000 rpm for 5 min and serially diluted up to 10^{-3}. An aliquot (1 ml) from each serial dilution was transferred into triplicate Petri dishes with Potato Dextrose Agar (PDA) (Himedia Ref M096) acidified with 10% tartaric Acid (Sigma-Aldrich, Schnelldorf, Germany) to pH 3.5 using a sterile micropipette. The inoculum was swirled gently and allowed to sit for 15 min. The plates were inverted and incubated at 25ºC for 72 h. Plates with between 30 to 300 colonies were selected for counting. The results were expressed as cfu/g of the sample.

3.15 Accelerated shelf-life test of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

Peroxide value was used in predicting the shelf life of wheat muffins enriched with *G. zambesina* caterpillar(s) flour at 0% (control wheat muffins), 5%, 10%, 15%, and 20% substitution levels (Prchalová *et al*., 2016). The enriched wheat muffins were stored at 25ºC, 37ºC, and 45ºC for 21 days. The formation of hydro peroxides was monitored within the 21 days evaluation period with tests done on days 0, 7, 14 and 21 of storage, respectively.

3.15.1 Peroxide value determination

The peroxide value of wheat muffins enriched with *G. zambesina* caterpillar(s) flour was determined by iodometric titration according to AOAC (2000) Method 965.33. Approximately, 0.3 g oil extracted from the enriched wheat muffins by Soxhlet extraction method AOAC (2000) Method 920.39 was weighed into a 250 ml glass stoppered Erlenmeyer. Accurately, 30 ml acetic acid chloroform solvent mixture was measured, added to the flask, and swirled to dissolve. Saturated potassium iodide (KI) solution (0.5 ml) was added using a Mohr pipet and left to stand for 1 min with occasional shaking after which, 30 ml of distilled water was added. The solution was slowly titrated with 0.01M sodium thiosulfate with vigorous shaking until the yellow colour was almost gone. Then, 0.5 ml 1% starch solution was added and titration continued, shaking vigorously until the blue colour disappeared. The peroxide value was calculated according to the formula:

$$PV_1\ [meq\ O_2/kg] = \frac{T \times M \times 100}{W} \qquad 16$$

where: PV_1 is peroxide value expressed in meq O_2 /kg, M is the molarity of sodium thiosulfate solution consumed in mol/L, T is the titre of the sodium thiosulfate solution, W is weighed portion of substance in grams

3.15.2 Data processing

As shown in Table 5, established was a regression plot of semi-logarithmic scale for the rate constant for each sample at the three temperatures (Ln k) against the absolute inverse of temperature ($\frac{1}{T}$) to obtain an Arrhenius equation based on:

$$Ln\ k = Lnk_o - \frac{E_a}{R}\left(\frac{1}{T}\right) \quad 17$$

$$\frac{1}{T} = \frac{1}{T^*} - \frac{1}{T_{ref}} \quad 18$$

where: k is the reaction rate constant; R is the molar gas constant (8.314 J/K/mol), T is the absolute temperature (K); E_a is the apparent activation energy (J/mol), and k_o is the pre-exponential factor; T_{ref} corresponding to the average of the temperature range used during the experiment where in this case was taken to be 37°C (310.15K) and T^* is the temperature to which prediction of shelf life is done, in this case, it was 22°C (295.15K).

Table 5: First order regression models for wheat muffin enriched with *G. zambesina* caterpillar(s) flour at different substitution levels stored at 25ºC, 37ºC and 47ºC.

Sample	Temperature	Regression equation	R^2
A	25ºC	Y=0.0347x+0.0749	0.9295
	37ºC	Y=0.0388x+0.0731	0.9465
	45ºC	Y=0.0426x+0.1219	0.8984
B	25ºC	Y=0.0311x+0.1089	0.8476
	37ºC	Y=0.032x+0.1365	0.7929
	45ºC	Y=0.0431x+0.1824	0.8025
C	25ºC	Y=0.0332x+0.0913	0.9018
	37ºC	Y=0.0378x+0.0774	0.9453
	45ºC	Y=0.0471x+0.087	0.9552
D	25ºC	Y=0.0269x+0.064	0.9277
	37ºC	Y=0.0327x+0.0872	0.9021
	45ºC	Y=0.0389x+0.1363	0.8497
E	25ºC	Y=0.0266x+0.0659	0.9094
	37ºC	Y=0.0337x+0.0477	0.9685
	45ºC	Y=0.0441x+0.094	0.9416

Note: A- 100% wheat flour; B- 95% wheat flour, 5% *G. zambesina* caterpillar(s) flour; C- 90% wheat flour, 10% *G. zambesina* caterpillar(s) flour; D-85% wheat flour, 15% *G. zambesina* caterpillar(s) flour; E- 80% wheat flour; 20% *G. zambesina* caterpillar(s) flour.

The Arrhenius parameters were obtained to estimate the shelf life according to the formula described by (Manzocco *et al*., 2012):

$$Shelf\ life = \frac{[A_{lim}]-[A_0]}{k_{ref}Exp\left[-\frac{E_a}{R}\left(\frac{1}{T^*}-\frac{1}{T_{ref}}\right)\right]} \quad 19$$

where: A_0 is the initial peroxide value at time zero; k_{ref} is the rate constant at the reference temperature; E_a is the apparent activation energy (J/mol); T^* is the temperature to which prediction

of shelf life is done, in this case, it was 22°C (295.15K); A_{lim} is the standard acceptable limit for peroxide value in processed foods which is 10.0 meq O_2/kg oil according to East African Standard (EAS 795:2013); $T_{ref,}$ corresponding to the average of the temperature range used during the experiment where in this case was taken to be 37°C (310.15K).

3.16 Sensory evaluation

Aroma, color, taste, texture, and the overall acceptability were the key parameters monitored during sensory evaluation. The sensory study was approved by the ethics review committee (ERC) of JOOUST (Appendix 4). Participants who voluntarily accepted to be part of the study were picked randomly and provided with a consent form with the full description of the product to read through and consent to the study. Each panelist was also provided with a sensory score card (appendix 1) with 5-point hedonic scale rating (1: dislike extremely, 2: dislike, 3: neither like nor dislike, 4: like and 5: like extremely) (Sharif & Sharif, 2017). A total of 30 semi-trained panelists made of students of JOOUST aged between 18-27 years participated in the sensory study (Kinyuru *et al*., 2009). The number of female participants was 20 whereas male participants were 10. Each panelist was randomly served with five coded samples of wheat muffins; EM-4 (100% wheat flour), EM-3 (95% wheat flour; 5% *G. zambesina* caterpillar(s) flour), EM-1 (90% wheat flour; 10% *G. zambesina* caterpillar(s) flour), EM-5 (85% wheat flour; 15% *G. zambesina* caterpillar(s) flour) and EM-2 (80% wheat flour; 20% *G. zambesina* caterpillar(s) flour). Potable water was also provided for rinsing the palates before and after tasting each sample. Participants were asked to rank each muffin in order of preference in terms of aroma, color, taste, texture, and overall acceptability.

3.17 Data analysis

Data obtained from this study was analyzed using Statistical Analysis System SAS® software version 8.3. Data was arranged in an excel sheet as per the variables and tested for normality using PROC UNIVARIATE and for homogeneity using Levene's method. T-test was used to compare the proximate compositions of the raw materials. PROC GLM was used to carry out an analysis of variance (ANOVA) to test the hypotheses of the study at 95% confidence level. Means separation was done using Tukey's Honestly Significant Difference (Tukey's HSD) method at ($p<0.05$). The results were presented as means ± standard deviations. Data output has been reported using tables and graphs.

CHAPTER FOUR: RESULTS

4.1 Introduction

In this chapter, the results for the study are presented using tables and graphs. Results on the characterization of the raw materials have been presented first. Results on the nutritional composition, microbiological assessment, shelf life and sensory properties of wheat muffins enriched with *G. zambesina* caterpillar(s) flour have been presented based on the study objectives.

4.2 Raw materials characterization

4.2.1 Physicochemical composition of wheat and *G. zambesina* caterpillar(s) flour

The physicochemical composition of the wheat and *G. zambesina* caterpillar(s) flour is presented in Table 6. There was significant difference ($p<0.05$) in the ash content, dry matter content, crude protein content, crude fat content, crude fibre content, and carbohydrate content of *G. zambesina* caterpillar(s) flour and wheat flour. *Gonimbrasia zambesina* caterpillar(s) flour was 64.05% digestible (*in vitro*).

Table 6: Proximate composition of *G. zambesina* caterpillar(s) flour and wheat flour in (%)

Flour	Proximate components (%)					
	Protein	**Fat**	**Carbohydrate**	**Fibre**	**Ash**	**Dry matter**
CF	59.33±0.30^{a}	14.87±0.26^{a}	1.17±0.39^{b}	15.50±0.25^{a}	3.46±0.20^{a}	94.33±0.06^{a}
WF	13.11±0.45^{b}	0.65±0.09^{b}	75.56±0.69^{a}	0.88±0.01^{b}	0.53±0.10^{b}	90.73±0.17^{b}
t-value	85.57	52.11	-93.74	59.16	13.24	-20.11
P-value	<.0001	<.0001	<.0001	<.0001	0.0002	<.0001

Key: CF= Caterpillar flour; WF= Wheat flour; Values are mean ± SD of triplicate analysis. Means with the different superscripts (alphabet) along the column are significantly different ($p<0.05$)

4.2.2 Mineral composition of *G. zambesina* caterpillar(s) flour

The mineral element concentrations of *G. zambesina* caterpillar(s) flour is presented in Figure 11. *Gonimbrasia zambesina* caterpillar(s) flour had high concentrations of potassium and phosphorus, respectively. Iron was the least in concentration among the elements analysed.

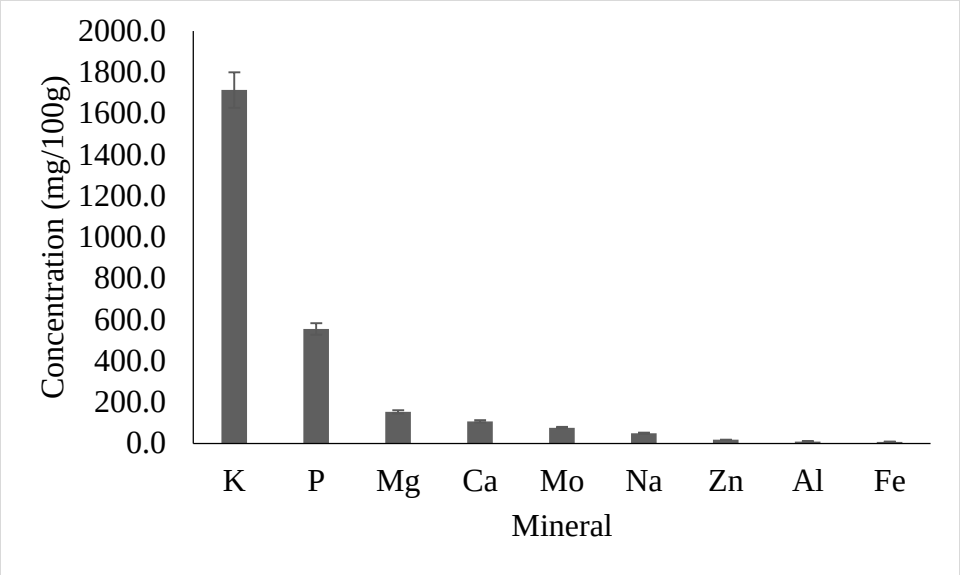


Figure 11: Concentration of minerals in *G. zambesina* caterpillar(s) flour

Note: Error bars represents percentage error

4.2.3 Retinol, α, and ɣ-tocopherol concentrations of *G. zambesina* caterpillar(s) flour

Retinol, α, and γ-tocopherol concentrations in *G. zambesina* caterpillar(s) flour are presented in Figure 12. The flour had 0.0 mg/100 g of retinol, 4.32 mg/100 g of α- tocopherol and 0.16 mg/100 g of γ-tocopherol concentrations.

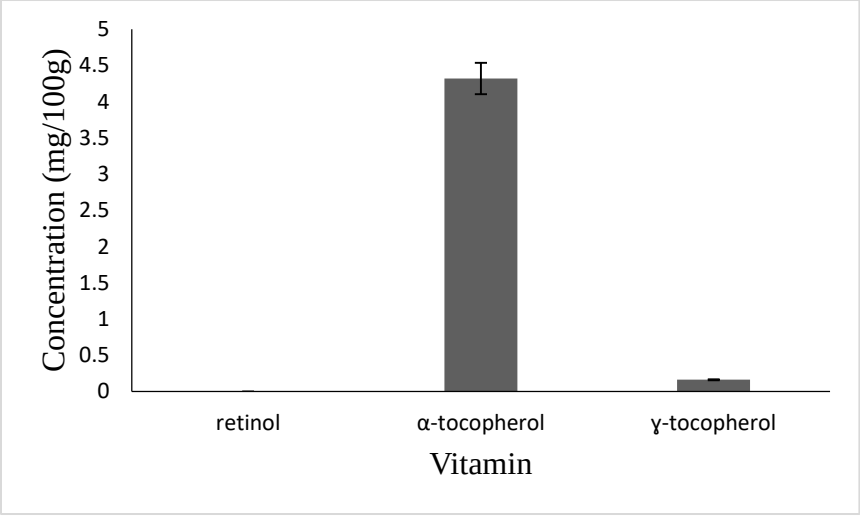


Figure 12: Retinol, α, and γ-tocopherol concentrations in *G. zambesina* caterpillar(s) flour

Note: Error bars represents percentage error

4.3 Wheat muffins enriched with *G. zambesina* caterpillar flour

4.3.1 Proximate composition

As shown in Table 7, there was significant difference ($p<0.05$) in crude protein, crude fibre, crude fat, and carbohydrate contents of wheat muffins enriched with *G. zambesina* caterpillar(s) flour. However, there was no significant difference ($p<0.05$) in dry matter content and ash content of wheat muffins enriched with *G. zambesina* caterpillar(s) flour. Crude protein, crude fat, crude fibre, and ash contents increased with increase in the substitution level. At 20% substitution level of wheat flour with *G. zambesina* caterpillar(s) flour resulted in 60.5% increase in protein content of the wheat muffins. The carbohydrate content decreased with increase in substitution level.

Table 7: Effect of substituting wheat flour with *G. zambesina* caterpillar(s) flour on the proximate composition of wheat muffins in (%)

Level of Substitution	Proximate components (%)					
	Protein	**Fat**	**Carbohydrate**	**Fibre**	**Ash**	**Dry matter**
0 %	19.67 ± 0.70^{d}	28.83 ± 0.64^{c}	47.00 ± 1.16^{a}	0.89 ± 0.00^{c}	0.93 ± 0.09^{a}	97.32 ± 0.18^{a}
5 %	25.47 ± 0.50^{bc}	29.75 ± 0.38^{bc}	39.96 ± 0.37^{b}	1.14 ± 0.00^{c}	1.11 ± 0.11^{a}	97.44 ± 0.14^{a}
10 %	28.23 ± 1.55^{b}	30.68 ± 0.64^{b}	35.40 ± 2.22^{bc}	1.82 ± 0.07^{b}	1.18 ± 0.01^{a}	97.31 ± 0.08^{a}
15 %	31.58 ± 0.44^{ab}	31.52 ± 0.51^{ab}	30.83 ± 0.49^{c}	2.04 ± 0.06^{b}	1.23 ± 0.26^{a}	97.19 ± 0.19^{a}
20 %	34.15 ± 0.54^{a}	32.62 ± 0.22^{a}	26.09 ± 0.96^{c}	2.67 ± 0.22^{a}	1.35 ± 0.20^{a}	96.87 ± 0.19^{a}
F(4,10) value	43.64	8.65	43.36	42.46	0.91	1.78
P-value	<.0001	0.0028	<.0001	<.0001	0.4924	0.2096

Key: LS=Level of substitution; Values are mean ± SD of triplicate analysis (n=3). Means with different superscripts (alphabet) along the column are significantly different ($p<0.05$)

4.3.2 *In vitro* protein digestibility

There was significant difference ($p<0.05$) in the *IVPD* of wheat muffins enriched with *G. zambesina* caterpillar(s) flour (Figure 13). The *IVPD* was high for wheat muffins enriched with *G. zambesina* caterpillar(s) flour at 5%, 10%, and 15% substitution levels. Control wheat muffins and muffins with 20% *G. zambesina* caterpillar(s) flour showed a similar *IVPD*.

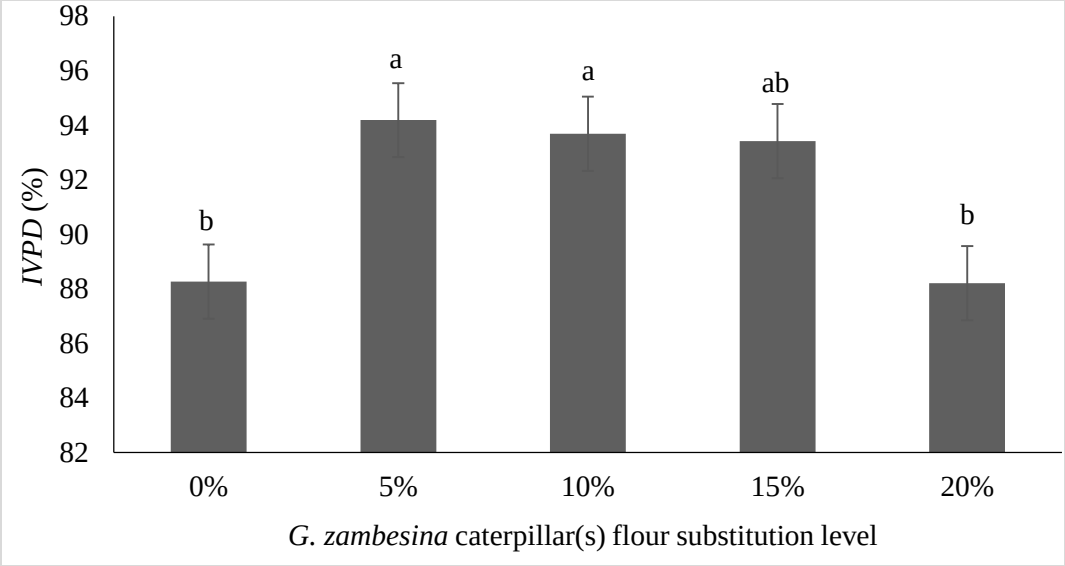


Figure 13: Effect of *G. zambesina* caterpillar(s) flour substitution levels on *IVDP* of wheat muffins

Note: Bars with different alphabet letters are significantly different ($p<0.05$); Error bars represents Standard Error

4.3.3 Mineral composition

The result presented in Table 8 shows that magnesium, phosphorus, potassium, sodium, and molybdenum concentrations of wheat muffins enriched with *G. zambesina* caterpillar(s) flour were significantly different ($p<0.05$). However, there was no significant difference ($p<0.05$) in calcium, aluminum, iron, and zinc concentrations of the enriched wheat muffins. Enriched wheat muffins were high in sodium, potassium, and phosphorus concentrations, respectively with zinc being the least. Generally, the concentrations of minerals in the wheat muffins increased with increase in substitution level of wheat flour with *G. zambesina* caterpillar(s) flour.

Table 8: Mineral concentration in wheat muffins enriched with *G. zambesina* caterpillar(s) flour (mg /100g)

LS	Mg	Ca	P	Al	K
0%	25.58±0.17^{b}	34.71±0.79^{a}	172.93±3.00^{b}	11.93±0.14^{a}	156.10±2.40^{c}
5%	26.66±0.31^{b}	35.27±0.75^{a}	174.77±1.06^{b}	12.33±0.74^{a}	161.59±5.16^{c}
10%	29.03±0.66^{b}	37.07±1.32^{a}	177.91±3.89^{ab}	12.62±0.27^{a}	197.36±4.35^{b}
15%	31.22±1.23^{ab}	38.88±2.25^{a}	185.28±4.51^{ab}	12.75±0.08^{a}	242.11±4.32^{a}
20%	35.50±1.96^{a}	41.20±2.07^{a}	194.19±1.20^{a}	13.14±0.25^{a}	273.14±9.25^{a}
F(4,5) value	13.26	2.91	8.12	1.49	83.13
P-value	0.0072	0.1358	0.0206	0.3323	<.0001

(Table 8 continued)

LS	Na	Fe	Zn	Mo
0%	202.83±1.82^{b}	4.49±0.19^{a}	2.31±0.01^{a}	33.34±1.48^{b}
5%	213.12±12.83^{ab}	4.67±0.31^{a}	2.90±0.06^{a}	36.25±0.24^{b}
10%	222.16±1.93^{ab}	4.76±0.03^{a}	3.34±0.13^{a}	37.37±2.10^{ab}
15%	234.79±11.16^{ab}	4.91±0.04^{a}	3.37±0.40^{a}	39.48±0.51^{ab}
20%	255.50±5.68^{a}	4.98±0.27^{a}	3.40±0.14^{a}	43.57±0.48^{a}
F(4,5) value	6.33	0.89	5.66	10.17
P-value	0.0341	0.5304	0.0424	0.0128

Key: LS= Level of substitution; Values are mean ± SD of triplicate analysis. Means with different superscript (alphabet) along the column are significantly different ($p<0.05$)

4.3.4 Retinol, α, and ɣ-tocopherol concentrations

As shown in Figure 14, concentrations of α-tocopherol in the enriched wheat muffins non-substantially increased with corresponding increase in the substitution level of wheat flour with *G. zambesina* caterpillar(s) flour. There was also no substantial increase in the concentrations of ɣ-tocopherol in the enriched wheat muffins with increase in substitution level. The concentrations of α-tocopherol and ɣ-tocopherol were highest at the 20% substitution level of wheat flour with *G. zambesina* caterpillar(s) flour (3.71 mg/100 g and 1.04 mg/100 g, respectively). Control wheat muffins had 3.11 mg/100 g concentrations of α-tocopherol and 0.98 mg/100 g concentration of ɣ-tocopherol. Retinol was present in the control wheat muffins and those enriched with *G. zambesina* caterpillar(s) flour.

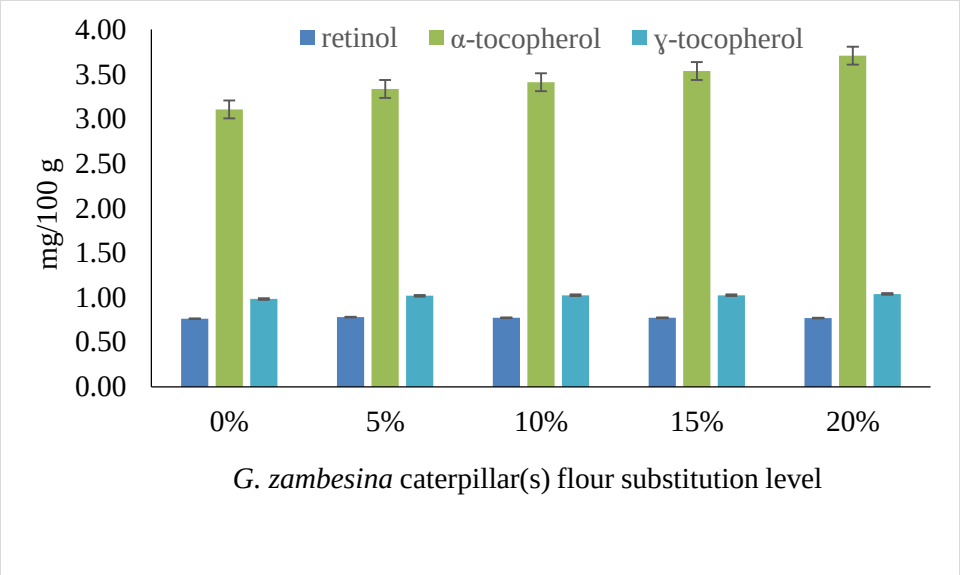


Figure 14: Retinol, α, and ɣ-tocopherol concentrations of wheat muffin enriched with *G. zambesina* caterpillar(s) flour

Note: Error bars represents standard error

4.4 Microbiological assessment

The microbiological quality of wheat muffins enriched with *G. zambesina* caterpillar(s) flour is shown in Table 10. The total viable count and total coliform count in all analysed samples was found to be <30 cfu/g. *Staphylococcus aureus* and *Salmonella* were not detected in all the samples analysed. Yeast and moulds were reported to be <30 cfu/g over the evaluation period.

Table 9: Microbiological quality of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

Level of Substitution	TVC (cfu/g)	TCC (cfu/g)	*Staphylococcus aureus*	Salmonella spp.	YM
0%	<30	<30	Not detected	Not detected	<30
5%	<30	<30	Not detected	Not detected	<30
10%	<30	<30	Not detected	Not detected	<30
15%	<30	<30	Not detected	Not detected	<30
20%	<30	<30	Not detected	Not detected	<30

Note: TVC= Total viable count; TCC=Total coliform count; YM= Yeasts and moulds

4.5 Accelerated Shelf life

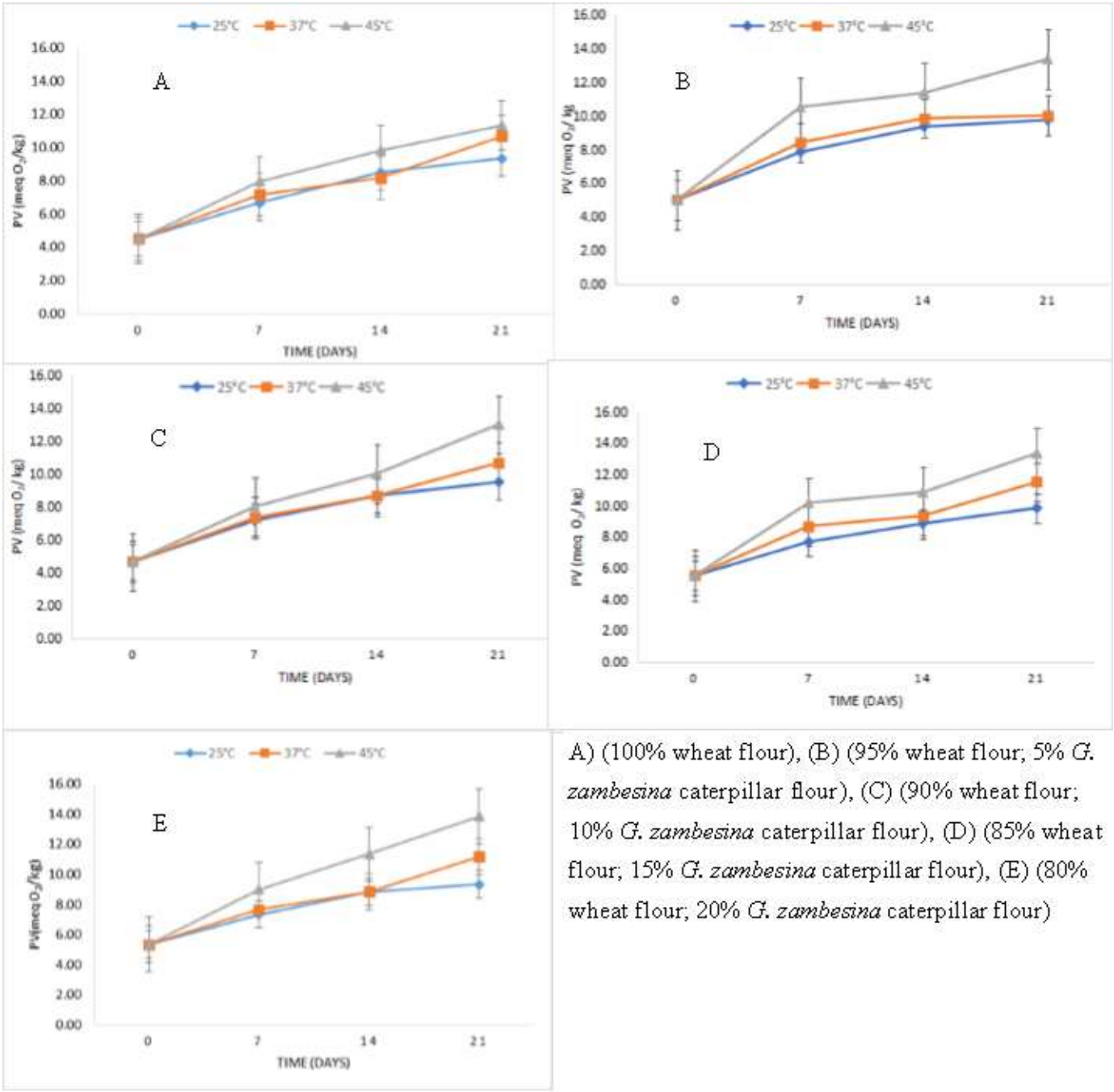
Figure 15 shows the peroxide values of oils extracted from the wheat muffins enriched with *G. zambesina* caterpillar(s) flour at different substitution levels. The wheat muffins were stored at

three different temperatures (25ºC, 37ºC, and 45ºC) for 21 days. Generally, there was an increase in peroxide value with increase in storage time across all the storage temperatures. The wheat muffins oil samples recorded initial peroxide values of between 4.49 to 5.50 meq O_2/kg. Samples stored at 45ºC had higher peroxide values than those stored at 25ºC and 37ºC. The oil quality of the control wheat muffins stored at 25ºC increased from 4.50 meq O_2/kg to 9.43 meq O_2/kg oil over a storage period of 21 days. At 37ºC, the peroxide value was less than 10 meq O_2/kg oil for tests done on day 0, 7, and 14.However, a peroxide value of 10.67 meq O_2/kg was recorded as the highest on day 21 of sample storage. At 45ºC, the peroxide value increased from 4.50 meq O_2/kg to 7.97 meq O_2/kg, 9.83 meq O_2/kg, and 11.34 meq O_2/kg on test day 0, 7, 14, and 21, respectively.

The initial peroxide value for 5% wheat muffins oil was 5.00 meq O_2/kg. At 25ºC storage temperature, the peroxide value was reported as 7.84 meq O_2/kg, 9.83 meq O_2/kg, and 9.73 meq O_2/kg on test days 7, 14, and 21, respectively .Peroxide values 8.40 meq O_2/kg, 9.83 meq O_2/kg and 10.0 meq O_2/kg on test days 7, 14 and 21, were further reported, respectively, at 37ºC storage temperature. Higher peroxide values were obtained at 45ºC storage temperatures as 10.50 meq O_2/kg, 11.34 meq O_2/kg, and 13.34 meq O_2/kg on test days 7, 14 and 21, respectively. The peroxide value obtained for the 10% wheat muffins oil before storage was 4.67 meq O_2/kg. On test day 7, 14, and 21, the peroxide values reported at 25ºC storage temperature were 7.17 meq O_2/ kg, 8.67 meq O_2/kg, and 9.50 meq O_2/kg, respectively. At 37ºC, the oil samples recorded peroxide values of 8.67 meq O_2/kg and 9.34 meq O_2/kg on test day 7 and 14, respectively. However, the peroxide value surpassed 10.0 meq O2/kg on test day 21. The peroxide values recorded at 45ºC were 8.00 meq O_2/kg, 10.0 meq O_2/kg, and 13.0 meq O_2/kg on days 7, 14, and 21, respectively.

Wheat muffins enriched with 15% *G. zambesina* caterpillar(s) flour and stored at 25ºC recorded peroxide values of 7.67 meq O_2/kg, 8.84 meq O_2/kg, and 9.83 meq O_2/kg on test days 7, 14, and 21 respectively. Storage at 37ºC resulted into peroxide values of 8.67 meq O_2/kg, 9.34 meq O_2/kg, and 11.50 meq O_2/ kg on test day 7, 14, and 21 respectively. Furthermore, samples stored at 45ºC recorded peroxide values of 10.17meq O_2/kg, 10.84meq O_2/kg, and 13.34 meq O_2/kg on test day 7, 14, and 21 respectively. The initial peroxide value for the 20% wheat muffins oil was 5.34 meq O_2/kg. However, storage at 25ºC led to a rise in the peroxide value to 7.34 meq O_2/kg, 8.83 meq O_2/kg, and 9.34 meq O_2/kg on test days 7, 14, and 21, respectively. At 37ºC, the peroxide values were 7.68 meq O_2/kg, 8.83 meq O_2/kg, and 11.17 meq O_2/kg on test day 7, 14, and 21, respectively.

Peroxide value tests on enriched wheat muffins oil samples stored at 45ºC were 9.00 meq O_2/kg, 11.83 meq O_2/kg and 13.83 meq O_2/kg on days 7, 14 and 21, respectively.



A) (100% wheat flour), (B) (95% wheat flour; 5% *G. zambesina* caterpillar flour), (C) (90% wheat flour; 10% *G. zambesina* caterpillar flour), (D) (85% wheat flour; 15% *G. zambesina* caterpillar flour), (E) (80% wheat flour; 20% *G. zambesina* caterpillar flour)

Figure 15: Representative graphs showing the PVs of wheat muffins enriched with *G. zambesina* caterpillar(s) at different substitution levels.

Table 9 shows that there was significant difference ($p<0.05$) in the shelf life of wheat muffins enriched with *G. zambesina* caterpillar(s) flour. Generally, the predicted shelf life of the enriched wheat muffins decreased with corresponding increase in substitution level. The estimated shelf life of the control wheat muffins was 120.0 days. Wheat muffins enriched with *G. zambesina* caterpillar(s) flour at 5%, 10%, 15%, and 20% substitution levels estimated shelf life's were 111.0 days, 103.0 days, 102.0 days, and 90.0 days, respectively.

Table 10: Predicted shelf life of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

Substitution level	Arrhenius equation	R^2	Shelf-life (days)
0%	Y= -962.37x- 3.2398	0.9934	120.0^{a}
5%	Y= -1424.0x- 3.3292	0.7023	111.0^{a}
10%	Y= -1597.3x- 3.2195	0.9234	103.0^{a}
15%	Y= -1726.7x- 3.3996	0.9903	102.0^{ab}
20%	Y= -2344.2x- 3.3414	0.9715	90.0^{b}

Note: Values with different superscript (alphabet) along the column are significantly different ($p<0.05$)

4.6 Sensory evaluation

There was significant difference ($p<0.05$) in aroma, colour, texture and overall acceptability of wheat muffins enriched with *G. zambesina* caterpillar(s) flour (Table 11). However, there was no significant difference ($p<0.05$) in taste among the enriched wheat muffins. EM-2 had the least scores for aroma, colour, taste, texture and overall acceptability. A decline in sensory scores was observed for aroma, colour and overall acceptability with increase in substitution level of wheat flour with *G. zambesina* caterpillar(s) flour. Generally, all the sensory parameters evaluated for wheat muffins enriched with *G. zambesina* caterpillar(s) flour at all the substitution levels showed an above average mean score rating.

Table 11: Sensory scores for wheat muffins enriched with *G. zambesina* caterpillar(s) flour on a 5-point hedonic scale

Level of substitution	Aroma	Colour	Taste	Texture	Overall acceptability
EM-4	4.63±0.49^{a}	4.29±0.91^{a}	4.17±0.87^{a}	4.40±0.86^{a}	4.47±0.97^{a}
EM-3	4.17±0.59^{ab}	4.17±0.59^{ab}	3.90±0.66^{a}	4.17±0.70^{ab}	4.17±0.75^{ab}
EM-1	3.57±0.77^{bc}	3.93±0.83^{b}	4.00±0.69^{a}	3.83±0.79^{bc}	3.83±0.75^{bc}
EM-5	3.20±1.06^{cd}	3.53±1.01^{b}	4.00±0.87^{a}	3.63±1.07^{c}	3.67±0.96^{c}
EM-2	2.80±1.13^{d}	3.97±1.07^{b}	3.90±0.66^{a}	3.37±1.33^{c}	3.53±0.90^{c}
F(4, 145) value	22.60	2.98	0.62	5.37	5.74
p-value	<.0001	0.0212	0.6498	0.0005	0.0003

Note: Comparison is column wise. Values are mean ± SD of triplicate analysis. Means with different superscript (alphabet) are significantly different ($p<0.05$)

As shown in Figure 16, the colour of wheat muffins enriched with *G. zambesina* caterpillar(s) flour became darker with corresponding increase in the substitution level. Substitution of wheat flour with *G. zambesina* caterpillar(s) flour at 20% substitution level produced the most dark wheat muffins.

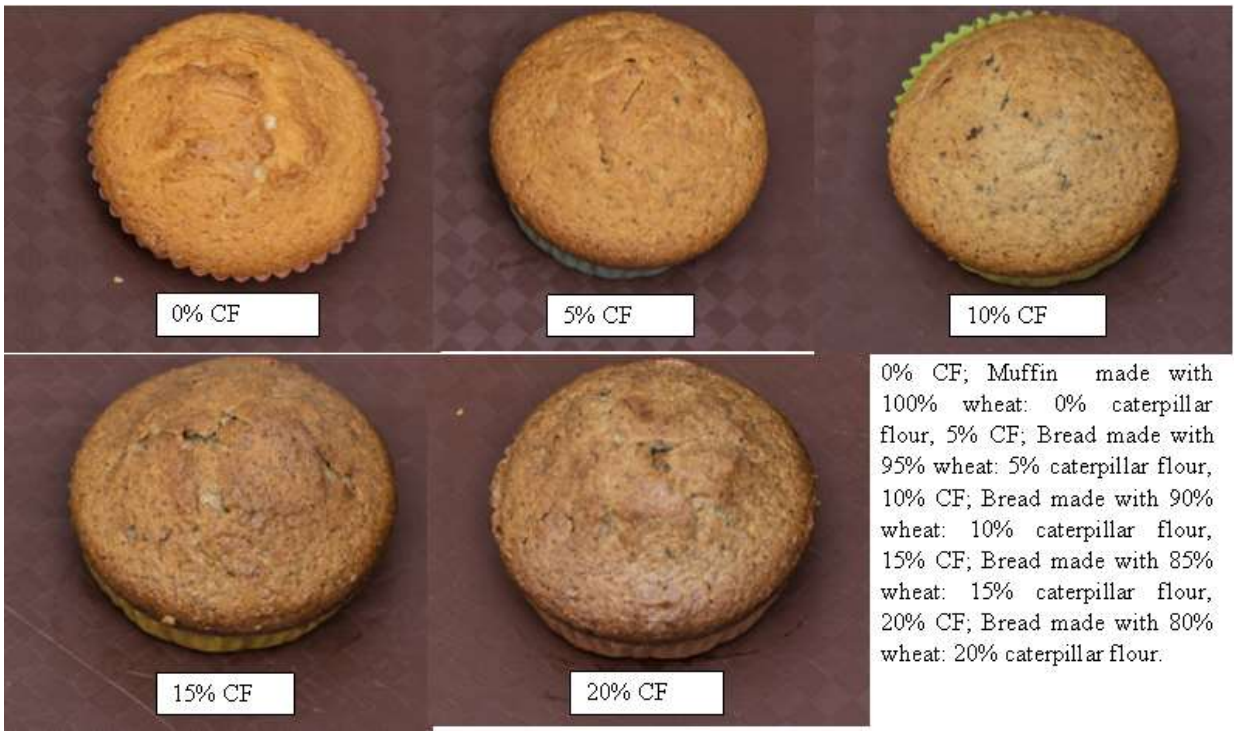


Figure 16: Wheat muffins enriched with *G. zambesina* caterpillar(s) flour at different substitution levels

4.7 The correlation coefficient for the sensory attributes of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

Table 12 shows correlation coefficient of sensory attributes of wheat muffins enriched with *G. zambesina* caterpillar(s) flour at different substitution levels. All the sensory attributes had a positive correlation and were significantly different ($p<0.05$). Among the sensory attributes, texture and overall acceptability had the strongest correlation coefficient (0.697) whereas taste and texture had the weakest correlation coefficient (0.215).

Table 12: Correlation coefficient for the sensory attributes of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

	Aroma	**Colour**	**Taste**	**Texture**	**Overall acceptability**
Aroma	1.000	0.238*	0.314*	0.301*	0.475*
Colour		1.000	0.261*	0.515*	0.551*
Taste			1.000	0.215*	0.365*
Texture				1.000	0.697*
Overall acceptability					1.000

Key: Values with asterisks (*) are significantly different ($p<0.05$)

CHAPTER FIVE: DISCUSSION

5.1 Introduction

In this chapter, the focus is on discussion of the results as presented in the previous chapter. The discussion on the characterization of the raw materials has been presented first. The findings of the study have then been discussed based on the study objectives and comparing the findings of this study with other studies.

5.2 Raw materials characterization

5.2.1 Physicochemical composition of wheat flour and *G. zambesina* caterpillar(s) flour

The protein content of *G. zambesina* caterpillar(s) flour was very high, (59.33±0.30%). This is in agreement with the review by Subramanian *et al*. (2017) in which it is reported that more than 50% of the bodyweight of the *G. zambesina* caterpillar is protein (dry mass). High protein contents have been reported in the lepidopterous larvae with and without evisceration. However, evisceration slightly increases the protein contents of edible caterpillars (Lautenschläger *et al*., 2017; Rumpold & Schlüter, 2013b, 2013a; Solomon & Prisca, 2012). The wheat flours' protein content of 13.11±0.45% on a dry matter basis was slightly higher than those reported by Victor *et al*. (2013) and Chen *et al*. (2021). This difference in protein contents could be due to the type of wheat flour used for baking. Since *G. zambesina* caterpillar(s) flour is higher in crude protein content than wheat flour, substituting wheat flour with the caterpillar(s) flour may increase the protein content of wheat flour.

The crude fibre content of *G. zambesina* caterpillar(s) flour was higher (15.50±0.25%) compared to wheat flour (0.88±0.01%). The high crude fibre content of *G. zambesina* caterpillar(s) flour can be attributed to a component of the exoskeleton known as chitin which is a polymer of N-acetyl-D-glucosamine which is present in insects in varying proportions (Dauda *et al*., 2014; Kim *et al*., 2019). The low fibre content of the wheat flour can be ascribed to the flour refining process during which, wheat bran which is a rich source of fibre is separated from wheat grain (P. Kumar et al., 2011). The fat content of *G. zambesina* caterpillar(s) flour was found to be 14.87±0.26% which fall within the range for the fat content of lepidopterous (5.25% -77.17%) (Rumpold & Schlüter, 2015). Compared to the fat content of other closely related caterpillars, *G. zambesina* caterpillar flour had a higher crude fat content (14.87±0.26%) than 10.85±0.65% and 4.68±0.01% reported for *Bunaea alcinoe* and *Cirina forda,* respectively (Dauda *et al*., 2014; Omotoso, 2006). Fat plays

an essential role in the human diet. The presence of fat makes food more palatable by absorbing and retaining the flavors therein. It also helps in the transportation of fat-soluble vitamins which is very essential nutrition-wise (Igbabul *et al*., 2014). The fat content of wheat flour was found to be too low (0.65±0.09%). This can be ascribed to the fact wheat germ which stores oil is just 3% of the whole wheat grain (Kumar *et al*., 2011).

The ash content of *G. zambesina* caterpillar(s) flour was 3.46±0.20% which was higher than that of wheat flour (0.53±0.10%). The low ash content of wheat flour can be attributed to the removal of the wheat bran during the flour refining process (Victor *et al*., 2013). In comparing the ash content of *G. zambesina* caterpillar(s) flour to those of other caterpillars, *G. zambesina* caterpillar was lower in ash content (3.46±0.20%) than *C. forda* (10.26±0.01%) and silkworm (*Bombyx mori* L.) (6.34±0.84%). Ash content of a product is a reflection of its mineral content (Omotoso, 2006, 2015). Carbohydrate was the major component of wheat flour (75.56±0.69%). This was higher than that of *G. zambesina* caterpillar(s) flour (1.17±0.39%). A comparison of the carbohydrate content of *G. zambesina* caterpillar(s) with other lepidopterous larva showed that the carbohydrate content of *G. zambesina* fell short being within the range of average insect's carbohydrates (Kim *et al*., 2019; Mlcek *et al*., 2014). The low carbohydrate content of *G. zambesina* caterpillar(s) can be ascribed to the removal of the gut content during processing. Evisceration significantly reduces carbohydrates originating from the host plant in edible caterpillars (Lautenschläger *et al*., 2017).

5.2.2 *In vitro* protein digestibility of *G. zambesina* caterpillar(s) flour

In vitro protein digestibility of *G. zambesina* caterpillar(s) flour was 64.05% which falls within the expected digestibility range of 45-66.9% for most insects with chitin (Marono *et al*., 2015). In general, animal proteins are 90-95% digestible (*in vitro*) which is much superior to plant proteins *IVPD* (75-80%) (Kouřimská & Adámková, 2016) (Sá *et al*. 2020). The *IVPD* of *G. zambesina* caterpillar(s) flour (64.05%) was higher than that of *G. belina* (53.3%) to which it is closely related. However, the *IVPD* of *G. zambesina* caterpillar(s) was lower compared to those of Orthopterans such as fresh dried termites (90.11%), green grasshopper (76.64%), and brown grasshopper (81.11%) (Kinyuru *et al*., 2010). The low *IVPD* of *G. zambesina* caterpillar(s) flour might be attributed to the presence of chitin that binds to cuticular proteins with high proportions of amino acids (Mba *et al*., 2019; Oonincx & Finke, 2020). Chitin is non-degradable and cannot be absorbed by the small intestine (Marono *et al*., 2015). It might further be ascribed to processing of *G.*

zambesina caterpillar(s) flour and the drying method which might have had an impact on *IVPD* of the caterpillar flour (Kinyuru *et al*., 2010; Oonincx & Finke, 2020).

5.2.3 Mineral composition of *G. zambesina* caterpillar(s) flour

Composition and concentration of minerals vary from one insect to another and often do not follow a particular pattern (Kinyuru, 2020). Comparing the mineral concentration of *G. zambesina* caterpillar(s) flour to that of another related caterpillar, *G. zambesina* caterpillar(s) had a higher zinc concentration (17.8 mg/100 g) than *G. belina* (8-14 mg/100 g). It was also found to have high potassium concentration (1740.0 mg/100 g) than *Cirina butyrospermi* caterpillar (1160 mg/100 g). However, the iron concentration of *G. zambesina* caterpillar(s) flour was lower than that of *C. butyrospermi* caterpillar (12.97 mg/100 g) and *G. belina* (31-77 mg/100 g) (Anvo *et al*., 2016; Kouřimská & Adámková, 2016). Dietary zinc and iron are essential in the human diet, especially for pregnant women, vegetarians, and malnourished individuals (Ayensu *et al*., 2019). Some of the essential roles dietary zinc play in the human body are; regulating cell growth, improving sperm quality in males, acting as a cofactor for certain enzymes, and it also plays part in cell gene expression (Khan *et al*., 2013). With a zinc concentration of 17.8 mg/100 g, *G. zambesina* caterpillar(s) flour has the potential to contribute to the zinc recommended daily intake for adults (12-15 mg/ day) RDA (Khan *et al*., 2013). The iron concentration of *G. zambesina* caterpillar(s) flour was lower than the recommended daily intake for adults (10-15 mg/ day) (Khan *et al*., 2013). Iron is essential in the human diet since it's responsible for the formation of hemoglobin. Thus, dietary iron intake is highly recommended for premenopausal women for replenishing blood lost during menstruation (Khan *et al*., 2013).

5.2.4 Retinol, α, and γ-tocopherol concentrations in *G. zambesina* caterpillar(s) flour

Lepidopterous species such as *Imbrasia oyemensis*, *Nudaurelia oyemensis, Ichthyodes truncata*, *Imbrasia epimethea, T. molitor, and* super worm *Z. mori* have been reported to have retinol (Kouřimská & Adámková, 2016). However, insects do not generally appear to contain much-preformed vitamin A (Kouřimská & Adámková, 2016). Retinol is most labile during culinary process (Lešková *et al*., 2006). Therefore, exposure of *G. zambesina* caterpillar(s) flour to factors/ conditions such as light, drying temperature, oxygen during processing and the duration of treatment might have enhanced the degradation of retinol (Belitz *et al*., 2009; Lešková *et al*., 2006). The concentration of α-tocopherol was found to be more in the *G. zambesina* caterpillar(s) (4.32

mg/100 g) than in the larvae of *T. molitor* (1.9 mg/100 g), *Imbrasia truncata* (0.52 ± 0.08 mg/100 g) and *I. epimethea* (0.41 ± 0.06 mg/100 g) (Kouřimská & Adámková, 2016; Mba *et al*., 2019). This difference in the concentration levels of α-tocopherol between *G. zambesina* caterpillar(s) and the related caterpillars may either increase or reduce depending on whether all the samples are analysed on dry or wet mass basis. Though present, the levels of concentration of γ-tocopherol in *G. zambesina* caterpillar(s) flour was low (0.16 mg/ 100 g). Generally, animal foods are not rich sources of vitamin E (Belitz *et al*., 2009). The presence of both α and γ-tocopherol concentration of *G. zambesina* caterpillar(s) flour implies that the flour has a potential to contribute to the dietary requirement for vitamin E. Consumption of *G. zambesina* caterpillar(s) as a source of vitamin A is recommended only when it's supplemented it with other sources of vitamin A since it deficient of the same.

5.3 Proximate composition of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

Substitution of wheat flour with *G. zambesina* caterpillar(s) flour at 5%, 10%, 15%, and 20% substitution levels resulted in a 29.5%, 43.5%, 60.5%, and 73.6% increase in protein content of enriched wheat muffins, respectively. This increase might be attributed to the addition of *G. zambesina* caterpillar(s) with a relatively high protein content (Table 6) to wheat flour. These findings are in agreement with observations by Ayensu *et al*. (2019) who reported an increase in protein content of biscuits fortified with palm weevil larvae (*Rhynchophorus phoenicis*) and orange-fleshed potato. Similarly, Kinyuru *et al*. (2009) reported an increase in protein content of wheat buns enriched with winged termites (*Macrotermes subhylanus*) flour with corresponding increase in substitution level. Zielińska *et al*. (2021) also reported a proportional increase in protein content of muffins enriched with cricket (*Gryllodes sigillatus*) and mealworm (*T. molitor*) flour.

The carbohydrate content of the wheat muffins decreased with increasing the substitution levels of wheat flour with *G. zambesina* caterpillar(s) flour. This was expected since wheat flour is the major component of wheat muffins and has a carbohydrate content of 75.56±0.69%. Hence, substituting it with *G. zambesina* caterpillar(s) flour with only 1.17±0.39% carbohydrate content would lower the overall carbohydrate content of the enriched wheat muffins. These findings were in agreement with that by de Oliveira *et al*. (2017) who reported a decrease in carbohydrate contents in bread enriched with cinereous cockroach (*Nauphoeta cinerea*) flour. Research by

Ayensu *et al*. (2019) on biscuits fortified with palm weevil larvae (*Rhynchophorus phoenicis*) and orange-fleshed potato also reported similar trends in carbohydrate contents of the biscuits.

The increase in crude fibre content of the enriched wheat muffins with corresponding increase in substitution level can be attributed to the results in Table 6. *Gonimbrasia zambesina* caterpillar(s) flour has higher fibre content (15.50±0.25%) than wheat flour (0.88±0.01%). The data obtained in this study are in agreement with the findings by Ayensu *et al*. (2019) on the crude fibre contents of biscuits fortified with palm weevil larvae (*R. phoenicis*) flour and orange-fleshed potato. Osimani *et al*. (2018) also reported similar findings on crude fibre contents of bread enriched with cricket flour (*Acheta domesticus*). Dietary fibre has an important role in the human diet. It is responsible for maintaining the health of the digestive system and for proper bowel movement, adding bulk to the diet, preventing absorption of excess cholesterol, and suppressing and delaying digestion of carbohydrates (Nantanga & Amakali, 2020; Princewill-Ogbonna *et al*., 2019). According to EFSA (2010), a dietary fibre intake of 25 g/ day is adequate for a normal laxation in adults. It further reports that dietary fibre intake of 2 g/ MJ fibre in food is adequate for laxation in children below 1 year. Substituting wheat flour with *G. zambesina* caterpillar(s) flour at 15% and 20% substitution levels resulted into wheat muffins with 2.04±0.06% and 2.67±0.22% fibre content, respectively. These, have the potential to contribute to the daily fibre intake threshold for adults and children. According to EFSA, for a particular food to be considered a high fibre food, it should provide 3 mg/ 100 g of dietary fibre (EFSA, 2010; Osimani *et al*., 2018). Based on the findings of this study, wheat muffins enriched with *G. zambesina* caterpillar(s) flour have got potential to contribute to dietary fibre intake.

An increase in the fat content of wheat muffins with corresponding increase in the substitution level of wheat flour with *G. zambesina* caterpillar(s) flour might be attributed to the higher fat content of *G. zambesina* caterpillar(s) flour than wheat flour's (Table 6). However, the generally high-fat content for both the reference wheat muffins and the enriched wheat muffins can be attributed to an equal quantity of melted margarine added according to the optimized wheat muffins formula (Table 2). These findings are in agreement with those reported by Ayensu *et al*. (2019) in their research on biscuits fortified with palm weevil larvae (*R. phoenicis*) and orange-fleshed sweet potato among pregnant women. Similarly, Zielińska *et al*., (2021) in their study

reported an increase in the fat contents of muffins enriched with cricket (*Gryllodes sigillatus*) and mealworm (*T. molitor*) flour.

There was a non-significant increasing trend in the ash contents of wheat muffins enriched with *G. zambesina* caterpillar(s) flour. These findings are in agreement with that by de Oliveira *et al*. (2017) on bread enriched with a cinereous cockroach (*Nauphoeta cinerea*) flour. Ayensu *et al*. (2019) also reported an increasing trend in the ash contents of biscuits fortified with palm weevil larvae (*R. phoenicis*) flour and orange-fleshed potato with corresponding increase in substitution level. Similarly, Osimani *et al*. (2018) also reported a linear relationship in ash content of bread enriched with cricket flour (*Acheta domesticus*) with an increase in substitution level. Furthermore, research by (Smarzyński et al., 2019) showed an increase in the ash content of enriched pork pâtés with increase in cricket powder. The ash content of any food product is an indicator of its mineral content (Khan *et al*., 2013). Therefore, it can be concluded that enriched wheat muffins had high mineral content than the control wheat muffins (Chen *et al*., 2021; Cheng & Bhat, 2016).

5.4 *In vitro* protein digestibility of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

The higher *IVPD* of wheat muffins enriched with *G. zambesina* caterpillar(s) flour at 5% substitution level compared to the reference wheat muffins might be ascribed to the addition of novel protein present in *G. zambesina* caterpillar(s) flour. This novel protein in *G. zambesina* caterpillar(s) flour was exposed to the digestive enzymes together with the wheat protein for digestion. It can be further argued that at 5% substitution level, the proportions of non-degradable chitin fibre in the blended flour were insignificant. That is to say, only a small portion of amino acids was bound with chitin. The decline in trend in *IVPD* of wheat muffins enriched with *G. zambesina* caterpillar(s) flour from 10% to 20% substitution levels can be attributed to increasing proportions of chitin fibre in *G. zambesina* caterpillar(s) flour with corresponding increase in substitution level. A similar observation was made by Dewi *et al*. (2020) who reported reduction in *IVPD* of baby biscuits fortified with wood grasshopper flour (*Melanoplus cinereus*) as an alternative complementary food for children at higher substitution levels. Chitin decreases protein digestibility in food since it contain anti-nutrients and it binds to protein which then become non-degradable by the digestive enzymes (Guerreiro *et al*., 2020; Marono *etal*., 2015). It also hampers

hydrolysis of proteins as it successfully eludes the digestive enzymes rendering it non-absorbable by the small intestines (Moyo *et al*. 2019).

5.5 Mineral content of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

The linear relationship observed between mineral concentrations of enriched wheat muffins with corresponding increase in the substitution levels can be ascribed to mineral concentrations of *G. zambesina* caterpillar(s) flour (Figure 10). These findings are in agreement with that by Ayensu *et al*. (2019) who reported increase in the mineral concentrations in biscuits fortified with palm weevil larvae (*R. phoenicis*) and orange-fleshed potato. Similarly, Bawa *et al*. (2020) also reported an increase in the mineral concentration of bread and cookie enriched with house cricket (*Acheta domesticus*) powder with increase in substitution level. The high concentrations of sodium, phosphorus, and potassium in the enriched wheat muffins might be attributed to the mineral contents of the soil in which wheat was planted. It might further be attributed to the concentrations of these minerals in *G. zambesina* caterpillar(s) flour as influenced by the host plant (*M. indica*) (Kumar *et al*., 2021). Both wheat flour and *G. zambesina* caterpillar(s) flour were used as ingredients in wheat muffins formulation hence they collectively contributed to the mineral concentrations of the enriched wheat muffins.

The RDA for potassium in adults is 2500 mg/day (Khan *et al*., 2013). Based on the findings of this study, enriching wheat muffins with *G. zambesina* caterpillar(s) flour has the potential to contribute to the daily recommended intake for potassium. Potassium plays a role in managing hypertension (Igbabul *et al*., 2014; Khan *et al*., 2013). Therefore, *G. zambesina* caterpillar(s) flour enriched wheat muffins might be recommended for people suffering from hypertension. Wheat muffins enriched with *G. zambesina* caterpillar(s) flour had lower sodium concentrations than the recommended daily intake (500 mg/day RDA) (Khan *et al*., 2013). Low intake of sodium reduce chances of dietary-induced hypertension. Pregnant women, children, and strict vegans in developing countries often suffer from iron and zinc deficiency (Igbabul *et al*., 2014; Khan *et al*., 2013; Solomon & Prisca, 2012). However, iron concentrations in wheat muffins enriched with *G. zambesina* caterpillar(s) flour were higher than in the control wheat muffins. Therefore, enriching wheat muffins with *G. zambesina* caterpillar(s) flour has the potential to contribute to the daily intake threshold for iron. The enriched wheat muffins might not be exclusively recommended to those suffering from zinc deficiency over reference wheat muffins. This is because statistically, they were not significantly different ($p<0.05$) in zinc concentrations. Dietary phosphorous in the

human diet is essential since it's responsible for maintaining serum phosphate levels. This reduce chances of hypophosphatemia in adults and children (Bawa *et al*., 2020). Therefore, *G. zambesina* caterpillar(s) flour enriched wheat muffins can be recommended to people suffering from hypophosphatemia due to their high concentrations of phosphorus.

5.6 Retinol, α, and γ-tocopherol contents of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

The increase in α-tocopherol and ɣ-tocopherol concentration with corresponding increase in substitution level in the enriched wheat muffins can be attributed to the presence of α-tocopherol and ɣ-tocopherol in *G. zambesina* caterpillar(s) flour (Figure 12). However, the increase was minimal and insignificant for ɣ-tocopherol concentration. This can be ascribed to the low concentrations of γ-tocopherol in *G. zambesina* caterpillar(s) flour used for substituting wheat flour. The presence of vitamin A in wheat muffins enriched with *G. zambesina* caterpillar(s) flour might be attributed to the addition of margarine fortified with vitamin A as an ingredient during formulation (Dary & Mora, 2002). Hydrogenated fats provide a suitable medium for vitamin A hence, are vitamin A fortified (Dary & Mora, 2002). It can further be attributed to the fortified wheat flour used since wheat grain, like other whole grain cereals is deficient in Vitamin A hence is often fortified (Klemm *et al*., 2010). The concentrations of retinol in the enriched wheat muffins were however low. This can be can be attributed to the antagonistic interaction of certain nutrients used in processing of the wheat muffins that have degraded the available retinol (Klemm *et al*., 2010).

5.7 Microbiological quality of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

Consumers are not only concerned about the nutritional benefits of edible insects and insect based products but also the safety of these products. From literature reviewed, edible insects are host to a range of spoilage and pathogenic microorganisms (Braide & Nwaoguikpe, 2011; Klunder *et al*., 2012; Ng'ang'a *et al*., 2019). The gut of edible insects are composed of micro biota that provides suitable medium for the growth of most spoilage and pathogenic microorganisms (Kouřimská & Adámková, 2016). However, enriching wheat muffins with *G. zambesina* caterpillar(s) flour during processing resulted to microbiologically safe wheat muffins for human consumption. This might be attributed to the fact that caterpillars whose flour was used in microbiological analysis were domesticated. This may have modulated the source to new microorganisms and the intrinsic ones in *G. zambesina* caterpillars (van der Fels-Klerx *et al*., 2018). These findings were similar to

that reported by Ayensu *et al*., (2019) in their research on the nutritional composition and acceptability of biscuits fortified with *R. phoenicis* larvae and orange-fleshed sweet potato in pregnant women.

The TVC for wheat muffins at all the substitution levels were below the $<10^5$ cfu/g which is the maximum permissible limit for insect-based bakery products (Das *et al*., 2020). This might be attributed to GMPs of the wheat muffins post baking and packaging in sterile polyethylene zip-lock bags (Vinayak Udyog, New Delhi, India) (Das *et al*., 2020). The absence of coliforms, *S. aureus*, and *Salmonella* in wheat muffins enriched with *G. zambesina* caterpillar(s) flour might be attributed to the dehulling of wheat during wheat flour processing which concentrates about 90% of microorganisms on the bran which then is separated from the germ and pollard (Sperber & GROUP, 2007; Victor *et al*., 2013). Wheat flour forms the major component of the wheat muffins. The absence of coliforms (indicator organism) in wheat muffins enriched with *G. zambesina* caterpillar(s) flour is evidence that GHPs and GMPs were adhered to during and post the processing (Martin *et al*., 2016). It might further be attributed to proper packaging which was done in sterilized polyethylene zip lock bags (Vinayak Udyog, New Delhi, India). Coliforms contaminations occur in bakery products due to poor post-process handling (Das *et al*., 2020).

Staphylococcus aureus and *Salmonella* were not detected in wheat muffins enriched with *G. zambesina* caterpillar flour which might also be ascribed to GHPs and GMPs. From the literature reviewed, egg content which was part of the ingredients for enriched wheat muffins harbour *Salmonella* spp (Das *et al*., 2020). Therefore, the absence of *Salmonella* in the enriched wheat muffins might further be attributed to the fact that *Salmonella* (enterobacteriaceae family) is sensitive to heat treatment (Das *et al*., 2020; Kouřimská & Adámková, 2016). Furthermore, degutting, washing and rinsing of the *G. zambesina* caterpillars which might have reduced the gut microorganism (Kouřimská & Adámková, 2016). For food product to meet the microbiological guidelines on safety, there should be no detection of *Salmonella* in 25 g of the sample tested (Nyangena *et al*., 2020).

On 21st day of storage, the yeast and moulds counts were lower than 30 cfu/g in all the enriched wheat muffins samples. This was way below the maximum acceptable limit for yeast and moulds in foods (1000 cfu/g) (Đurovic´ *et al*., 2021). Yeast and moulds are associated with high sugar foods such as wheat muffins which are often responsible for their spoilage thereby limiting their

shelf life (Đurovic´ *et al*., 2021; Saeed *et al*., 2019). Therefore, based on the findings of this study, wheat muffins enriched with *G. zambesina* caterpillar(s) flour are safe microbiologically safe for human consumption over a storage period of 21 days.

5.8 Shelf life of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

The initial peroxide values of wheat muffins enriched with *G. zambesina* caterpillar(s) flour at different substitution levels were generally higher than that of fresh amaranth seed oil (0.487 meq O_2 /kg oil) and fresh coconut oil (0.24 to 0.49 meq O_2 /kg oil) (Gichau *et al*., 2019). This can be attributed to primary oxidation process which might have set off after processing of the *G. zambesina* enriched wheat muffins (Gichau *et al*., 2019). It might also be ascribed to the high baking temperature (180ºC). A linear relationship exists between the rate of oxidation and temperature (Flick *et al*., 1992). Furthermore, the addition of sodium bicarbonate (metal ion) as an ingredient and exposure of wheat muffins enriched with *G. zambesina* caterpillar(s) flour to atmospheric oxygen before packaging might have catalyzed the oxidative reaction. Metal ions and exposure to oxygen enhances primary oxidation of lipids (Flick *et al*., 1992; Gichau *et al*., 2019).

The oxidative level of oils is reflected in the number of hydro peroxides formed in it which ultimately informs the tendency of an oil to become rancid (Moigradean *et al*., 2012). A food product is classified as a low oxidative state when its peroxide value is between 1-5 meq O_2/kg, a moderate oxidative state when it has a peroxide value between 5-10 meq O_2/kg, and a high oxidative state when its peroxide value is above 10 meq O_2/kg (Gichau *et al*., 2019; Moigradean *et al*., 2012). According to the East African Standard for refined oils, the peroxide value limit is 10 meq O_2/kg oil (EAS 795:2013) (Gichau *et al*., 2019). However, the peroxide value limit according to Codex Alimentarius is 15 meq O_2/kg oil (Moigradean *et al*., 2012). The East African Standards on cereal-based foods for infants and young children require that the developed product should be free from rancidity. A food product with a peroxide value of 20-40 meq O_2/kg exhibits a noticeable rancid taste and odour (Gichau *et al*., 2019). In this study, 9.83 meq O_2/kg, 11.50 meq O_2/kg, and 13.83 meq O_2/kg were the highest peroxide values recorded at 25ºC, 37ºC, and 45ºC, respectively among the oil samples analysed.

The increase in the peroxide values of *G. zambesina* caterpillar(s) flour enriched wheat muffins with corresponding increase in storage temperature might be ascribed to the linear relationship between temperature and the rate of oxidation in fats (Flick *et al*., 1992). Exposure of lipids to

higher temperatures enhances the rate of lipid oxidation while at lower temperatures, the rate of lipid oxidation is slow (Farhoosh & Hoseini-Yazdi, 2013). The higher peroxide values for the enriched wheat muffins reported on test day 21 might be attributed to the fact that as lipid oxidation takes place over a period of time, there is buildup of the hydro peroxides in the food product which leads to deterioration in quality (Flick *et al*., 1992). Wheat muffins enriched with *G. zambesina* caterpillar(s) flour at higher substitution levels were expected to have high peroxide values since Lepidoptera's larva flour are rich in unsaturated fatty acids (Van Huis, 2015). Unsaturated fatty acids are broken down by oxygen through an autolytic free radical mechanism (Moigradean *et al*., 2012; Smith *et al*., 2004). Higher substitution levels of wheat flour with *G. zambesina* caterpillar(s) flour translated to higher quantities of unsaturated fatty acids in the enriched wheat muffins.

In the estimation of the shelf life of *G. zambesina* caterpillar(s) flour enriched wheat muffin, the quality reference estimation for a maximum peroxide value was taken to be 10 meq O_2/ kg. The control wheat muffins had longer shelf life. This might be ascribed to the fact that no unsaturated fatty acid from the caterpillar was used in its formulation. At lower substitution levels, the shelf life of wheat muffins were relatively longer. This can be attributed to the small quantities of unsaturated fatty acids in *G. zambesina* caterpillar(s) flour at low substitution levels (Moigradean *et al*., 2012). The decrease in the shelf life of enriched wheat muffins with corresponding increase in substitution level might be attributed to the increase in unsaturated fats with corresponding increase in the substitution level. Based on these findings, the bakery industry can attempt enriching wheat products with *G. zambesina* caterpillar(s) flour at lower substitution levels (5% and 10%) without compromising their shelf life.

5.9 Sensory properties of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

The colour of wheat muffins enriched with *G. zambesina* caterpillar(s) flour became more dark brown with corresponding increase in the substitution level of wheat flour with *G. zambesina* caterpillar(s) flour (Figure 15). This findings is in agreement with that by Kinyuru *et al*. (2009) who reported that wheat buns enriched with *M. subhylanus* became more dark brown at higher substitution levels. The brown colour that forms during baking of wheat products is attributed to the Maillard reaction and caramelization of sugars (Kinyuru *et al*., 2009; Purlis, 2010). Maillard reaction is a spontaneous and naturally occurring non-enzymatic chemical reaction between the reducing sugars, amino acids peptides, and proteins causing browning (Oliver *et al*., 2006; Purlis,

2010). Just as the other insects' flour, *G. zambesina* caterpillar(s) flour contain compounds responsible for the Maillard reaction (Kouřimská & Adámková, 2016). The overall mean rating in colour for all the enriched wheat muffins were above average with the least mean rating of 3.53±1.01 reported for wheat muffins enriched at 15% substitution level. These high (above average) mean ratings for colour can be attributed to changing food systems among the middle class as their consumption behavior is fast shifting towards the utilization of healthy foods e.g brown bread (Gillespie & Bold, 2017). The highest mean rating score for colour (4.29±0.91) attained by wheat muffins enriched at 0% substitution level can be ascribed to the striking similarity between the control wheat muffins with the muffins consumer often meet on supermarket shelves.

Most panelists remarked that EM-5 (15% enriched wheat muffins) and EM-2 (20% enriched wheat muffins) had rough textures. This was also evident in the mean rating score for texture for 15% enriched wheat muffins (3.63±1.07) and 20% enriched wheat muffins (3.37±1.33). These scores were lower than those for wheat muffins at lower substitution levels. The rough texture of wheat muffins at higher substitution levels might be attributed to the addition of *G. zambesina* caterpillar(s) flour to wheat muffins during formulation. *Gonimbrasia zambesina* caterpillars have got a hard exoskeleton and spikes which are not finely grounded during flour processing. The presence of these hard exoskeleton particles in the flour might have affected the texture of the wheat muffins (Kouřimská & Adámková, 2016; Mishyna et al., 2020). Furthermore, the high fibre content of the *G. zambesina* caterpillar(s) flour might have also contributed to the rough texture. This is because the low fibre component, wheat flour was substituted with a high fibre component, *G. zambesina* caterpillar(s) flour. Despite grinding *G. zambesina* caterpillar(s) and sieving through a 250µm sieve, the panelists were able to point out detectable particles in wheat muffins enriched with *G. zambesina* caterpillar(s) flour at 5%, 10%, 15%, and 20% substitution levels. This can be ascribed to hard irregular-shaped particles that might have escaped through the 250µm sieve during sieving. These hard irregular particles can easily be detected in the mouth and by touching. These findings were similar to those by Tortoe, (2014) who reported in their research on assessing the sensory characteristics and consumer preference of yam-cowpea-soybeans porridge in the Accra metropolitan area that detectable hard particles were present.

A decrease in the mean rating for aroma with corresponding increase in the substitution level was observed. A similar observation was made by Kinyuru *et al*. (2009) in their research in which wheat buns enriched with *Macrotermes subhylanus* flour had higher ratings for aroma at lower substitution levels (0% and 5%) compared to wheat buns at 15% and 20% substitution levels. Panelists pointed out a unique aroma in wheat muffins at 15% and 20% substitution levels of wheat flour with *G. zambesina* caterpillar(s) flour. This unique aroma in enriched wheat muffins can be attributed to the addition of *G. zambesina* caterpillar(s) flour which might have introduced an insect-like smell in the wheat muffins. However, this is not a strange phenomenon since in a study by Ayieko *et al*. (2010), it was similarly observed that enriching wheat products with mayfly resulted in cookies having a unique aroma/ flavour. The presence of this unique aroma/flavour in insects can therefore be used as a justification for the panelist's low mean rating score for the aroma of wheat muffins enriched at 20% substitution level (2.80±1.13). This low mean rating can be further attributed to the fact that majority of the panelists might have not had prior history of entomophagy. This might have made it difficult for them to appreciate the unique aroma/ flavour associated with edible insects (Ayieko *et al*., 2010). The general mean ratings for aroma of the wheat muffins except that at 20% substitution level is an indication that at lower substitution levels, consumers are more likely to accept the *G. zambesina* caterpillar(s) flour enriched wheat muffins.

High mean ratings were observed for taste at 0% (4.17±0.87), 5% (3.90±0.66), 10% (4.00±0.69), 15% (4.00±0.87) and 20% (3.90±0.66) substitution levels. The taste can be attributed to addition of egg, margarine, and sugar used as ingredients in the wheat muffins formulation which might have influenced the taste of the wheat muffins. Similar observation was also made by Kinyuru *et al*. (2009) in their research on process development, nutrition and sensory qualities of wheat buns enriched with *M. subhylanus*.

The significant difference ($p<0.05$) in the overall acceptability can be attributed to the addition of *G. zambesina* caterpillar(s) flour. The highest mean rating (4.47±0.97) for the control wheat muffins might be attributed to the fact that they were actually what most consumers interact with on shelves and in food stores. As expected, wheat muffins enriched at 20% substitution level of wheat flour with *G. zambesina* caterpillar(s) flour received the least mean rating (3.53±0.90) for overall acceptability. This might be ascribed to the differences in attributes such as colour, aroma, and texture observed between control wheat muffins (0%) and wheat muffins enriched at 20%

substitution level of wheat flour with *G. zambesina* caterpillar(s) flour. These sensory attributes are capable of informing a consumer's decision to accept or reject a food product. The mean rating for wheat muffins at 0%, 5%, and 10% substitution levels of wheat flour with *G .zambesina* caterpillar(s) flour; 4.47±0.97, 4.17±0.75, and 3.83±0.75 respectively, were comparably high. Hence, 10% wheat muffins can be recommended for large-scale production since it is also richer in crude protein.

5.10 The correlation coefficient for the sensory attributes of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

The implication of a strong correlation coefficient between texture and overall acceptability is that texture influenced the consumer's decision to accept wheat muffins enriched with *G. zambesina* caterpillar(s) flour. These findings are in agreement with the findings by Gitau *et al*. (2019) who reported that texture was the most influencing factor in the overall acceptability of legume-cereal root porridge. The texture of the wheat muffins was mainly influenced by the substitution of wheat flour with *G. zambesina* caterpillar(s) flour which is rich in insoluble fibre. Colour and overall acceptability had a correlation coefficient of 0.551. Taste and overall acceptability had a weak correlation coefficient (0.365) which implied that taste least influenced the consumer's overall acceptability for the enriched wheat muffins.

CHAPTER SIX: CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

6.1 Conclusions

1. Substitution of wheat flour with *G. zambesina* caterpillar(s) flour increases the protein, fibre and fat content of wheat muffins with an increase in substitution levels. The addition of *G. zambesina* caterpillar(s) flour to wheat flour also affects the *IVPD* of the enriched wheat muffins with muffins enriched at 5%, 10% and 15% showing high *IVPD*. The increase in α and γ tocopherol with an increase in substitution level is non-substantial while the presence of retinol in the enriched wheat muffins is attributed to another vitamin A fortified ingredient but not the addition of *G. zambesina* caterpillar(s) flour. The mineral content of the enriched wheat muffins increases with an increase in the substitution levels hence enriched wheat muffins have the potential to contribute to the RDAs for various minerals.

2. Substitution of wheat flour with *G. zambesina* caterpillar(s) flour does not affect the microbiological quality of the enriched wheat muffins. The TVC, TCC, yeast and moulds were below the maximum acceptable limits while *Salmonella* and *S. aureus* were not detected. This phenomenon is associated more with the baking temperature-time combination and the post-process handling of the enriched wheat muffins.

3. The peroxide values of wheat muffins increase with an increase in the substitution level of wheat flour with *G. zambesina* caterpillar(s) flour and the storage period. The formation of hydro peroxides affects the shelf life of *G. zambesina* caterpillar(s) flour-enriched wheat muffins by significantly reducing their shelf life. Wheat muffins enriched at lower substitution levels have longer shelf life than those substituted at higher substitution levels.

4. Substitution of wheat flour with *G. zambesina* caterpillar(s) flour affects the sensory properties of wheat muffins. Wheat muffins at lower substitution levels are most preferred by consumers in terms of aroma, colour, texture and overall acceptability to those at higher substitution levels. Taste is not influenced by the substitution of wheat flour with *G. zambesina* caterpillar(s) flour.

6.2 Recommendations

The study recommends that;

1. *Gonimbrasia zambesina* caterpillar(s) flour should be adopted as an enrichment ingredient to refined wheat flour in the bakery industry to improve the protein, fibre and mineral contents of wheat muffins.

2. Adherence to GHPs during processing and post-process should be highly considered if *G. zambesina* caterpillar(s) flour-enriched wheat muffins with microbiological quality below the maximum acceptable limits are to be achieved. Microbiological contamination in baked products often occurs due to poor post-process handling.

3. The maximum substitution level for the substitution of wheat flour with *G. zambesina* caterpillar (s) flour should be 15% since substitution levels above 15% have proved to significantly reduce the shelf life of wheat muffins enriched with *G. zambesina* caterpillar(s) flour.

4. Enriching wheat muffins with *G. zambesina* caterpillar(s) flour should be done at 5% and 10 % substitution levels since enriched wheat muffins were more preferred by consumers in terms of aroma, colour, texture and overall acceptability at these levels than 15% and 20% substitution levels.

6.3 Suggestions for further research

1. Further research to establish the amino acid profile and presence of anti-nutrients in *G. zambesina* caterpillar(s) flour which was an enrichment ingredient in wheat muffins. This is because *the IVPD* of 20% wheat muffins were lower than those of 5%, 10% and 15% substitution levels despite having the highest protein content

2. Future work on the use of moisture content, microbiological changes and other chemical changes such as para-anisidine value (p-*AV*) and total oxidation value (TOTOX) to determine the shelf life of wheat muffins enriched with *G. zambesina* caterpillar(s) flour

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APPENDICES

Appendix 1: Consent form

Code: …….

Date: …….

Gender……

Age…….

Sensory evaluation of wheat muffins enriched with African emperor moth (*Gonimbrasia zambesina*) caterpillar flour

You are invited to participate in sensory evaluation of wheat muffins enriched with *Gonimbrasia zambesina* caterpillar flour. Kindly, keenly read through this form and ask any questions that you may have concerning the study before accepting to be enrolled. Note that your participation in the study is voluntary and you are free to withdraw at any time. Confidentiality of your assessment is guaranteed. Kindly fill in your details in the section below.

Declaration

I have read the explanation provided for this study and all my concerns about the study have been addressed adequately. I have no known food sensitivities and I am under no medications which restricts my choice of food.

Name : ……. Signature : ……

Appendix 2 : Wheat muffins sensory score card on a 5-point hedonic scale

Instructions

You have been provided with five coded samples (EM-1, EM-2, EM-3, EM-4 and EM-5) of wheat muffins enriched with *G. zambesina* caterpillar flour. Take a sip of water to clean your palate before and after tasting each sample. While tasting, hold the sample in the mouth for approximately 5secs as you gently chew the sample. (NB: Ensure you have tasted each of the coded muffin samples provided). Indicate your preference in the column using appropriate number against each attribute as follows:

- 5- Like extremely
- 4 - Like
- 3- Neither like nor dislike
- 2- Dislike
- 1-Dislike extremely

Attributes	**Sample Codes**				
	EM-4	**EM-3**	**EM-1**	**EM-5**	**EM-2**
Colour					
Aroma					
Texture					
Taste					
Overall Acceptability					

Remark……...

……

……

……

Appendix 3: NACOSTI research permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **336266**

Date of Issue: **18/January/2021**

RESEARCH LICENSE

[[permit_photo]]

This is to Certify that Mr.. Fedinand Opondo Ouma of Jaramogi Oginga Odinga University of Science and Technology, has been licensed to conduct research in Nakuru on the topic: NUTRITIONAL, MICROBIAL AND SENSORY PROPERTIES OF WHEAT MUFFINS ENRICHED WITH AFRICAN EMPEROR MOTH (Gonimbrasia zambesina) CATERPILLAR FLOUR for the period ending : 18/January/2022.

License No: **NACOSTI/P/21/8512**

336266

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix 4: Ethical clearance permit

JARAMOGI OGINGA ODINGA
UNIVERSITY OF SCIENCE AND TECHNOLOGY

DIVISION OF RESEARCH, INNOVATION AND OUTREACH
JOOUST-ETHICS REVIEW OFFICE

Tel. 057-2501804
Email: erc@jooust.ac.ke
Website: www.jooust.ac.ke

P.O. BOX 210 - 40601
BONDO

OUR REF: JOOUST/DVC-RIO/ERC/E2

24th November, 2020

Fedinand Opondo Ouma
A451/4134/2019
JOOUST

Dear Mr. Ouma,

RE: APPROVAL TO CONDUCT RESEARCH TITLED "NUTRITIONAL, MICROBIAL AND SENSORY PROPERTIES OF WHEAT MUFFINS ENRICHED WITH AFRICAN EMPEROR MOTH (GONIMBRASIA ZAMBESINA) CATERPILLAR FLOUR"

This is to inform you that JOOUST ERC has reviewed and approved your above research proposal. Your application approval number is **ERC/23/11/20-17.** The approval period is from 23rd November, 2020 – 22nd November, 2021.

This approval is subject to compliance with the following requirements:

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations and violations) are submitted for review and approval by JOOUST IERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to NACOSTI IERC within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks of affected safety or welfare of study participants and others or affect the integrity of the research must be reported to NACOSTI IERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to JOOUST IERC.

Prior to commencing your study, you will be expected to obtain a research permit from National Commission for Science, Technology and Innovation (NACOSTI) https:oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

Prof. Francis Anga'wa
Chairman, JOOUST ERC

Copy to: Deputy Vice-Chancellor, RIO Director, BPS Dean, SAFS

Appendix 5: Publications and conference presentation

Publications

Scientific Research Publishing

Food and Nutrition Sciences, 2022, 13, 734-749
https://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Microbiological Assessment and Shelf-Life Determination of Wheat Muffins Enriched with Domesticated African Emperor Moth (*Gonimbrasia zambesina* Walker) Caterpillar Flour

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Published: August 22, 2022

Abstract

African emperor moth (*Gonimbrasia zambesina*) caterpillars are considered healthy food as they are rich in protein and unsaturated fats. In Kenya, *G. zambesina* caterpillars are predominantly found along the coastal region, where they emerge in during the short and the long rains. The caterpillars forage in the wild on mango (*Mangifera indica*) leaves and *Euclea natalensis* (Ericales: Ebenaceae) leaves. The caterpillars are consumed whole or may be transformed into fine flour. The caterpillars' flour can be utilized in the bakery industry for the enrichment of bakery products since wheat (the major component of bakery products) is low in protein. However, consumers are concerned about the microbiological quality of bakery products enriched with insect flour. There are also concerns about the effect of these insects' flour on the shelf life of bakery products since they have unsaturated fats. Therefore, this study evaluated the microbial quality and shelf life of wheat muffins enriched with *G. zambesina* caterpillar flour at 0%, 5%, 10%, 15%, and 20%. For all the samples analyzed, total viable count (TVC) was <30 cfu/g, total coliform count (TCC) was <30 cfu/g, *Salmonella* spp, and *Staphylococcus aureus* were not detected. The colonies for yeast and moulds were <30 cfu/g throughout the evaluation time of 21 days. The PVs of wheat muffins increased with an increase in the substitution level. The PVs of enriched wheat muffins increased with an increase in storage time and temperature. The shelf-life of the wheat muffins decreased with an increase in the substitution level of wheat flour with *G. zambesina* caterpillar flour. The predicted shelf life of 0%, 5%, 10%, 15%, and 20% wheat muffins was 120.0 days, 111.0 days, 103.0 days,

International Journal of Tropical Insect Science (2022) 42:3097–3105
https://doi.org/10.1007/s42690-022-00848-w

ORIGINAL RESEARCH ARTICLE

Nutritional composition and sensory Properties of wheat muffins enriched with *Gonimbrasia zambesina*, walker caterpillar flour

Fedinand Opondo Ouma[1] · Alice Nakhumicha Muriithi[1] · Joseph Ochieng' Anyango[2]

Received: 29 October 2021 / Accepted: 4 July 2022 / Published online: 3 August 2022

Abstract

Sub-Saharan Africa still bears the greatest forms of malnutrition. Attention is shifting to the use of edible insects in forms which are acceptable to people irrespective of their social status and level of civilization in efforts to alleviate protein malnutrition. *Gonimbrasia zambesina* (Lepidoptera: Saturniidae) caterpillars emerge seasonally in the coastal part of Kenya and despite their rich nutritional profile, their consumption is low. This study was thus undertaken to evaluate the effect of substituting wheat flour with *G. zambesina* caterpillar flour at 0%, 5%, 10%, 15% and 20% substitution levels on the nutritional composition and sensory properties of wheat muffins. Substituting wheat flour with *G. zambesina* caterpillar flour resulted in significantly high protein, fat and fibre contents of enriched wheat muffins. There was also an increasing trend in the ash, minerals and tocopherol content. *Invitro* protein digestibility significantly decreased from 10 to 20% substitution levels. There was a significant difference ($p < 0.05$) in the carbohydrate contents of enriched wheat muffins. The sensory scores for colour, texture, aroma and the overall acceptability of wheat muffins decreased with increasing substitution levels. At 10% substitution level, wheat muffins had significantly higher nutritional content than control wheat muffins (0%) and were comparable to muffins enriched with 5% *G. zambesina* caterpillar flour in terms of overall acceptability. Thus, enriching wheat muffins with *G. zambesina* caterpillar flour at 10% substitution level has the potential to contribute to improved protein nutrition since they have a higher protein content than the control wheat muffin and are 88.8% digestible (in vitro).

Keywords Malnutrition · Protein quality · Edible insects · Baking · Food acceptability · Enrichment

Conference presentation:

Opondo, F., Nakhumicha, A., & Anyango, J. (2022, April). The Nutritional Composition and Sensory Properties of Wheat Muffins Enriched with *Gonimbrasia zambesina* Caterpillar Flour. In *Egerton University International Conference*.

Appendix 6: Analysis of Variance on the effect of substituting wheat flour with *G. zambesina* caterpillar(s) flour on proximate composition of wheat muffins

Dependent Variable: ASH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.28568261	0.07142065	0.91	0.4924
Error	10	0.78133510	0.07813351		
Corrected Total	14	1.06701770			

R-Square	Coeff Var	Root MSE	ASH Mean
0.267739	24.10339	0.279524	1.159686

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	0.28568261	0.07142065	0.91	0.4924

Dependent Variable: CP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	379.2245850	94.8061463	43.64	<.0001
Error	10	21.7257036	2.1725704		
Corrected Total	14	400.9502886			

R-Square	Coeff Var	Root MSE	CP Mean
0.945814	5.298201	1.473964	27.82009

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	379.2245850	94.8061463	43.64	<.0001

Dependent Variable: FAT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	26.17733333	6.54433333	8.65	0.0028
Error	10	7.56166667	0.75616667		

Corrected Total	14	33.73900000			

R-Square	Coeff Var	Root MSE	FAT Mean
0.775878	2.834350	0.869578	30.68000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	26.17733333	6.54433333	8.65	0.0028

Dependent Variable: FIBRE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	6.09146600	1.52286650	42.46	<.0001
Error	10	0.35862971	0.03586297		
Corrected Total	14	6.45009570			

R-Square	Coeff Var	Root MSE	FIBRE Mean
0.944399	11.06999	0.189375	1.710708

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	6.09146600	1.52286650	42.46	<.0001

Dependent Variable: CHO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	785.8742278	196.4685570	43.36	<.0001
Error	10	45.3154831	4.5315483		
Corrected Total	14	831.1897109			

R-Square	Coeff Var	Root MSE	CHO Mean
0.945481	5.937089	2.128743	35.85500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	785.8742278	196.4685570	43.36	<.0001

Dependent Variable: DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.55529074	0.13882268	1.78	0.2096
Error	10	0.78034902	0.07803490		
Corrected Total	14	1.33563976			

R-Square	Coeff Var	Root MSE	DM Mean
0.415749	0.287319	0.279347	97.22548

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	0.55529074	0.13882268	1.78	0.2096

Dependent Variable: IVPD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	99.7533566	24.9383392	7.16	0.0055
Error	10	34.8437259	3.4843726		
Corrected Total	14	134.5970825			

R-Square	Coeff Var	Root MSE	IVPD Mean
0.741126	2.036158	1.866647	91.67497

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	99.75335662	24.93833916	7.16	0.0055

Appendix 7: Analysis of variance on the effect of substituting wheat flour with *G. zambesina* caterpillar(s) flour on the minerals concentration of wheat muffins

Dependent Variable: Mg

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	125.0560423	31.2640106	13.26	0.0072
Error	5	11.7898273	2.3579655		
Corrected Total	9	136.8458697			

R-Square	Coeff Var	Root MSE	Mg Mean
0.913846	5.188181	1.535567	29.59740

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	125.0560423	31.2640106	13.26	0.0072

Dependent Variable: Fe

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.29725540	0.07431385	0.89	0.5304
Error	5	0.41598341	0.08319668		
Corrected Total	9	0.71323880			

R-Square	Coeff Var	Root MSE	Fe Mean
0.416768	6.059491	0.288438	4.760109

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	0.29725540	0.07431385	0.89	0.5304

Dependent Variable: Ca

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	57.12702498	14.28175625	2.91	0.1358

Error	5	24.50848379	4.90169676	
Corrected Total	9	81.63550877		

R-Square	Coeff Var	Root MSE	Ca Mean
0.699782	5.915425	2.213978	37.42719

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	57.12702498	14.28175625	2.91	0.1358

Dependent Variable: Cu

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5384.750224	1346.187556	30.49	0.0010
Error	5	220.735393	44.147079		
Corrected Total	9	5605.485617			

R-Square	Coeff Var	Root MSE	Cu Mean
0.960622	4.172595	6.644327	159.2373

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	5384.750224	1346.187556	30.49	0.0010

Dependent Variable: Po

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	611.3710355	152.8427589	8.12	0.0206
Error	5	94.0759882	18.8151976		
Corrected Total	9	705.4470236			

R-Square	Coeff Var	Root MSE	Po Mean
0.866643	2.396275	4.337649	181.0163

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	611.3710355	152.8427589	8.12	0.0206

Dependent Variable: Zn

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.77958260	0.44489565	5.66	0.0424
Error	5	0.39299389	0.07859878		
Corrected Total	9	2.17257649			

R-Square	Coeff Var	Root MSE	Zn Mean
0.819112	9.149521	0.280355	3.064147

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	1.77958260	0.44489565	5.66	0.0424

Dependent Variable: Mo

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	116.5937354	29.1484339	10.17	0.0128
Error	5	14.3247407	2.8649481		
Corrected Total	9	130.9184761			

R-Square	Coeff Var	Root MSE	Mo Mean
0.890583	4.453797	1.692616	38.00388

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	116.5937354	29.1484339	10.17	0.0128

Dependent Variable: Al

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.66432155	0.41608039	1.49	0.3323
Error	5	1.39881762	0.27976352		
Corrected Total	9	3.06313916			

R-Square	Coeff Var	Root MSE	Al Mean
0.543339	4.212805	0.528927	12.55522

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	1.66432155	0.41608039	1.49	0.3323

Dependent Variable: K

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	20695.78835	5173.94709	83.13	<.0001
Error	5	311.19147	62.23829		
Corrected Total	9	21006.97982			

R-Square	Coeff Var	Root MSE	K Mean
0.985186	3.828563	7.889125	206.0597

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	20695.78835	5173.94709	83.13	<.0001

Dependent Variable: Na

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3329.534258	832.383565	6.33	0.0341
Error	5	657.018530	131.403706		
Corrected Total	9	3986.552788			

R-Square	Coeff Var	Root MSE	Na Mean
0.835191	5.079397	11.46315	225.6792

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sample	4	3329.534258	832.383565	6.33	0.0341