The impact of composition and techno-functional properties of edible insect (*Macrotermes subhyalinus*, *Gonimbrasia belina* and *Hermetia illucens*) flours on the nutritional and sensorial profile of biscuits

By

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DECLARATION

I**, Nthabeleng Vanqa**, declare that the content of this thesis represents my unaided work and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

N. Vanqa	05 December 2022
Signature	**Date**

ABSTRACT

In response to a growing demand for protein, the food industry is exploring alternative protein sources for human consumption. The aim of this research was to explore the potential use of insect flour as a protein-rich ingredient for biscuits and monitor the sensorial acceptability of the product for human consumption. *Macrotermes subhyalinus* (Madzhulu), *Gonimbrasia belina* (Mashonzha) and *Hermetia illucens* (black soldier fly), were ground. Analyses were conducted to determine the three edible insect flours' nutritional, physicochemical, technological and antioxidant properties. A relatively high protein was obtained for the edible insect flours ranging from 34.90 to 52.74%. A significant difference ($p < 0.05$) was observed for the fat within the edible insect flours. The three edible insect flours showed no significant difference ($p > 0.05$) in their carbohydrate content and ranged from 22.33 to 28.10%, respectively. There was a significant difference ($p < 0.05$) in the lightness of the edible insect flours, with *G. belina* (57.95) being the lighter in colour. In the comparison of the redness of the three edible insect flours, there was no significant difference ($p > 0.05$); however, *M. subhyalinus* (5.72) was redder compared to *G. belina* (3.92) and *H. illucens* (4.46), respectively. The foam capacity (FC) and foam stability (FS) of all three edible insect flours were not significantly different ($p > 0.05$). Moreover, *H. illucens* (3.63%) displayed low antioxidant activity against the DPPH radical, while *M. subhyalinus* (55.37%) displayed the highest activity. Principal component analysis was applied to the edible insect flours' techno-functional properties and antioxidant indices. Principal component 1 (PC1) accounted for 51.39% of the total variability, while component 2 accounted for 24.71%. Regarding PC1, the FS, Oil binding capacity (OBC) and FC were responsible for the major differences in the edible insect flours. The findings revealed that edible insect flours are a good source of antioxidants and can be used as an alternative protein source and a potential novel food additive due to their techno-functional qualities. Due to their valuable nutritional value, edible insect flours were a good choice for traditional food supplementation. Composite flour and biscuits were made by partially substituting wheat flour with 5%, 10%, 15% and 20% (w/w) edible insect (*M.subhyalinus*, *G. belina* and *H. illucens)* flour. Wheat biscuit (100%) was used as a control. An acceptability study of the biscuit sample was conducted among fifty (n=50) untrained using a five-point facial hedonic scale. The evaluation of the biscuits among the panellist was based on the following parameters: appearance, colour, aroma, taste, texture, and overall liking on a 5-point hedonic scale, with one (1) representing the least score (dislike very much) and five (5) the highest score (like very much). The results revealed that all the biscuits formulated with insect flours might be labelled as “high in protein” products following Regulation no. 1924/2006, ranging from 12.13 to 20.63%. With regards to optical properties, all of the biscuits were placed in the first quadrant of the chromatic diagram, but the biscuits with insect flour showed a significantly lower value of the b* coordinate 27.91 to 29.83 than the control (31.27 ± 1.09), which led to a darkening of the samples regardless the concentration used. As a result, the colour difference for biscuits was not significantly different ($p > 0.05$) . Textural quality revealed that hardness decreased as the concentration of edible insect addition increased. Based on the data obtained, for sensory it was concluded that the enriched of biscuit to up to 10% was preferable as there were no significant differences ($p > 0.05$) in these samples and the control. Moreover, most panellists perceived the biscuits containing insects as too dark at 20%. Furthermore, studies should be carried out to analyse the influence of different structural components and flavourings, such as chocolate, in the formulation of biscuits with insect powders to improve their sensory attributes. The use of *M. subhyalinus*, *G. belina* and *H. illucens* flour might offer a new opportunity for reformulating bakery products, such as biscuits and improving their properties

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DEDICATION

Dedicated to my late grandmothers, Nobonile Mbolekwana and Limakatso Pisto, I am certain these are the days you would have loved to live for. To my family for their endless support, for being proud of me, and for everything they have done for me. To Dr Vusi V. Mshayisa for his excellent supervision, for being my inspiration source, for always believing in me, and for ensuring that I aim for excellence in everything I do. To all my friends, thank you for your endless support. Above all, to God almighty, for it is not by my might that this work is completed but by his grace.

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GLOSSARY

Terms/ Acronyms/ Abbreviations	Definition/ Explanation
$K_2S_2O_5$	Potassium metabisulphite
ABTS	2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
AOAC	Association of Official Analytical Chemists
BSFL	Black soldier fly larvae
DPPH	2, 2-diphenyl-picrylhydrazyl
EC	Emulsion capacity
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organisation
ES	Emulsion stability
FC	Foam capacity
FS	Foam stability
pH	Potential of hydrogen
OBC	Oil binding capacity
RP	Reducing power
PCA	Principal component analysis
WBC	Water holding capacity
WHO	World Health Organisation

CHAPTER 1: MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

Global food demand is rapidly rising (Tilman *et al.*, 2011). The trend is expected to continue for decades, propelled by the world population, which is anticipated to reach nine billion by 2050 (FAO, 2009; Van-Huis *et al.*, 2013; Orsi *et al.*, 2019). In addition to the challenges raised by these projections, there are also large-scale environmental changes and the need to maintain food supplies for an increasingly growing and expectant world population (Garcia-Segovia *et al.*, 2020). There is particular interest in the projected demand for protein, with projections that the world may require double the amount of animal-derived protein by 2050 (Henchion *et al.*, 2017).

Currently, plant-based protein (cereals), animal-based protein (meat) and dairy provide the main source of protein. A large portion of dietary protein intake on a global level comes from plant-based proteins, which are essential to both human and animal health (Shewry & Halford, 2002; van der Spiegel *et al.*, 2013). Nevertheless, plant-based proteins lack certain essential amino acids and are less digestible than animal-based proteins (Gravel & Doyen, 2020). The second most important source of protein comes from meat Livestock products are crucial agricultural commodities for global food security since they constitute 17% of global calorie consumption and 33% of global protein consumption (Rojas-Downing *et al.*, 2017). However, globally, meat production significantly contributes to land use changes from an environmental standpoint (Hurrell & Egli, 2010; Henchion *et al.*, 2017). This is partly because it is widely acknowledged that animal-based protein sources emit more greenhouse gases (GHG) than plant-based sources, which are linked to climate change (Henchion *et al.*, 2017). Due to this fact, there may be a shortage of resources; conventional protein sources may not suffice for the global population, which may be an obstacle to achieving sustainable development goals, including ending hunger and poverty, achieving gender equality, and improving health and education. Therefore, in light of the expected global food shortage and other environmental and economic challenges, finding sustainable food sources, particularly alternative protein sources, is one of the most promising ways to ensure global sustainability (Poma *et al.*, 2017; Govorushko, 2019; Patel *et al.*, 2019).

Thus, insect proteins have received more attention in recent years in the food industry because of their high protein content (average of 40% and up to 70% dry weight), mineral and vitamin content, and interesting polyunsaturated to saturated fatty acid ratio (Rumpold & Schlüter, 2013; Zielińska *et al.*, 2015; Gravel & Doyen, 2020). Moreover, Edible insects have fewer environmental impacts in comparison with conventional livestock because they emit fewer greenhouse gases, use less water, and convert more feed into biomass (Sriprablom *et al.*, 2022).

While the mentioned advantages are present, it is evident that In Western countries, neophobia, revulsion, and non-acceptance have been cited as important barriers to the consumption of insects as food (Caparros Megido *et al.*, 2016). Despite this aversion, recent studies show that consumers are willing to consume edible insects if they are in an unrecognizable form (Melgar-Lalanne *et al.*, 2019), for instance, if they are used in flours or powders or if they are added to various items such as cookies, sausages and biscuits. Consumers are most likely to purchase those foodstuffs (Hartmann & Siegrist, 2016). This necessitates a thorough understanding of their physicochemical and antioxidant properties as well as their techno-functional properties and an elaboration of consumers' perceptions and motivations before promoting the entomophagy (Jantzen da Silva Lucas *et al.*, 2020). Biscuits are popular, ready-to-eat convenient and affordable snacks that are widely consumed by all age groups in many countries and have been used for nutrient supplementation in many cases (Ayensu *et al.*, 2019). Their principal ingredients are wheat flour, shortening, and sugar. These ingredients result in products high in carbohydrates but low in protein and micronutrient contents (Ayensu *et al.*, 2019).

Currently, the edible insects which are considered innovative ingredients are *Tenebrio molitor* and *Musca domestica* are included in cookies to enrich protein and provide health benefits (Sriprablom *et al.*, 2022). No available studies have reported improving the nutritive values of biscuits by incorporating edible insect flours such as *G.belina, H. illucens* and *M. subhyalinus* in their formulations. To the best of our knowledge, edible insects like *G. belina, H. illucens and M. subhylanus* have limited studies for their nutritional enhancement or their effect on the functional and physicochemical properties of biscuits.

In this study, the edible insect flours from (*G. belina*, *H. illucens* and *M. subhyalinus*) were investigated as novel ingredients. The objectives of this research were two-fold. The first objective was to determine the edible insect flours' proximate, antioxidant, techno-functional, and physicochemical properties. The second objective was to produce protein-enriched biscuits with those edible insect flours and to evaluate the functional and physicochemical properties and sensorial profile of the protein-enriched biscuits.

1.2 Statement of the research problem

Although edible insects are considered an unappealing food source for a large part of the population (mainly in developed countries and some urban areas in developing countries), the FAO claims that insects could mitigate this lack of nutritive resources (Patel, 2019). For thousands of years, insects have been a common food in the diet of a large population (mainly in Africa) (Van-Huis, 2013; Hlongwane *et al.*, 2020).

Compared with conventional livestock, "mini livestock," i.e. insects, have several advantages (1) they have higher reproduction rates and food conversion efficiencies than those of poultry, pig and cattle; (2) they require less water; (3) they emit low levels of greenhouse gases; and (4) owing to their lack of similarity to humans, they have a lower risk of producing pathogens that are threatening to human health (Van-Huis *et al.*, 2013; Barroso *et al.*, 2017). However, there exists a lack of information on the use of these insects and their application in snack goods. Therefore, this study aimed to characterize the edible insect flours *(G. belina, H. illucens* and *M. subhyalinus*) for their proximate, techno-functional and antioxidant properties, and further use of the edible insect flours as potential functional ingredients for application in biscuits.

1.3 Research Objectives

1.4 Broad objectives

The aim of this study was to determine the proximate, techno-functional, and antioxidant properties of three edible insect flours (*G. belina*, *H. illucens* and *M subhyalinus*) with the view to apply the edible insect flours in the production of biscuits. The independent variables were (three (3) edible insect flours) and the dependent variables were (proximate, techno-functional and antioxidant properties).

1.5 Specific objectives

The specific objectives of this research were to:

1. Determine the proximate (Ash, moisture, crude fat, crude protein, carbohydrates, and energy) composition of (*G. belina*, *H. illucens* and *M. subhyalinus*) flours.
2. Determine the techno-functional properties (Water and oil binding capacity, emulsion capacity and stability, foam capacity and stability, solubility) of the. (*G. belina*, *H. illucens* and *M. subhyalinus*) flours.
3. Determine the antioxidant 2,2-diphenyl-1-picrylhydrazyl (DPPH-RS), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acids (ABTS-RS), Metal Chelation and Reducing power (RP) properties of the (*G. belina*, *H. illucens* and *M. subhyalinus*) flours.
4. Determine the physicochemical properties (Colour attributes, bulk density, water activity and pH) of (*G. belina*, *H. illucens* and *M. subhyalinus*) flours.
5. Determine the microbial properties of insect flours and biscuits in terms of total plate count, yeast, and mould.

6. Evaluate the proximate nutritional and physicochemical properties of the biscuits enriched with edible insect flours.
7. Establish consumer acceptability using a sensory panellist of a minimum of 50 panellists to establish consumer perception and preference for edible insect-based biscuits.

1.6 Hypotheses

The following hypotheses were tested in the study:

1. Edible insect flours will exhibit high nutritional and techno-functional properties.
2. Edible insect flour will provide alternative protein sources.
3. According to Biró *et al.* (2020), the use of edible insect flours will significantly influence the proximate composition of the biscuits.
4. The use of edible insect flours will influence the functional properties of the biscuits.

1.7 Delimitations of the research

Three (3) different edible insect species flours were used (*G. belina* and *M. subhyalinus*), which were procured from Limpopo province, South Africa (SA) and *H. illucens* flour, which was sourced from Western Cape province, SA). Four (4) insect flour concentrations were used in supplementing whole wheat flour (0%, 5%, 10%, 15% and 20%).

1.8 Significance of the research

Given the current state of food security, with the need for more animal-derived protein. There is a need to find alternative protein sources. This research may generate knowledge on the chemistry of edible insect flours. In addition, it may reveal the potential of edible insect flour as a source of protein. The study may demonstrate the commercial potential for edible insect flours. The successful development of the snack good may promote the use of underutilized edible insect flours. In addition, contribute directly to the sustainable development goals which is to achieve zero hunger, clean water and sanitation, life on land and climate action in the country. The success of this study may also provide food nutrition, security, and sustainability, as edible insects are highly nutritious.

Edible insects are commonly cultivated in the rural areas of South Africa, where they are most popular. Therefore, increasing the production of edible insects would improve rural livelihoods and poverty eradication. In addition, this could lead to an increase in exports of the material, which may boost the economy as it opens new and improved investment opportunities.

1.9 Expected outcomes

Extensive knowledge of the nutritional, techno-functional and antioxidant properties of edible insect flours and application of the flours in the production of biscuits. The findings of the research being published in a peer-reviewed accredited journal, and a poster or oral presentation will be presented at a local/international conference. The attainment of the master's degree was also expected from this study.

1.10 Ethical consideration

Before data collection, approval was obtained from the Cape Peninsula University of Technology Ethics Committee regarding animal (insect) usage in the study. The Committee requires that research participants are protected from any potential negative consequences that may result from participating in the study.

1.11 Thesis overview

This thesis is comprised of five chapters. The chapters are structured in article format, with each one being an individual entity. Figure 1.1 illustrates the structure of the thesis.

Chapter one (1) gives the motivation and design behind the study, which outlines the statement of the research problem, objectives, hypotheses, delimitations of the study, significance of the study and the expected research outcomes.

Chapter two (2) is the literature review which highlights the background information on the current state of food security and consumption of edible insects, their nutritional composition, techno-functional properties and known antioxidant properties and potential use as food ingredients in food processing.

Chapter three (3) is the first research chapter detailing the proximate, techno-functional, physicochemical and antioxidant properties of the Mopane worm flour, Black soldier fly flour and Madzhulu flour. In this chapter, the edible insects were ground into a flour form. Chapter four is the second research chapter focusing on the production of biscuits using edible insect flours at various concentrations (5%, 10%, 15% and 20%). The proximate and physicochemical properties of the flours were also determined. The organoleptic properties of the edible insect were determined to evaluate consumers' overall acceptability of the biscuit.

Chapter five (5) summarises all findings and general conclusions of the research.

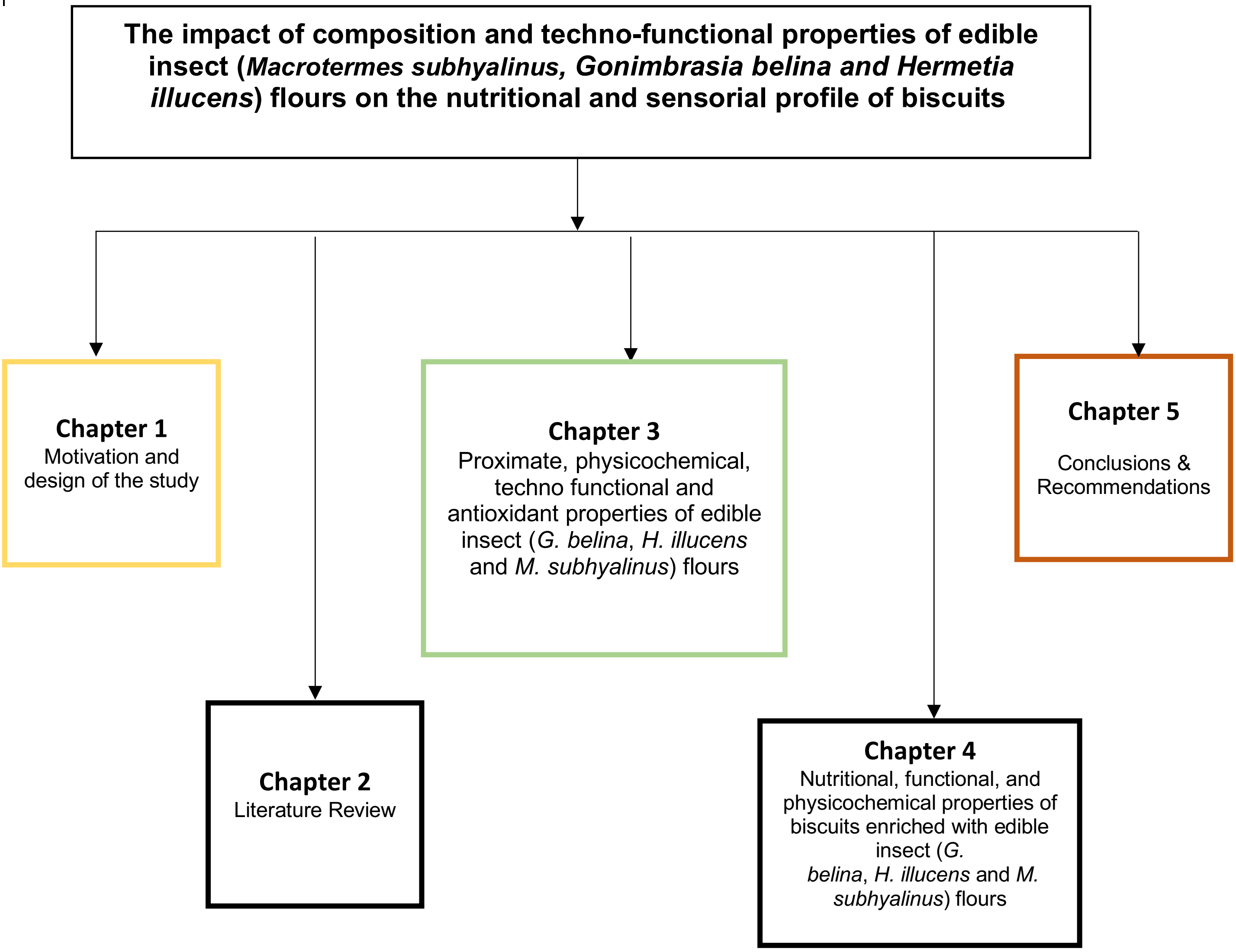

Figure 1.1 Research study overview

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CHAPTER 2: LITERATURE REVIEW

Abstract

In an attempt to find alternatives to conventional protein sources, which are overexploited, expensive and environmentally hazardous, the potential of edible insects as a novel ingredient in high-value-added products has been investigated for over a decade. In this review, we evaluate the state of insects as an alternative protein source, from production to consumption. As noted in this review, insect proteins can be used as functional ingredients in food preparation. Despite this, more comparative studies are needed to assess the effectiveness of various insect proteins compared to conventional proteins, regardless of how they are processed. To integrate these technologies into large-scale industries, more research is needed to optimize processing methods to optimize cost-effectiveness, functionality, tastiness, and sustainability while ensuring consumer safety.

Keywords: Edible insects, nutrition, functional properties, antioxidants, applications, future prospects

2.1 Introduction

In the face of climate change, food insecurity affects more than one (1) billion people worldwide and is confronted by rising food demand, food prices and an increasing global population (El Bilali *et al.*, 2020). The committee of world food security characterized food security as "a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious foods that meet their dietary needs and food preferences for a healthy life" (Barrett, 2010; Gahukar, 2011). Furthermore, food security is commonly conceptualized as resting on four pillars: access, availability, stability and utilization, while the nutritional dimension is considered integral to the concept of food security (van Huis, 2015). Food security is increasingly becoming an issue for humans. Currently, one (1) billion people suffer from food insecurity (El Bilali *et al.*, 2020) due to rising populations and a decrease in food supplies due to increased consumption growth (Gahukar, 2011). Climate change adds further pressure to these already dismaying challenges (Nelson *et al.*, 2010), and this is since food production is so reliant on local temperature and precipitation, any changes necessitate farmers to adapt their operations, which necessitates the use of resources that may otherwise be employed for other purposes (Nelson *et al.*, 2010). Climate change may force farmers across the world to adapt. While some farmers may benefit from the changes, current research shows that many will face significant hurdles in productivity and risk management. This raises critical issues for today's food system for the environment and the overall sustainability goals (Guiné *et al.*, 2021). In line with the UN's sustainable development goal of eradicating hunger, Establishing food security, Developing life below water, and Advance life on land, good health and well-being, which have been identified as critical to fulfilling by the year 2030 (Guiné *et al.*, 2021) Figure 2.1. Intensified efforts are needed towards improving food value by cost e actively processing edible materials in such a way that the nutritional components are enhanced to meet the daily recommended dietary allowances and providing foods that can confer health benefits(Mariutti *et al.*, 2021; Ordoñez-Araque and Egas-Montenegro, 2021).

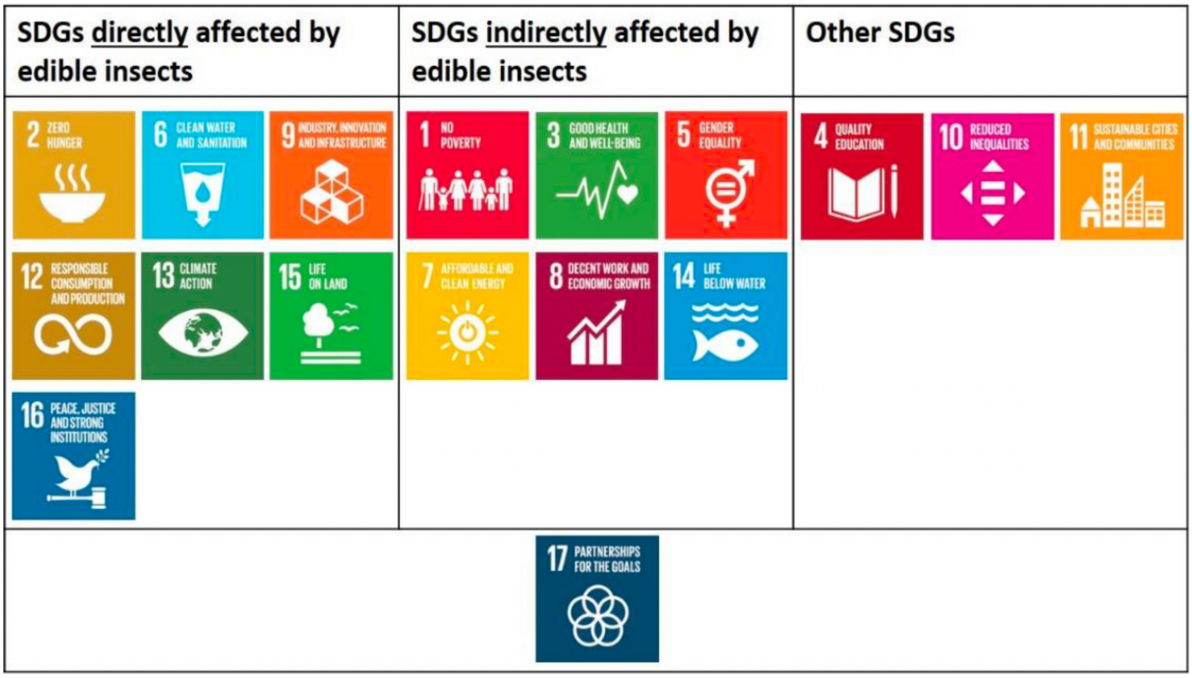

Figure 2.1 An interactive map showing the relationship between edible insects and sustainable development goals (Moruzzo et al., 2021)

Solving these challenges further necessitates re-evaluating food production practices and consumption patterns by introducing sustainable, safe and efficient food sources (Orsi *et al.*, 2019; Imathiu, 2020). The Food and Agricultural Organization of the United Nations (FAO) has advocated for the potential use of edible insects as a viable option for ensuring both global food and feed security (Sogari *et al.*, 2017; Orsi *et al.*, 2019). Insects are multicellular eukaryotes of the phylum Arthropoda, which has the largest number of species on earth (Kewuyemi *et al.*, 2020). On a global scale, the common and most consumed insects include the beetles (Coleoptera) (31%), which sums up to around one-third of the total available insect (Raheem *et al.*, 2019) followed by the Hymenoptera (22%), Orthoptera (17%), Lepidoptera (16%), Heteroptera (7%), Homoptera (5%), Isoptera (3%), Diptera (2%), and others (4%) (Van Huis, 2003). Lepidoptera is eaten as caterpillars and Hymenoptera are harvested and eaten whilst in their larval or pupal stages. Both adults and larvae of Colepterans are eaten, while the Orthoptera, Isoptera and Hemiptera orders are eaten mostly as mature (Raheem *et al.*, 2019). It is crucial to understand that Insects are devoured at different life stages and with various techniques of preparation including raw, fried, boiled, roasted or ground (Dobermann *et al.*, 2017). Henceforth, insects are consumed more in the tropics than in temperate areas of the world (Raheem *et al.*, 2019). This shows that entomophagy has long been practiced by humans with distinct preference for some insect species as a form of delicacy and strong ties with ethnicity. This however does not necessarily seem to be the case as some ethnic groups see the consumption of insects or certain types of insects as a taboo (Meyer-Rochow, 2009).

Despite significant advances in food processing techniques and significant contributions to converting farm produce into edible food products, new sources of proteins must be searched, being insect one possibility. The adaptation of the legal system to the new trends in the fight against hunger and climate change through food alternatives is quite slow, although some progress has been made. Thus, in 2015, a regulation was published to establish the requirements for novel foods, including insects (Regulation [EU] 2015/2283). A few years later, Commission Regulation (EU) 2017/893 of May 24, 2017, was published (Ortolá *et al.*, 2022). Bakery products are one of the most promising since the added ground insects can improve not only the nutritional quality of the dough but also the technological parameters and sensory properties of the final products (Lucas-González *et al.*, 2019; Biró *et al.*, 2020; Zielińska and Pankiewicz, 2020).

The availability of healthy, sustainable food in modern society is becoming increasingly limited. It is therefore imperative that we find new alternatives to traditional food sources in order to maintain the development of humanity in the long run. Insects could be a nice option. It has been demonstrated in numerous studies that edible insects can replace traditional sources of nutrition with proteins, lipids, and other nutrients (Sosa & Fogliano, 2017). As a result of insect consumption as food and feed, human demand for animal protein decreases, reducing the use of natural resources (Poma *et al.*, 2017). Due to their high nutritional content, edible insects can be used as a highly nutritious food source at any stage of growth. It is particularly popular with consumers to consume immature insects like larvae and pupae due to their high protein and fat content (Tang *et al.*, 2019). Compared to other food sources, edible insects are not only nutritious but also delicious, healthy, and environmentally friendly (Jantzen da Silva Lucas *et al.*, 2020).

In spite of their nutritional value and health benefits, edible insects are not widely accepted or used. Recently, the edible insect sector has gained considerable attention from companies and governments, and edible insect rules are being created and adopted worldwide (Lähteenmäki-Uutela *et al.*, 2021). However, most laws focus more on nutrient content and safety than on the functional compounds found in edible insects (Legendre & Baker, 2022).

Through a narrative approach (semi-systematic), this review evaluated the available studies and examined the nutritional content of common edible insects in Africa, their health benefits, and the possibility of insects for the production of edible insect foods of varying kinds, which can, in turn, contribute to food security and enrich the continent's food basket.

2.2 Common edible insects in Africa

In many parts of the African continent, the consumption of insects is part of the cultural practice (Kelemu *et al.*, 2015). Moreover, several studies have highlighted the importance of insects in the diet of communities in the African continent (Agea *et al.*, 2008; Mutungi *et al.*, 2019; Hlongwane *et al.*, 2021). The exact number of edible insects in Africa is still under revision despite several attempts, a single community alone has been reported to consume different kinds of insect species (Kelemu *et al.*, 2015). In total, approximately 470 species of insects are consumed in Africa (Raheem *et al.*, 2019), four of which are commonly found in parts of South Africa and are summarized in Table 2.1. In South Africa, the consumption of insects is popular in the provinces of Mpumalanga, Northwest, Limpopo, and Gauteng (Madibela *et al.*, 2009). Grasshoppers, winged termites, stink-bugs, jewel beetles and white grubs are among the most consumed insects. Mopane worm is one of the insect species that is reportedly consumed in Botswana (Obopile & Seeletso, 2013). In a study conducted by Mutungi (2019) in Uganda, grasshoppers contribute about 16,100 Kcal and 513 g of protein per person per annum.

Table 2.1 Nutrient composition of common edible insects in South Africa.

Insect	**Crude protein (%)**	**Ash (%)**	**Fat (%)**	**Carbohydrate (%)**	**Moisture (%)**	**Reference**
Gonimbrasia belina **(Caterpillar)**	≥ 38 to 56.8	6.9	16.4	13	83.1	(Amadi & Kiin-Kabari, 2016)
Mealworm (Coleoptera)	46.44	2.86	32.7	NA	5.33	(Ravzanaadii *et al.*, 2012)
Black soldier fly (Diptera)	45.82	6.85	25.78	17.41	4.14	(Zozo *et al.*, 2022)

***Macrotermes natalensis* (Isoptera)**	22.1	3.0	21.4	NA	4.1	(Hlongwane *et al.*, 2020)

NA (Not applicable)

According to Mutungi *et al.*(2019), in the Central African Republic, insect consumption was said to account for 15% of the protein intake of the Gbaya people. Furthermore, it has been recorded that 95% of forest people in the Central Africa Republic depend on insects to meet their protein needs. Furthermore, the sale of edible insects is a crucial income source in Zambia, Nigeria, Zimbabwe, Botswana and South Africa, where inter-country trade involving mopane caterpillars across these countries is significant (Mutungi *et al.*, 2019). Despite this, it is essential to highlight that very little knowledge exists about their nutritional, techno-functional and antioxidant properties, as well as their potential use as an alternative protein source as an ingredient in the production of snack goods. Thus, supplying further impetus for this research. In the following sections, three commonly consumed insects (*Gonimbrasia belina, Hermetia illucens* and *Macrotermes subhyalinus*) in South Africa have been selected as shown in Figure 2.2. They will be discussed below as potential protein alternative sources which might mitigate the lack of nutritive sources (Baiano, 2020).

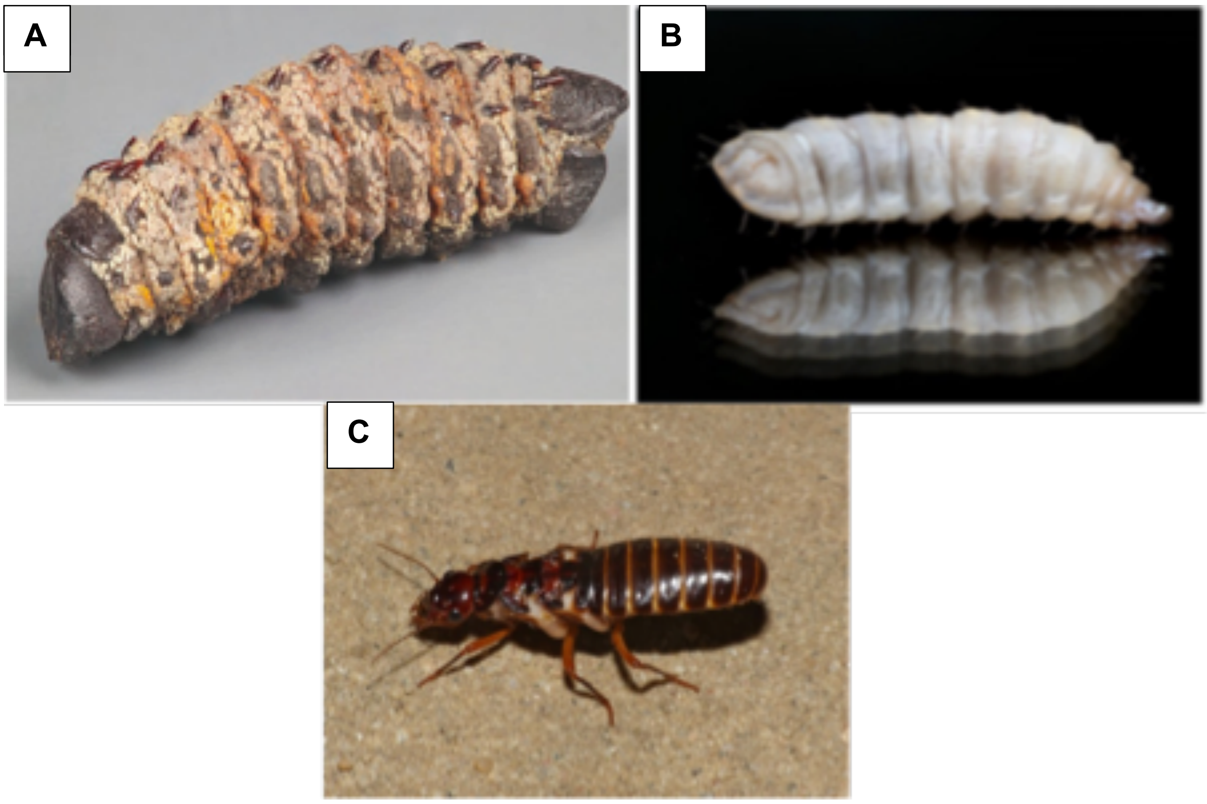

Figure 2.2 Common African edible insects (**A**) Dried mopane catepillar *G. belina* Photo credits and copyright: hands Smid, (**B**) Larva of the black soldier fly (*H. illucens).* Photo credits and copyright: hands Smid, (**C**) Reproductive Termite (*M. subhyalinus*)

Gonimbrasia belina (G. belina) is a species of the emperor moth (Lepidoptera) commonly known as the mopane worm, amacimbi (in IsiZulu) or *Mashonzha* (in Tshivenda), which feeds on fresh mopane tree leaves *Colophospermum* mopane (Baiyegunhi & Oppong, 2016) and the tree is disseminated widely in South Africa (Potgieter, 2015). Figure 2.2 shows the large, vividly coloured, spiny caterpillar that can be found where its host tree grows (Illgner & Nel, 2000). Mopane worms are consumed mostly in rural areas, where the commercialization of mopane worms is beneficial to rural areas. Cash earned by the selling of mopane worms aids poverty alleviation, improves livelihoods, and allows rural people to participate in a burgeoning cash economy (Baiyegunhi & Oppong, 2016), and to a lesser extent in the urban areas in countries such as Namibia, Botswana, Zambia, and Zimbabwe, as well as the South Africa provinces of Limpopo and Mpumalanga (Potgieter *et al.*, 2012).*G. belina*, in essence, necessitates ecological harvesting to assure a good yield the following season (Kwiri *et al.*, 2014). Harvesting of mopane worms takes place primarily from November through January, with a modest second harvest in April or May following good rains. The amount of mopane worms harvested varies depending on the amount of rain received in each area (Nantanga & Amakali, 2020). Although the mopane worm is only available during certain seasons, it is a readily available and inexpensive source of protein. It has a higher protein, fat, carbohydrate, and important mineral content than beef and chicken (Moreki *et al.*, 2012)

Moreover, a study conducted by Madibela *et al.* (2009) reported that degutting enhances the worms' crude protein concentration by 10%. Although *G.belina* has been used as a daily meal in South Africa due to its nutritional content, it has yet to be employed as a protein source in enriched blended meals (Kwiri *et al.*, 2014). Moreover, very little information exists about the techno-functional properties and antioxidant properties of the mopane worm; hence it is essential to expand knowledge on these properties as protein sources will not be enough by 2050, and therefore it is important that these properties are well-known to combat *G. belina* as an alternative ingredient for protein food sources.

Hermetia illucens (*H.illucens*) (Diptera, Stratiomyidae), commonly known as the black soldier fly Figure 2.2 is widely distributed throughout the tropics and warm temperate regions (Sheppard *et al.*, 2002). It is a very common species in Brazil, where it occurs both in natural and man-modified habitats, and various authors have studied its biology (Pujol-Luz *et al.*, 2008). It has, however, been discovered as far north as the Czech Republic (Hora & Roháček, 2013; Gligorescu *et al.*, 2019). In subtropical regions, the species has three generations per year, while in temperate regions, there is only one generation per year (Sheppard *et al.*, 1994;

Benelli *et al.*, 2014). Adult flies are only used for reproduction and have a life span of about 8–20 days. This species has great potential for large-scale production and can effectively transform low-value organic waste into high-value chemicals (Almeida *et al.*, 2020). In research conducted by Gobbi *et al*. (2013) reported that the black soldier fly (BSF) can lower the volume of organic matter by 42% to 56 %. Moreover, it has been reported by various authors that *H. illucens* has an incredible source of nutrients (De Marco *et al.*, 2015; Liland *et al.*, 2017; Spranghers *et al.*, 2017; Wang & Shelomi, 2017). For example, It could be considered a beneficial alternative to conventional protein sources, such as soybean meal, in terms of protein content it has (37 – 63%), amino acid profile, fat content (7 – 39%), and other macro-and micronutrients (Gariglio *et al.*, 2019). However, little knowledge is still known about the techno-functional and antioxidant properties of *H. illucens*. Therefore, more data is needed as this could be helpful in the commercialization of *H. illucens* as a suitable ingredient for food products.

Macrotermes subhyalinus (M. subhyalinus) belongs to the Isoptera order Figure 2.3 (Kelemu *et al.*, 2015) and occurs across the northern savannah region from West to East Africa. In West Africa, its distribution stretches from coconut plantations along the coast to the semiarid northern regions (Zinov'ev & Sole, 2004). The most recorded species is the *Macrotermes falciger,* with a protein content of (43.26%), crude fat (43.0%), and carbohydrate of (328 %) (Siulapwa *et al*., 2012); not enough information is available about the species of M*. subhyalinus* as much as it is commonly consumed in Southern Africa. Not so much information has been reported on *M. subhyalinus* however, Kinyuru *et al.* (2009) reported on the wheat buns enriched with *M.subhylanus* and discovered that The development of wheat termite buns with 5% termite concentration contributed significantly to the recommended daily intake (RDI) of the specific nutrients analyzed. They showed to provide 34.7% of the protein RDI on the consumption of 100g of the product per day. Furthermore, in another study by Kinyuru *et al.* (2013) on the nutrient composition of four species of winged termites consumed in western Kenya. *M.subhylanus* was reported to contain 39.34g/100g protein, 44.82 g/100g fat. Therefore, more research, especially in terms of the nutritional, techno-functional and antioxidant properties of the specie, to know much about its composition before it is applied as a food ingredient.

2.3 Nutritional composition and health benefits of edible insects

The nutritional and other edible food constituents contribute significantly to sustaining a healthy lifestyle. Studies have dealt with the nutritional composition of edible insects in terms of their quality. Protein, fat and essential micronutrients with levels close to those reported in other food sources (Kouřimská & Adámková, 2016; Meyer-Rochow & Hakko, 2018).

Repeating an earlier call by Meyer-Rochow (1975), edible insects are considered the main solution to the challenges of meeting the growing global demand for animal protein that is generally sought after for its high nutritional value. In comparison to meat, insects seem to be healthier and more nutritious. Additionally, insects still have a wide range of nutritional content. According to some statistics, insects are healthier than meat (Payne *et al*., 2016). For this reason, insects are considered to be a meat substitute. For people who are over-nourished, eating insects may exacerbate the over-nutrition, but in cases of malnutrition, eating insects may be a good source of supplementary nutrients (Payne *et al.*, 2016). For example, the larvae of palm weevil, one of Asia's best-known edible insects in Africa. Research has shown that the palm weevil (*Rhychophorus phoenicis*) larvae contain up to 66.3% total protein and 37.1% oil in dry weight. In addition, palm weevil larvae are an excellent source of potassium and phosphorus at 1025 and 658 mg/100 g, respectively (Elemo *et al.*, 2011).

In addition, the cookies with palm weevil larvae are more nutritious and at the same time have high sensory evaluation scores and acceptability(Ayensu *et al.*, 2019). The high nutritional properties of edible insects are gaining attention. Recent studies on the nutritional composition of insects indicate that nutrient-rich foods are receiving a great deal of attention from nutritionists and nutritionists.

2.3.1 Proximate composition of edible insect species

The proximate composition of 14 edible insects is shown in Table 2.2. Different units are used for representation due to the different reference sources and the fact that unit conversions affect the completeness of the data. As shown in Table 2.2 the material composition of insects varies considerably between species. In dry matter, protein and fat are the more abundant substances. The protein content ranged from 9.30% to 52.74% and the fat content ranged from 6.80% to 58.80%. It can also be seen from Table 2.2 that most insects contain less ash since they do not have the calcified skeleton that vertebrates have.

Table 2.2 Proximate composition of insect matter.

Edible insect (Scientific name)	Moisture	Fat	Ash	Protein	Fibre	Reference
Gonimbrasia Belina	5.68	14.04	11.38	46.70	NA	(Payne *et al.*, 2016; Vanqa *et al.*, 2022)
Hermetia Illucens	5.76	27.93	7.50	34.90	NA	(Vanqa *et al.*, 2022)

Macrotermes Subhylanus	6.40	6.36	6.41	52.74	NA	(Vanqa *et al.*, 2022)
Zonocerus variegatus	2.61	NA	1.20	26.80	2.40	(Banjo *et al.*, 2006)
Analeptes trifasciata	2.19	NA	4.21	29.62	1.96	(Banjo *et al.*, 2006)
Anaphe infracta	2.73	NA	1.60	20.00	2.40	(Banjo *et al.*, 2006)
Cirina Forda	4.40	NA	3.20	20.20	2.30	(Banjo *et al.*, 2006; Rumpold & Schlüter, 2013)
Locusta migratoria	4.2	38.10	2.30	48.70	8.80	(Turck *et al.*, 2021)
Alphitobius diapherinus	2.74	25.90	3.50	58.76	6.08	(Turck *et al.*, 2022)
Rhynchophorus ferrugineus	67.9	58.80	2.40	18.00	NA	(Chinarak *et al.*, 2020)
Tenebrio molitor	61.00	16.80	1.20	18.40	5.40	(Finke, 2002)
Galleria mellonela	58.50	24.90	0.60	14.10	12.50	(Finke, 2002)
Bombyx mori	82.70	1.40	2.20	9.30	2.20	(Finke, 2002)
Acheta domesticus	69.20	6.80	1.10	20.50	10.00	(Finke, 2002, 2015)

NA (Not applicable)

2.3.1.1 The amino acid composition of edible insect species

As a vital component of life, protein is highly demanded by humans. Amino acids make up proteins, which are organic compounds. They are essential and non-essential nutrients in food and contribute to its physical and sensory properties (Van-Huis *et al.*, 2013). The nutritional value of edible insects is determined by several factors: Protein quality, which is determined by the kind of amino acids present (essential or non-essential) and if the quality meets human requirements; and protein digestibility, which relates to the ability of the amino acids contained in the diet to be digested (Van-Huis *et al.*, 2013; Van-Huis, 2020). Insects are particularly rich in protein, with amounts that are equivalent to beef and milk Figure 2.3 (Shockley & Dossey, 2013). In a recent study, it was reported that eating 50 grams of *Eulepida Mashona* (beetle) and *Henicus whellani* (cricket) could contribute to 30% of recommended daily protein consumption (Manditsera *et al.*, 2019). On average, insect protein content varies between

35% and 60% dry weight or 10% to 25% fresh (Schlüter *et al.*, 2017). When compared to plant protein sources such as dry soybeans, which have a protein content of 35.8%, insects have a lot of potential as an alternative protein source. As an example, in a study conducted by Finke & Oonincx, (2013) the Housefly (*Musca domestica*) larvae and pupae had a crude protein digestibility of 70 to 80%. While Tang *et al.* (2019) reported *Tenebrio molitor* to contain 48.00 % crude protein and *H.illucens* 49.00 %. Furthermore, Jantzen da Silva Lucas *et al*., (2020) confirmed that insect proteins are easily digestible; in vitro data revealed that digestion time varies depending on the proportion of the proteins studied. Therefore, insects are both feasible and necessary as a source of protein for humans (Zhou *et al.*, 2022).

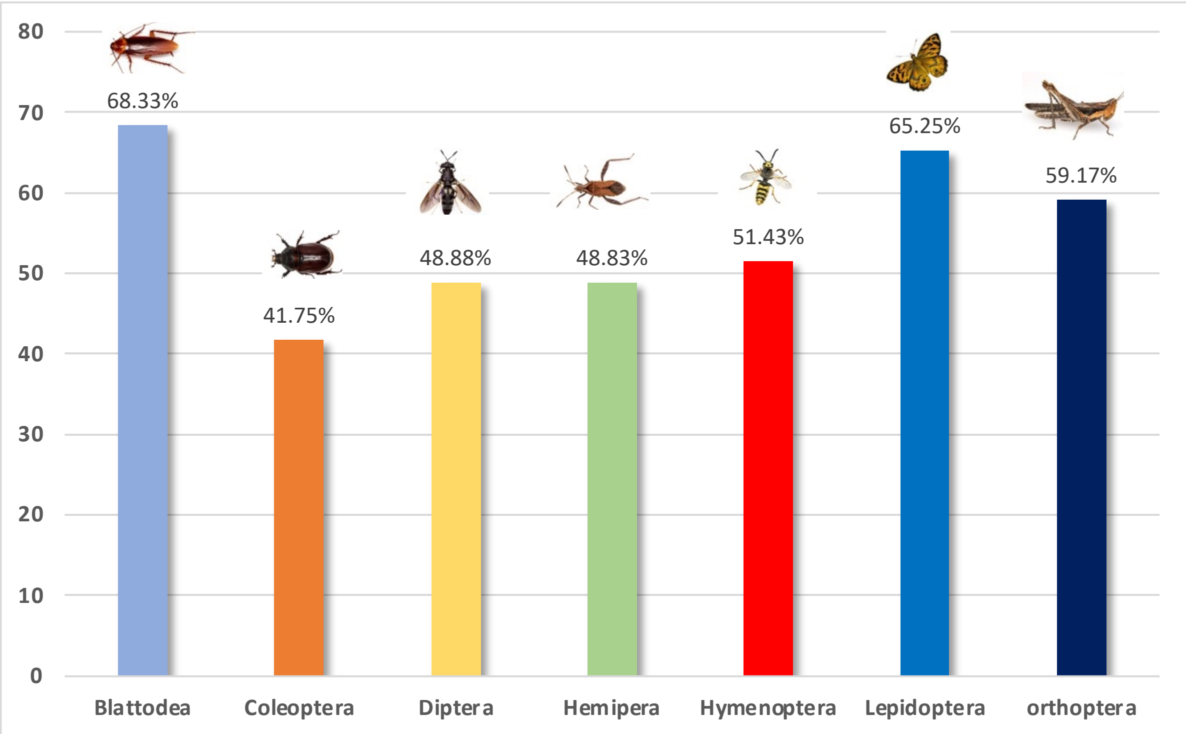

Figure 2.3 Protein content of edible insects from common orders (Zhou *et al.*, 2022)

Protein is made up of over 20 amino acids, among the 20 amino acids contained in protein, eight cannot be synthesized in the human body and must be taken in from outside (Zhou *et al.*, 2022) The amino acid composition of 10 edible insects is listed in Table 2.3. It is clear that insect proteins contain not only a wide range of amino acids but also an abundance of essential amino acids which are nutritionally valuable amino acids, including phenylalanine and tyrosine (Kouřimská & Adámková, 2016). Some insects contain large amounts of lysine, tryptophan, and threonine, which some cereal proteins lack. For example, in Angola, nutritional supplements are obtained through eating *Macrotermes subhyalinus* termites (Sogbesan & Ugwumba, 2008). In Papua New Guinea, tubers, which contain low levels of protein and amino acid lysine and leucine, are commonly eaten. Hence, the nutritional gap could be filled by consuming the larvae of *Rhynchophorus* family beetles, which contain high amounts of lysine (Kouřimská & Adámková, 2016). However, tubers contain a relatively high proportion of tryptophan, and valuable aromatic amino acids, which are not abundant in the larvae (Van-Huis *et al.*, 2013). According to an analysis of almost a hundred edible insect species, the content of essential amino acids represents 46–96% of the total amino acid content.

Table 2.3 Amino Acid composition of selected insect species

Scientific name	Lle	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val	Arg	Ala	Asp	Glu	Reference
Antherea pernyi	79.5	32.4	45.4	14.7	1.5	81	20.6	46.4	40.5	66.3	41.2	62.6	64.1	127.	(Zhou & Han, 2006)
Bombyx mori	57	83	75	46	14	51	54	54	6	56	68	55	109	149	(Zhou & Han, 2006)
Hermetia illucens	7.62	12.1	11.9	3.37	1.02	7.56	12.1	6.82	3.00	12.9	12.3	12.2	16.5	19.7	(Finke, 2013)
Musca domestica	8.1	12.4	12.6	5.84	1.4	7.91	9.26	7.54	2.4	11	12.1	11.7	16.3	21.1	(Finke, 2013)
Tenebrio molitor	8.6	14.3	11.2	2.6	1.5	7.5	14.3	6.4	1.7	12.2	10.3	13.7	16.2	22.8	(Finke, 2002, 2015; Tang *et al.*, 2018)
Acheta domesticus	9.4	20.5	11.0	3.0	1.7	6.5	10.0	7.4	1.3	10.7	12.5	18.0	17.2	21.5	(Finke, 2002)(Finke, 2015)(Ritvanen *et al.*, 2020)
Gryllus bimaculatus	9.2	16.5	11.4	3.5	1.6	7.4	11.7	8.1	2.2	13.6	11.4	19.3	19.7	24.4	(Ritvanen *et al.*, 2020)
Macrotermes falciger	18.9	31.6	37.2	37.2	8.2	1.3	19.7	34.4	19.5	3.5	21.7	27.4	37.3	46.8	(Siulapwa *et al.*, 2012)
Imbrasia belina	22.0	35	36.0	36	9.0	NA	25	36.0	27.0	7.0	NA	NA	NA	NA	(Rapatsa and Moyo, 2017)
Rhynchophorus ferrugineus	8	12	11	2	1	7	21	8	1	10	4	11	25	25	(Chinarak *et al.*, 2020)

NA (Not applicable)

2.3.1.2 Insect fat

In food, fat is the most energy-dense macronutrient. It is made up of triglycerides, which all contain a glycerol molecule as well as three fatty acids. Saturated, unsaturated, and essential fatty acids and some specific fatty acids such as linoleic acid and α- linolenic acid (Van-Huis *et al.*, 2013; Paul *et al.*, 2017). Thus, insect fats provide nutrition and health benefits to humans (Zhou *et al.*, 2022). Fat is the second most important fraction of edible insect nutrient composition (de Castro *et al.*, 2018; Kim *et al.*, 2019). Insect fat contains up to 43% of their dry weight which may also be affected the type of specie. habitat (Raksakantong *et al.*, 2010; Barroso *et al*., 2017) As an instance, Orthoptera contains 13.41% while Coleoptera is averaging at 33.40%(Rumpold & Schlüter, 2013). Live *termites* have 350 kcal/100 g and are composed of 28% of fat, being the second most consumed insect in the world, after locust. Moreover, Analyses of insect fatty acids demonstrate that insects contain both linoleic (omega-6) and α -linolenic (omega-3) acids (Bessa *et al*., 2020), Both of which are vital to brain function (Tzompa-Sosa *et al*., 2014; Janssen *et al*., 2017).This is especially significant in nations where fish, an excellent source of omega-3 fatty acids, is unavailable (Simopoulos, 2002). A study conducted by Wang *et al*. (2004) analyzing the common adult field cricket (*Gryllus testaceus* Walker) established that in most areas of China, the crickets contained unsaturated acid in high quantities with 77.51% of their composition containing valuable oleic (29.58%), linoleic (37.82%), and α-linolenic (10.12%) acid. In a Czech Republic study by Adamkova *et al.*(2016) assessing fatty acid profiles of edible Coleoptera, mealworms also contained the highest content of essential α-linolenic acid, with the second most abundant being essential linoleic acid (30.5%) out of three edible beetle species (giant mealworm larvae *Zophobas morio*, mealworm larvae *Tenebrio molitor(T. molitor)*, and lesser mealworm larvae *Alphitobius diaperinus*).

Fatty acid composition directly influences the nutritional quality of fats in food. Therefore, the composition of saturated fat (SFA), monounsaturated fat (MUFA), and polyunsaturated fat (PUFA) in insect fat determines its nutritional quality. There is a strong association between human health and unsaturated fatty acids(Tang *et al.*, 2018; Köhler *et al.*, 2019).

2.3.1.3 Carbohydrates of edible insect species

Food insects contain carbohydrates as another important nutrient component. Carbohydrates are the most abundant biomolecule in the organic compound class found in all living species (Khowala *et al.*, 2008). In insects, carbohydrates occur mainly as chitin and glycogen (Kim *et al.*, 2019) They present the common source of energy consumed by humans (Jørgensen, 2009).as an example, crickets (*Gryllus bimaculatus* De Geer) have a polysaccharide and carbohydrate content of 0.3% of their fresh weight (Finke & Oonincx, 2013). In a study by Rumpold & Schlüter (2013) on 236 insect species, Insects are rich in carbohydrates including the cricket *Brachytrupes* sp. (Orthoptera) with 2.33–85.30% and *Myrmecosistus melliger*, with 77.00–77.73%. Furthermore, Mlcek *et al* (2014). Showed that the carbohydrate contents of edible insects generally ranged from 6.71 % in sting bugs to 15.98 % in cicada. As mentioned previously chitin is the key component of carbohydrates in insects (Kim *et al.*, 2019) The carbohydrate polymer chitin can be found in the exoskeleton of invertebrates, protozoa, fungi, and algae, and is thought to be the most prevalent in nature. An insect's cuticle consists of chitin in a matrix with proteins, lipids, and other compounds (Finke & Oonincx, 2013) .which may have 'anti-nutrient' properties due to potential negative effects on protein digestibility It has a caloric value that varies depending on the species. Insects contain 1–7% chitin (Finke, 2007). Even though chitin is widely thought to be indigestible by humans, chitinolytic enzymes produced by bacteria from human gastrointestinal tracts have recently been discovered, showing that chitin and chitosan can be digested (Belluco *et al.*, 2013; Dobermann *et al.*, 2017).

2.3.1.4 Vitamins and minerals of edible insect species

While micronutrient levels differ greatly between insect species, some micronutrients are consistently higher in (Dobermann *et al.*, 2017). Vitamins and minerals are commonly found in insects, but these compounds are synthesized in insects and ingested by them to enrich their bodies. Vitamins and minerals are vital components in the metabolic processes of human and animals, and their paucity may have adverse health effects (Kinyuru *et al.*, 2015). Table 2.4 shows vitamins which are present in edible insects.

Insects contain several minerals, including iron, zinc, potassium, sodium, calcium, phosphorus, magnesium, manganese, and copper, as well as lipophilic vitamins including riboflavin, and pantothenic acid, biotin, and, in some cases, folic acid(de Castro *et al.*, 2018). Based on the study conducted by (Rumpold & Schlüter, 2013), 23 of the 77 insects studied for magnesium content were found to be properly supplied. True bugs (Hemiptera) and some members of the Orthoptera order (grasshoppers, crickets, locusts) are particularly high in magnesium. The large caterpillar of the moth *G.belina* has high iron content (31–77 mg per 100 g), and so does the grasshopper *Locusta migratoria* (8–20mgper 100 g of dry matter) (Kouřimská & Adámková, 2016). While in a study by Kim *et al* (2017) on five edible insects, the mineral content amounts varied from 349.2 to 2282 mg/kg dry weight, with *Protaetia brevibarsis alleinensis* exhibiting the highest levels and *T. molitor* exhibiting the lowest.

Table 2.4 Vitamin composition of edible insects.

Scientific name	A (IU/kg)	B1(mg/kg)	B2(mg/kg	B6(mg/kg	B12(mg/kg	C(mg/kg	E(mg/kg	reference
Hermetia Illucens	<1000	7.7	16.2	6.01	55.8	<10.0	6.2	(Finke, 2013)
Musca domestica	<1000	11.3	77.2	1.72	6.0	<10.0	29.7	(Finke, 2013)
Tenebrio molitor	<1000	1.2	16.1	5.8	NA	24	<0.5	(Finke, 2002, 2015; Nowak *et al.*, 2016)
Bombyx mori	1580	3.3	9.4	1.6	NA	<10.0	8.9	(Finke, 2002)
Acheta domesticus	<1000	0.4	34.1	2.3	NA	30.0	19.7	(Finke, 2002; Nowak *et al.*, 2016)
Macrotermes messubhylanus	<1000	NA	41.8	2.7	NA	7.3	NA	(Kinyuru *et al.*, 2010)
Rhynchophorus ferrugi-neus	NA	NA	NA	NA	NA	NA	18.8	(Chinarak *et al.*, 2020)

NA (Not applicable)

Currently, information on the relative bioavailability of different insect species is limited, especially on the bioavailability of minerals. (de Castro *et al.*, 2018). Latunde-Dada *et al*.(2016) reported the first study on the bio-accessibility of minerals found in commonly eaten insects. According to the authors, insects can supply substantial minerals and iron to human diets. Grasshoppers, crickets, mealworms, and buffalo worms can be great sources of Fe, Ca, Cu, Mg, Mn, and Zn. When consumed in the right quantities and forms, grasshoppers, crickets, mealworms, and buffalo worms can supply an excellent source of Fe, Ca, Cu, Mg, Mn, and Zn to humans. As insects contain a wide range of vitamins and minerals, most of the insects available are good sources of the vitamins and minerals that the human body needs (Kinyuru *et al.*, 2015). Thus, we can consider dietary insects for the purpose of micronutrient supplementation.

2.4 Techno-functional properties

2.4.1 Solubility

Techno-functional qualities such as (foaming, gelation & emulsions) are crucial in food systems, and solubility is one of the most important (Gravel & Doyen, 2020). Protein solubility is a thermodynamic property defined as the concentration of protein in a saturated solution in equilibrium with a solid phase, either crystalline or amorphous, under certain conditions. There are several factors which affect protein solubility; these factors include extrinsic and intrinsic factors. Extrinsic factors that influence protein solubility include pH, ionic strength, temperature, and the presence of various solvent additives. The intrinsic factors that influence protein solubility are defined primarily by the amino acids on the protein surface, but a detailed understanding of how one can alter the intrinsic properties of a protein to increase its solubility is lacking (Kramer *et al.*, 2012). Protein structure also affects solubility; a less impermeable protein configuration favours interaction with water and, so, solubilization. Reducing protein size can also improve protein solubility. Hydrolysis is believed to increase the repulsive forces and hydration between proteins as smaller peptide fragments with more ionizable groups are released, hence the solubility increases (Gould & Wolf, 2018). The study of protein solubility in insect's flours and protein derivatives are still in its infancy, especially in Mashonzha and Madzhulu; however, there are few studies which are available on edible insect protein hydrolysates. Bußler *et al.* (2016) successfully increased the solubility of *Hermetia illucens* proteins when increasing the temperature to 50–60 °C. In a study conducted by Purschke *et al.* (2018) on *Locusta migratoria*, hydrolysis improved the solubility a similar result was observed by Hall *et al.* (2017) on *Gryllodes sigillatus*.

Solubility is also dependent on pH. Near the isoelectric point (pI), a protein's net charge is neutral, which favours protein-protein interactions and is detrimental to water-protein interactions, resulting in protein precipitation. Extreme pH values yield charged protein surfaces that repel each other and increase the solubility (Sathe *et al.*, 2018). The pI of food proteins is usually between 3 and 6. For example, milk caseins have a pI of 4.6 (Gravel & Doyen, 2020). Insect proteins show the same trend. The minimum solubility of *Tenebrio molitor*, *Hermetia illucens*, *Locusta migratoria*, *Patanga succinta*, *Chondracris rosea,* and *Schistocerca gregaria* protein concentrates occurred at pH 4 and maximum solubility at alkaline pH (Gravel & Doyen, 2020).

2.4.2 Water binding and oil binding capacity

Water-holding capacity (WHC), and water-binding capacity (WBC) are properties associated with the ability of a protein matrix to retain as much water as possible per gram of sample material against gravity, whether it be bound or physically entrapped water (Gravel & Doyen, 2020). According to Ozyurt & Ötles, (2016) reported that the amount of water bound or retained by a known weight of fibre under defined conditions might be termed WHC. WHC depends on protein-water interactions that affect the protein function (Cha *et al.*, 2020). This functional property is highly integrated with gelation properties. Likewise, WBC is increased by heat-induced denaturation. This functional property is also associated with improved texture and moisture, which is of great importance in the food formulation (Aryee *et al.*, 2018). As reported by Foh *et al* (2012) that interactions between proteins and water or oil are important in food systems, as they affect the flavour and texture of food. WHC is mainly measured in 2 ways: (a) dispersing the sample in excess water in certain conditions (time and temperature), followed by the removal of the unabsorbed water using pressure, filtration, or centrifugation and then measurement of the water-bound by gravimetry or (b) measuring the water uptake in conditions of limited water. The WHC can thus be estimated as the amount of water released from the sample (water retention measurement) or as the amount of water absorbed or bound by the sample (water absorption measurement) (Villemejane *et al.*, 2013).

Oil holding capacity (OHC) is the amount of oil retained by the fibres after mixing, incubation with oil and centrifugation. The oil absorption of cereal derivatives, e.g., wheat bran, is related mainly to the surface properties of the bran particles, but may also be related to the overall charge density and to the hydrophilic nature of the constituents, e.g., alginate and fucan, of the algae (Ozyurt & Ötles, 2016).

2.4.3 Emulsion stability and emulsion capacity

The purpose of emulsion science in the food industry is to develop food quality and production techniques by benefiting from emulsion principles (Serdaroğlu *et al.*, 2015). In general, "emulsion" is described as a structure created through the dispersion of one of two (2) iimmiscible liquids within the other one in the form of little droplets (generally oil and water) (McClements *et al.*, 2007; Brodkorb *et al.*, 2019). Emulsions may be divided conventionally into two water-in-oil emulsions (W/O) and oil-in-water (O/W) emulsions in accordance with their continuous and dispersed phases (Serdaroğlu *et al.*, 2015). Many food emulsions consist of three main zones with different physicochemical characteristics: "disperse phase" generating droplets, "continuous phase" surrounding droplets and interface (McClements *et al.*, 2007).

2.4.4 Foam stability

Similar to the emulsifying properties of proteins, foaming properties also depend on interfacial properties. By electrostatic and steric repulsive forces, proteins adsorb to the air–water interface and stabilize the foam bubbles. Foaming properties are often expressed in terms of foaming capacity and foam stability (Kutzli *et al.*, 2021). Proteins with a high water solubility are essential for serving as a good foam stabilizer(Ivanova *et al.*, 2018) Increased solubility upon glycation is attributed to increased hydrophilicity and enhanced hydrogen-bonding capacity of the protein due to the covalent attachment of hydrophilic carbohydrates and the modification of the protein net charge, contributing to greater repulsion between protein molecules (Zhang *et al.*, 2019; Kutzli *et al.*, 2021). Further factors that influence the foaming properties of a protein are its molecular structure and flexibility(Foegeding *et al.*, 2006).

2.5 Antioxidant properties

Antioxidants are thought to play a critical function in the body's defense system against reactive oxygen species (ROS) (Boxin *et al.*, 2002). In another term antioxidant is "any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate" (Yadav *et al.*, 2016). Antioxidants are classified in two categories: (1) primary or chain-breaking antioxidants, especially acting by scavenging reactive oxygen species/reactive nitrogen species (ROS/RNS) Figure 2.4 and (2) secondary or preventive antioxidants, that suppress the oxidation promoters such as metal ions, singlet oxygen, pro-oxidative enzymes and other antioxidants, commonly operating by transition metal ion chelation (Shahidi & Zhong, 2015). Moreover, it’s important to note that antioxidant activity and antioxidant capacity are different terms. The antioxidant activity is proportional to the rate constant of an antioxidant against a given free radical, whereas the antioxidant activity is proportional to the antioxidant activity (Pisoschi *et al.*, 2016).

The number of moles of a specific free radical scavenged by an enzyme is referred to as capacity-specific antioxidants found in the combination under investigation (Shahidi & Zhong, 2015). Global current challenges include a deficiency in nutritious dietary protein and the accumulation of organic waste. The most important task is the search for new sources of protein for human nutrition and animal feed, and the disposal of food waste is the most important environmental problem. It is believed that by 2050 the protein of industrially cultivated insects can reach 15% of the total amount of protein produced in the world (Ushakova *et al.*, 2019). However, most of the attention devoted to insects as a food source focuses on protein content. Very limited data published about the antioxidant properties of edible insects are available, and most studies are based on insects caught in the wild therefore more knowledge is acquired (Zielińska *et al.*, 2017). A variety of active ingredients in edible insects have been reported to possess antioxidant activity (Zhou *et al.*, 2022).

In the study, grasshoppers, silkworms, and crickets were found to have the highest antioxidant activity, five times higher than fresh orange juice, while fat-soluble extracts from silkworms, evening cicadas, and African caterpillars were found to have the highest antioxidant activity, twice that of olive oil.(Di Mattia *et al.*, 2019). Orange juice and olive oil are known to be functional foods that regulate antioxidant activity in the body due to their high antioxidant activity (Zamora-Ros *et al.*, 2013; Foroudi *et al.*, 2014). So, it is theoretically feasible to use insects to develop antioxidant-functional foods(Zhou *et al.*, 2022).

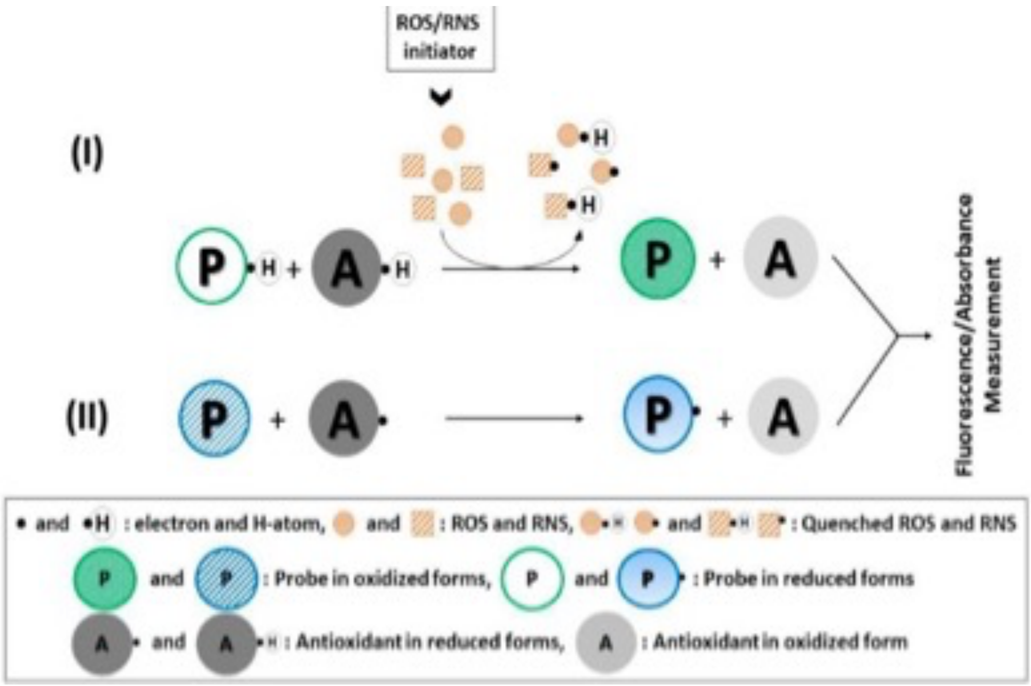

Figure 2.4 I) Direct (competitive) antioxidant assay, involving a fluorogenic or chromogenic probe and biologically relevant ROS/RNS, and (II) indirect (non-competitive) antioxidant assay, in which physiological redox reactions (i.e., oxidant−antioxidant interaction) (Apak, 2019)

2.5.1 2,2-diphenyl-1-picrylhydrazyl (DPPH)

DPPH radical scavenging assay is among the most frequently used methods and offers the first approach for evaluating the antioxidant activity (Prior *et al.*, 2005). DPPH is a stable chromogen radical with a deep purple colour. It is commercially available and does not need to be generated prior to the assay. The DPPH scavenging assay is based on the electron donation of antioxidants to neutralize DPPH radicals. The reaction is accompanied by the colour change of the DPPH measured at 517 nm, and the discolouration acts as an indicator of antioxidant efficacy. The antioxidant activity by the DPPH scavenging method is often reported as EC_{50} which is defined as the effective concentration of the antioxidant necessary to decrease the initial DPPH concentration by 50% (Shahidi & Zhong, 2015). The disadvantage of the DPPH assay is that the evaluation of antioxidant capacity by the change in DPPH absorbance must be carefully evaluated since the absorbance of DPPH after reaction with an analyzed sample may be diminished by some other factors (pH, O2, light, type of solvent, etc.) (Ozcelik *et al.*, 2003; Xie & Schaich, 2014). Furthermore, since the ionization of phenols – and consequently the reaction rates are highly influenced by solvent composition and pH, the DPPH assay is not adequate to ranking antioxidant compounds and natural extracts (Ozcelik *et al.*, 2003).

The aqueous extract of housefly larvae prepared using the decoction method was reported to have significant antioxidant activity, with a DPPH radical scavenging activity of 75.4% at a level of 5 mg/mL(Li *et al.*, 2017). Another study prepared silkworm larvae protein hydrolysate using gastrointestinal enzymes, which also exhibited relatively high DPPH radical scavenging activity (IC50 = 57.91 _g/mL)(Wu *et al.*, 2011).

2.5.2 2,2-azinobis 3 ethylbensothioazoline-6 sulfonate

In the improved version, ABTS•-, the oxidant, was generated by persulfate oxidation of 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS2-). Specifically, 7 mmol of ABTS ammonium was dissolved in water and treated with 2.45 mmol of potassium persulfate, and the mixture was then allowed to stand at room temperature for 12-16 h to give a dark blue solution. This solution was diluted (Huang *et al.*, 2005). The strong points of this assay are that it can be applied for both water-soluble and lipid-soluble antioxidants. This assay is the basis for more elaborate assays, which add more time points, more samples, and more complex calculations. It has been adapted for clinical studies, based on more complex conditions (Pinchuk *et al.*, 2012).

2.5.3 Metal chelation

Transition metal ions are known to stimulate lipid oxidation via Fenton reaction and by decomposing lipid hydroperoxides into more reactive peroxyl and alkoxyl radicals. Generally, metal chelation capacity is determined by measuring the chelating effect of antioxidants on the ferrous ion (Antolovich *et al.*, 2002). Ferrous sulphate and ferrozine are the most used sources of ferrous ions. A loss of absorbance at 485 nm (for ferrous sulphate) or 562 nm (for ferrozine) after the addition of antioxidants represents the formation of a metal antioxidant complex, and the metal chelation capacity of the added antioxidant can be quantified spectrophotometrically (Schlesier *et al.*, 2002)

2.5.4 Ferric reducing power

The FRAP assay is a typical ET-based method that measures the reduction of ferric ion (Fe^{3+}) ligand complex to the in- tensely blue-coloured ferrous (Fe^{2+}) complex by antioxidants in acidic media. Antioxidant activity is determined as the increase of absorbance at 593 nm figure 2.5, and results are expressed as micromolar Fe^{2+} equivalents or relative to an antioxidant standard (Antolovich *et al.*, 2002). Unlike other ET-based methods, FRAP assay is carried out under acidic pH conditions (pH 3.6) to maintain iron solubility and more importantly, drive electron transfer. This will increase the redox potential, causing a shift in the dominant reaction mechanism (Shahidi & Zhong, 2015). As reported by Zielińska et al (2017), The highest value of reducing power was noted for the cricket *A. annulipes* hydrolysate (0.652), and the *Z. morio* (0.522) and *G. portentosa* (0.485) hydrolysates had a high value as well. This suggests that the hydrolysates obtained from some edible insect protein can be used as compounds able to donate electrons and thus showing antioxidant activity (Zielińska *et al.*, 2017).

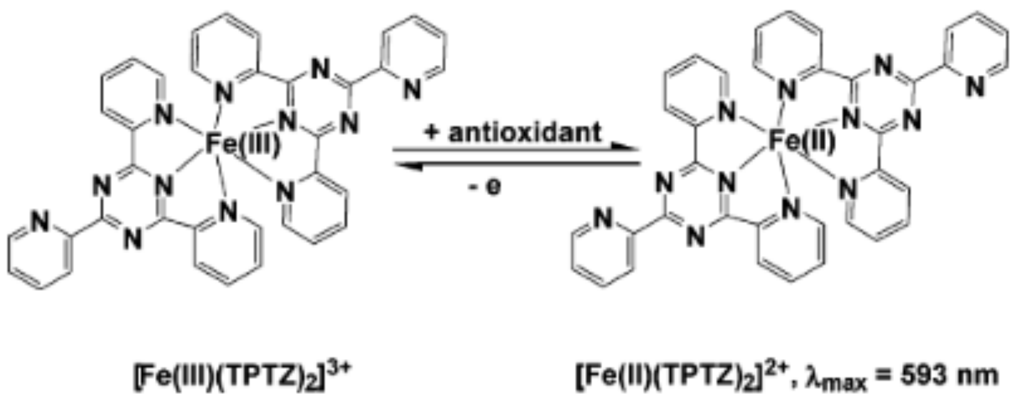

Figure 2.5 FRAP antioxidant mechanism(Amarowicz & Pegg, 2019).

2.6 Microbiological flora of edible insects

The path each food takes from primary production to the final product to be consumed hosts a specific micro- and mycobiome, and edible insects are no exception (Mariod, 2020). Nevertheless, edible insects should be carefully monitored for microbiological hazards as with any other food (van der Spiegel *et al.*, 2013; Megido *et al.*, 2017). Consequently, insects provide a favourable microbial growing environment by providing nutrients and moisture (Klunder *et al.*, 2012; Van-Huis *et al.*, 2013). For instance, as reported by Megido *et al.* (2017) there are cases of botulism and other foodborne illnesses linked to the consumption of insects stored in poor conditions in Africa

A limited number of studies have examined the microbiological hazards presented by the insect consumption (Abu-Ghannam & Crowley, 2006). Gonimbrasia belina (Westwood 1849) (Lepidoptera: Saturniidae), otherwise known as the mopane worm, was the first one performed in Africa. Prior to consumption, the caterpillar undergoes a 24-hour fasting period, followed by 15 to 30 minutes of blanching and one to three days of sun drying (Mpuchane *et al.*, 2000; Simpanya *et al.*, 2000). Within the processed caterpillars, several species from seven genera of bacteria and five genera of fungi have been identified(Simpanya *et al.*, 2000; Megido *et al.*, 2017).

Two types of microbiota should be considered when assessing the microbial community of insects: those that are intrinsically associated with insects as part of their lifestyle and those that are introduced during farming and processing and carried forward (van der Fels-Klerx *et al.*, 2018). In both cases, the farming environment plays a significant role, because it can both modulate intrinsic microorganisms and also be the source for new ones(van der Fels-Klerx *et al.*, 2018). Klunder, Wolkers-Rooijackers, Korpela, and Nout (2012) were the first, to our knowledge, to report on the edible insects from a food safety perspective, with an exploratory study. Mealworm (*T. molitor*) (farmed)and cricket, *Acheta domesticus* (farmed) and *Brachytrupes s*pp. (harvested from the wild) from Lao PDR were analysed in different conditions: fresh, boiled, roasted, and fresh and stored. High counts of total viable bacteria, Enterobacteriaceae and spore-forming bacteria were isolated from the fresh insects, with an increase after crushing, attributed to the release of intestinal microbiota. In addition, Garofalo *et al*. (2017) studied the microbial composition of marketed processed edible insects, including powdered small crickets (*A. domesticus*), whole-dried small crickets, whole-dried locusts (*L. migratoria*), and whole-dried mealworm (*T. molitor*) larvae. The samples were purchased from a company and shipped in plastic bags by international transport. The authors observed a strong variation in the microbial composition of the different species. Counts of total mesophilic aerobes, Enterobacteriaceae, lactic acid bacteria, Clostridium perfringens spores, yeasts, and moulds were relatively low.

2.7 Incorporation of edible insects in common food

To date, baked foods represent the most important nutrient vehicle due to their wide acceptance. Thus, recent studies are utilizing such products as a means of incorporating edible insects to prepare acceptable foods. A handful of trials have utilized insects suitable for the production of baked foods (e.g., buns, biscuits, cookies) in Africa (Ayieko *et al.*, 2016; Ogunlakin *et al.*, 2018) Fermented edible insects have also been well processed into intermediate food products such as flour, paste and sauces through back slopping and controlled fermentation (Kewuyemi *et al.*, 2020). Table 2.5 shows the most recent studies of edible insect flours and the effect it has on the quality and sensorial profile of baked goods.

In a study on increasing the nutritional quality of bread through the incorporation of innovative ingredients, the substitution of cricket powder prior to fermentation and subsequent baking to obtain bread resulted in a higher nutritional profile in terms of fatty acid composition, protein content and occurrence of essential amino acids (Osimani *et al.*, 2018). The authors reported higher titratable acidity after fermentation in doughs and bread containing cricket powder, attributing this to the higher ash content of the cricket powder which may have had an influence on dough burring capacity. Compared with other substituted ratios, bread enriched with 10% cricket powder reportedly showed discrete acceptability by untrained panellists, with the study suggesting that foods, where insects are not directly visible, could be more successfully marketed (Osimani *et al.*, 2018). Even though probands blindfolded and unable to smell had trouble to identify insects by taste alone (Benno Meyer-Rochow, 2018). Similar observations were also demonstrated by González et al. (2019). Another cereal-based staple product, a maize tortilla, has been enriched by replacing nixtamalized maize flour with 6.5% of yellow mealworm larva flour (*T. molitor*). Maize tortillas' protein, fat, amount of essential amino acids, and polyunsaturated acids increased. Tortillas were darker than the control; consequently, a larva was dried to avoid darker colour (Acosta-Estrada *et al.*, 2021). More studies have been reported on baked products by Kinyuru (2009).

Table 2.5 Effect of edible insect on a number of technological and sensorial properties of some bakery products.

Edible insect	Bakery product	Using ratio	Results	Reference
Mullberry silkworm pupae (*Bombyx mori*), Locust	Biscuit	15%	Weight ↓,width↑,thickness↑, spread ratio↓ , sensory properties↔ (expect aroma)	(Akande *et al.*, 2020)
Termite (*Macrotermes nigeriensis*)	Biscuit	5,10,15,20 and 25%	Weight↑,diameter↑, spread ratio↑, breaking strength↓,sensory properties: taste↓, a aroma↔,texture↔,overall acceptability↓	(Ogunlakin *et al.*, 2018)
Grasshopper (*Melanoplus cinereus*)	Baby biscuit	5,7 and 10%	Sensory properties, taste↑, aroma↑ , colour↑ , texture↑	(Dewi *et al.*, 2020)
Termite (*Macrotermes belliscosus*)	Cookie	5,10 and 15%	Weight↓ ,diameter↓, thickness↑, spread factor↓, Colour: L*↓, a*↑, b*↑, texture: hardness↓, fracturability↑	(Awobusuyi et al., 2020)
Cricket, termite (Soldier, termite, winged termite)	Cracker	8%	Sensory properties: taste↓ , flavour↓r, aroma↓, colour ↓, appearance↓, overall acceptability ↓	(Akullo *et al.*, 2018)

↓ indicates an increment that is statistically different, ↑ indicates a decrease that is statistically different, ↔ indicates an increment or decrease that is not statistically different.

2.8 Consumer acceptance of edible insects

There is a growing market for edible insects with a recent forecast suggesting that the edible The insect market will be worth approximately 8 billion US dollars by 2030. While this is interesting to note, there are still issues of neophobia (unwillingness to consume novel products) and food safety challenges with insects (Imathiu, 2020). In addition, are issues of unfamiliarity, sociocultural influence, prejudice, flavour, disgust and emotional concerns with consumers (Tan *et al.*, 2015; Gmuer *et al.*, 2016; Kewuyemi *et al.*, 2020). Moreover, whether edible or not, insects are generally perceived as annoying pests or dangerous vectors of disease. Such a mindset influences food choices with sight possibly playing a role in this. This is substantiated by findings of blindfolded sensory taste experiments in which participants had difficulty identifying edible insect products (Meyer-Rochow & Hakko, 2018). Although consumers cannot necessarily be blindfolded when purchasing food products, processing methods such as fermentation singly and in combination with other techniques can help promote the consumption of edible insects as subsequent products will be in an unrecognizable form. Innovative processing technologies could also be applied, as demonstrated in a study where three-dimensional (3D) printed snacks containing edible insects were produced (Severini *et al.*, 2018). Such products could be more appealing, leading to better acceptability.

2.9 Future prospects and conclusion

The growing human population in the continent, which is expected to be over 8 billion in 2025, would increase the demand for food protein sources for human consumption. The processes of converting edible insects into nutritious and healthy foods could, in part, contribute significantly towards achieving a food-secure nation and thus attaining the UN’s millennium development goal of zero hunger in Africa. In the Western world, varying innovative insect-based foods such as extract, paste, powder sauces and insects have been prepared and demonstrated promising prospects in terms of nutritious and healthy diets. However, a major problem associated with insect use as a food source is its sustainability. There is an alarming rate of habitat destruction, making it harder to find edible insects. A traditional agricultural practice in Nigeria and most African countries, crop rotation, has almost been abandoned due to the use of fertilizers, pesticides, and herbicides. This may lead to the disappearance of edible insects since crop rotation disrupts disease cycles and insect pest lifecycles (Amadi & Kiin-Kabari, 2016). In addition, a huge concern is ensuring optimum Microbial stability and further research to establish the suitability of incorporating the edible insect Intermediates into other food mixtures to enable different modes of utilization and to provide options for highly demanding consumers. Consuming edible insect-based foods could be explored as a viable way of addressing food insecurity in Africa as these insects are of high nutritional quality, readily available, cheap/affordable, and their propagation is associated with negligible environmental footprints. While consumer issues may be challenging, this can be surmounted by incorporating edible insects into ready-to-eat snacks/products that would mask their appearance and increase acceptance of this product. Furthermore, to ensure the food security prospect of edible insects, much is still needed to be done in the areas of agricultural productivity. Achieving food security while balancing the risk of depleting edible insect resources and addressing the 'disgust factor is still a challenge, as Gahukar (2020) points out in his review so eloquently.

To better apply edible insects to daily eating in the future, several obstacles must be resolved. First and foremost, as a foundation for their development and use, edible insect use as food or medicine needs to be supported by international and national laws and policies. Second, researchers should focus more on undiscovered food insect species because there are probably many undiscovered edible bug species and those with therapeutic characteristics. In addition, the nutritional value of edible insects should be standardized, and the selection and compounding of different insects may lead to insect products with higher nutritional value and better therapeutic effects. In addition, special attention should be paid to the safety and stability of edible insects, and the development of non-toxic and safe insect products is the goal we are pursuing (Schlüter *et al.*, 2017). Overall, edible insects have a significant role to play in sustaining human health and supplying nourishment, and they could represent the future of both food research and the food industry.

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CHAPTER 3: PROXIMATE, PHYSICOCHEMICAL, TECHNO-FUNCTIONAL AND ANTIOXIDANT PROPERTIES OF THREE EDIBLE INSECT (*GONIMBRASIA BELINA*, *HERMETIA ILLUCENS* AND *MACROTERMES SUBHYLANUS*) FLOURS

Abstract

In this study, edible insect flours from *G. belina* (*mashonzha*), *H. illucens* (black soldier fly larvae and *M. subhylanus* (*madzhulu*) were prepared and assessed in terms of proximal, physicochemical, techno-functional and antioxidant properties. The crude protein of the edible insect flours varied between 34.90–52.74%. The crude fat of the insect flours differed significantly ($p < 0.05$), with *H. illucens* (27.93%) having the highest crude fat. *G. belina* was lighter (L*) and yellower (+b*) compared to *H. illucens* and *M. subhylanus,* and there was no significant difference ($p > 0.05$) in the redness (+a*) of the edible insect flours. There were no significant differences ($p > 0.05$) in foam capacity and foam stability of all three edible insect flours. Moreover, the antioxidant activity against the DPPH radical was low for *H. illucens* (3.63%), with *M. subhylanus* (55.37%) exhibiting the highest DPPH radical. Principal component analysis (PCA) was applied to the edible insect flours' techno-functional properties and antioxidant indices. Principal component one (1) PC1 accounted for 51.39% of the total variability, while component two (2) accounted for 24.71%. In terms of PC1, the FS, OBC and FC were responsible for the major differences in the edible insect flours. The findings revealed that edible insect flours are a good source of antioxidants and can be used as an alternative protein source and a potential novel food additive due to their techno-functional qualities.

Keywords: Edible insect flours; *G. belina*; *H. illucens*; *M. subhylanus*; nutritional properties; techno-functional properties; antioxidant activity; metal chelation; Mashonzha; Madzhulu; black soldier fly.

3.1 Introduction

As vast as the challenge is to feed 9 billion people by 2050, increasing food availability is insufficient due to increasingly limited resources, such as agriculturally cultivable land (Gahukar, 2011). This, without a doubt, calls for innovative, alternative ways of ensuring that adequate, quality, safe and nutritious foods are available and accessible to all people at all times (Imathiu, 2020). As early as 1975, Meyer-Rochow (1975) argued and proposed that edible insects could play a role in alleviating food security and combating protein deficiency in some underdeveloped countries. Over the last two (2) decades, there has been a renewed (Van-Huis *et al.*, 2013; Hlongwane *et al.*, 2021) The FAO report titled "*Edible Insects: Future Prospects for Food and Feed*" (Van-Huis *et al.*, 2013) and other scientific literature seems to have re-invigorated the earlier call made by Meyer-Rochow in 1975. This is because, compared to conventional protein sources, edible insects have an excellent feed conversion ratio; a source of protein, fat and minerals, and this characteristic is particularly valuable given that future protein consumption is expected to increase with a declining food supply (Kim *et al.*, 2019; Raheem *et al.*, 2019).

Entomophagy, the practice of consuming insects, has been practised worldwide for centuries, yet it has only recently gained momentum in Western cultures (Bußler *et al.*, 2016).. Insects are consumed prominently in Latin America, Asia, and Africa (Raheem *et al.*, 2019). People throughout the world have been consuming insects as a regular part of their diets for millennia (Van-Huis *et al.*, 2013). Considering the growing population worldwide and the increasing demand for additional sources of proteins, edible insects are seen as an economical alternative and as a sustainable source of nutrients and bioactive compounds (González *et al.*, 2019). *H. illucens* (black soldier fly*), G. belina* (*mashonzha*) and *M.subhylanus* (*madzhulu)* are among edible insect species that have gained attention as alternative sources of protein*;* the latter two are indigenous to parts of South Africa and play a vital role in food security, rural livelihoods, and poverty eradication (Hlongwane *et al.*, 2021). Black soldier fly larvae are commercially produced in South Africa by one of the largest industrial insect processing companies, AgriProtein. The European Food Safety Authority (EFSA) is currently considering black soldier fly as a novel ingredient to be used in food.

Gonimbrasia belina is an emperor moth species indigenous to Southern Africa's warmer areas. It is a giant edible caterpillar, known as the *mashonzha* (Tshivenda), *madora* (in Shona) or mopane worm or *amacimbi* (IsiNdebele), which mainly feeds on mopane tree leaves but not exclusively. For millions in the region, *mashonzha is* a significant source of protein. Emperor moth *G. belina* caterpillars are a significant natural resource for rural individuals residing in Botswana, Namibia, northern South Africa, and southern Zimbabwe's mopane forests (Hlongwane *et al.*, 2020).

Macrotermes subhylanus, known as *madzhulu* in Tshivenda and isusu in Nigeria are termites and are gregarious insects most common during the rainy season (Netshifhefhe *et al.*, 2018; Hlongwane *et al.*, 2021). They are the second most eaten insects in South Africa and are harvested during the rainy season. At the same time, *mashonzha* and *madzhulu* are sold at informal markets predominantly in the Limpopo and KwaZulu-Natal provinces, and in other parts of South Africa are considered a delicacy.

In addition to insects, algae and in vitro meat have also been considered as potential alternatives to conventional sources (Hall *et al.*, 2017). The inclusion of insects among these alternatives is highly recommended since they are widely incorporated in food cultures worldwide and have excellent nutritional qualities.

Nevertheless, it is essential to highlight that food neophobia is still directed to the consumption of edible insects, especially in Western and urban societies. However, Schösler *et al*. (2012) reported that edible insects, if incorporated in foods in a less obvious form, such as food ingredients (flours, powders, or pastes) in products that are indistinguishable from familiar food items, consumers would accept them. This indicates that insects could be used as food ingredients in the food supply chain, particularly in areas where traditional approaches are unlikely to be adopted owing to a lack of sensory appeal, and insect flour is one way to incorporate insects into food production systems(Akande *et al.*, 2020; Biró *et al.*, 2020).

Therefore, it is crucial to note that the first step to large-scale industrial success is the exploration of the nutritional, techno-functional and antioxidant properties of proposed edible insect ingredients. Currently, available literature on the application of insect flour mainly focuses on *T. molitor (mealworm*) (González *et al.*, 2019; Lucas-González *et al.*, 2019). There has been little attention paid to the nutritional, techno-functional and antioxidant properties of *mashonzha*, black soldier fly larvae and *madzhulu)* edible insect flours from South Africa.

Therefore, the aim of this study was to establish the proximate composition, physicochemical, techno-functional properties, and antioxidant activity of edible insect flours obtained from *mashonzha*, black soldier fly larvae and m*adzhulu* with the view to find alternative protein sources for human consumption.

3.2 Materials and methods

3.2.1 Source of materials

The edible insects were sourced from different provinces of South Africa: *mashonzha* (*G. belina*) and *madzhulu* (*M. subhylanus*) were sourced in the Vhembe district, Limpopo province, and the black soldier fly larvae (*H. illucens*) were sourced from Cape Town, Western Cape province, South Africa. *M. subhylanus* and *G. belina* were collected from the fields of the Vhembe district. Both edible insects were light trapped causing them to fall in large swarms that were collected and put in a clean container. The chemical reagents, 2,2 diphenyl-1-picrylhydrazyl (DPPH), 2,2′ azobis (2-methyl, 2,2-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), Ferric (III) chloride, ethylenediaminetetraacetic acid (EDTA), tertiary butyl hydroquinone (TBHQ), ferrous (II) chloride and thiobarbituric acid (TBA) were obtained from Merk (Sigma-Aldrich, Kempton Park, South Africa). All the chemicals used in this study were of analytical grade, and chemical reagents were prepared according to standard analytical procedures. Prepared reagents were stored under conditions that prevented deterioration or contamination. The water used in the study was ultrapure water purified with a Milli-Q water purification system (Millipore, Microsep, Bellville, South Africa).

The ethics committee of the faculty of applied sciences gave its approval to the study (215062965/05/2021).

3.2.2 Preparation of insect flour

Representative samples of sun-dried *Mashonzha* (hereinafter indicated as *G. belina*) and *Madzhulu* (hereinafter indicated as *M. subhylanus*) edible insects were purchased from street vendors from the Vhembe district (Limpopo province, South Africa). Black soldier fly larvae (hereinafter indicated as *H. illucens*) reared on clean larvae was purchased from AgriProtein (Cape Town, South Africa), and the flour was prepared following the method described by Zozo *et al*. (2021) and freeze-dried (Wizard 2.0, SP Scientific, Johannesburg, South Africa). The dried edible insects were subjected to a grinding/ milling process using a laboratory blender (Bamix, Checkers, Cape Town, South Africa). The flours were stored at room temperature (21°C) under conditions that prevented deterioration

3.3 Proximate composition analysis

Proximate composition, i.e., moisture (925.10), crude protein (920.87), crude fat (932.06), and ash content (923.03) of the insect flours were determined following standard methods recommended by the Association of Official Analytical Chemists (AOAC,2015) The crude protein determination was performed using Dumas (TruSpec^{TM} Leco Carbon/Hydrogen/Nitrogen Series, Leco Africa) which was calibrated with EDTA according to Zozo *et al*. (2021). The crude protein was subsequently calculated by multiplying nitrogen content by a protein-to-nitrogen conversion factor of 5.60 as recommended by Janssen *et al.* (2017). Moisture percentage was calculated by drying the sample in a vacuum oven at 100 °C for two (2) hours. The dried sample was placed into a desiccator, allowed to cool, and then re-weighed. The process was repeated until a constant weight was obtained. Crude fat was calculated by drying fats after extraction in a Soxhlet assembly using petroleum ether. The ash percentage was calculated by combusting the samples in a silica crucible placed in a muffle furnace at 550 °C. The percentage of carbohydrates on a dry basis was determined according to the method described by Cebeci *et al.*,(2020) equation 3.1. The energy was calculated using the formula equation 3.2 (Farzana & Mohajan, 2015).

%Carbohydrate = 100- (%moisture+% protein+% fat+%Ash) *Equation 3.1*

Energy $\left(\frac{Kcal}{100g}\right) = 4\ (\%\ \text{Carbohydrate} + \%\ \text{Protein}) + (9 \times \%\text{fat})$ *Equation 3.2*

3.4 Determination of physicochemical properties

3.4.1 Determination of colour attributes

The colour attributes of the edible insect flours was measured using spectrophotometry (Model CM-5, Konica Minolta Sensing, Tokyo, Japan) as described by (Larouche *et al.*, 2019), set at standard observer 10° and D65. The instrument was zero calibrated using a black tile (L* = 5.49, a* = −7.08, b* = 4.66) and white calibration was performed using a white tile (L* = 93.41, a* = −1.18, b* = 0.75). Edible insect flour samples were evenly placed in a petri-dish (30 mm diameter), and reflectance was measured for L*a*b* colour scales. The L* coordinate is lightness, 100 represents white and closer to 0 represents black Measurements for each sample were performed in triplicate at three different positions in the samples, with the results recorded in L* (lightness), a* (chromaticity coordinate +a* = red and −a* = green), b* (chromaticity coordinate +b* = yellow and −b* = blue).

3.4.2 Determination of bulk density

The procedure was described by Mintah *et al*. (2020) with some modifications. Five (5) g of the sample was transferred into a weighed measuring cylinder (50 mL) (W_1) and then compressed by tapping until sample volume remained constant. The tube was again weighed (W_2) the new volume (V_1) was noted and the density (g/mL) was measured using equation 3.3:

$$\text{Bulk Density} = \frac{W_2 - W_1}{V1}$$ *Equation 3.3*

3.4.3 Evaluation of water activity

The water activity (A_w) of edible insect flours was measured using the method described by Benamara *et al*. (2016) with minor modifications. Salt humidity standards of 53, 75 and 90% relative humidity were used to calibrate the measurement cell. Five (5 g) of the insect flours was transferred into a sample dish and placed inside the (AW SPRINT TH500, Novasina analyser, Zurich, Switzerland), and the cell measuring protection filter was immediately closed. The reading was observed after a period of 60 to 80 s

3.5 Determination of techno-functional properties

3.5.1 Determination of water binding capacity and oil binding capacity

The water-binding capacity (WBC) of the edible insect flours was determined according to Mshayisa & van-Wyk (2021) with slight modifications. Briefly, a 0.5 g sample was mixed with 2.5 mL deionized water, vortexed for 60 s (Vortex-Genie 2, Scientific Industries, Bohemia, NY, USA), and centrifuged for 20 min at 3220 g at room temperature. The supernatant was removed by decantation and drainage of the residual non-bound water by placing the centrifugation tube upside-down on filter paper for 60 min. WBC was calculated using equation 3.4:

$$\mathrm{WBC} = \frac{\mathrm{m_1} - \mathrm{m_0}}{\mathrm{m_0}}$$ *Equation 3.4*

where m_0 is the initial weight, m_1 is the final weight. The oil binding capacity (OBC) was analysed using sunflower oil instead of deionized water. Except for the vortexing step (120 s), the experimental procedure was performed in analogy to the WBC assay. OBC was similarly calculated using equation 3.4.

3.5.2 Determination of emulsion capacity and emulsion stability

Emulsifying properties were determined according to the method of Mshayisa & van Wyk (2021) The samples were dispersed in distilled water 1% (w/v), and 15 mL of the dispersion was homogenized with 15 mL of vegetable oil at a speed of 10,000 rpm for 3 min. Subsequently, the samples were centrifuged (Thermo Electron Corporation Jouan MR1812, Waltham, MA, USA) at 3220 g for 5 min and the volume of the individual layers were read. Emulsion stability was evaluated by heating the emulsion for 30 min at 80 °C. Then, the samples were centrifuged at 3200 g for 5 min. The emulsifying capacity (%) was expressed as a percentage of the volume of the emulsified layer (mL) against the volume of the whole layer (mL). Emulsion capacity and emulsion stability were calculated using equation 3.5:

$$\%\ \text{Emulsion capacity (EA)} = \frac{\mathrm{Ve}}{\mathrm{V}} \times 100$$ *Equation 3.5*

3.5.3 Determination of foam capacity and foam stability

Foaming capacity (FC) and foam stability (FS) were determined according to the method of Zielinska *et al*. (2018). First, 20 mL of a 1% sample was homogenized in a high shear homogenizer mixer (Polytron PT 2500E, United Scientific, Cape Town, South Africa) at a speed of 10,000 rpm for 4 min. The whipped sample was then immediately transferred into a graduated cylinder. The total volume was read at time zero and 30 min after homogenization. The foaming capacity and foam stability were calculated using equation 3.6 and 3.7 respectively:

$$\%\ \text{Foaming capacity (FC)} = \frac{V_0 - V}{\mathrm{V}} \times 100$$ *Equation 3.6*

$$\%\ \text{Foaming stability (FS)} = \frac{\mathrm{V_{30}}}{V_0} \times 100$$ *Equation 3.7*

where: V—volume before whipping (mL), V_0—volume after whipping (mL), V_{30}—volume after standing (mL)

3.6 Determination of antioxidant activity

3.6.1 Preparation of edible insect extract

Two (2) g of the edible insect flours was mixed with 40 mL Milli-Q water in a 50 mL centrifuge tube. The edible insect flour solution was centrifuged (Thermo Electron Corporation Jouan MR1812, Waltham, MA, USA) at room temperature for 15 min at 8000 rpm, and the supernatant was collected and stored at 4°C until further analysis and the pellet was discarded.

3.6.2 Determination of DPPH radical scavenging activity

The antioxidant activity of the extract was determined by the 1,1-diphenyl-2-picryl-hydrazyl radical scavenging (DPPH-RS) assay according to the method of Vhangani & van-Wyk (2013). The method uses a stable chromogen radical, DPPH in ethanol, which gives a deep purple colour. The reaction mixture was prepared by reacting 2 mL of edible insect extract with 4 mL of DPPH (0.12 mM) in 95% in ethanol. The reaction mixture was incubated for 30 min in the dark, and then the absorbance of the resulting solutions was measured at 517 nm using a spectrophotometer (Lambda 25, Perkin Elmer, Singapore). The control was prepared similarly, except that Milli-Q water was used, and TBHQ (0.1%) was used as a positive control. The percentage of inhibition was calculated using equation 3.8:

$$\%\ \mathrm{DPPH-RS} = \frac{A_{0\,(517\mathrm{nm})} - A_{1(517\mathrm{nm})}}{A_{0(517\mathrm{nm})}} \times 100 \qquad \textit{Equation 3.8}$$

where: A_0 is the absorbance of the negative control (water) at 517 nm and A_1 is the absorbance of the edible insect extract at 517 nm test sample.

3.6.3 Determination of ABTS+ radical scavenging

The experiment was performed according to the method of Chatsuwan *et al*. (2018) and Mshayisa and van Wyk (2021). The 2,2-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt ($ABTS^{\bullet+}$) radical was produced by reacting 7.4 mM ABTS stock solution with 2.45 mM potassium persulphate at a ratio of 1:1 (*v*/*v*). The mixture was allowed to react for 12–16 h at room temperature in the dark. This working solution of $ABTS^{\bullet+}$ solution was diluted with 95% ethanol at a ratio of 1:50 (*v*/*v*) in order to obtain an absorbance of 1.00 at 734 nm. A fresh $ABTS^{\bullet+}$ solution was prepared daily for each assay. The reaction mixture contained 0.15 mL of edible insect extract solution and 2.85 mL of $ABTS^{\bullet+}$ solution. The mixture was incubated at room temperature for 6 min in the dark. Then, the absorbance was measured at 734 nm in a spectrophotometer (Lambda 25, Perkin Elmer, Singapore). The control was prepared in the same manner, except that distilled water was used instead of the sample, and TBHQ (0.1%)

was used as a positive control. The scavenging activity was determined according to equation 3.9:

$$\% \text{ ABTS} - \text{RS} = \frac{A_{control(730mn)} - A_{sample(730nm)}}{A_{control(730nm)}} \times 100 \qquad \textit{Equation 3.9}$$

where: $A_{control}$ is the absorbance of the control (water) at 730 nm and A_{sample} is the absorbance of edible insect extract at 730 nm.

3.6.4 Determination of metal chelating activity

The chelating effect on ferrous ions of the prepared extracts was estimated by the method of Sudan *et al*. (2014) with slight modifications. Briefly, 1 mL of each edible insect extract was mixed with 1.85 mL of Milli-Q water and 0.05 mL of 2 mM $FeCl_2$. Next, the reaction was initiated by the addition of 0.1 mL of 5 mM ferrozine into the mixture, which was then left at room temperature for 10 min and the absorbance of the mixture was determined at 562 nm using a spectrophotometer (Lambda 25, Perkin Elmer, Singapore). The percentage of chelating activity was calculated using equation 3.10:

$$\% \text{ Chelating activity } \frac{A_0 - A_1}{A_0} \times 100 \qquad \textit{Equation 3.10}$$

where: A_0 is the absorbance of the negative control (water) control and A_1 is the absorbance of the edible insect extract

3.6.5 Determination of reducing power

The reducing power was determined according to the method of Athukorala *et al*. (2006). First, 1.0 mL aliquots of edible insect were mixed with 2.5 mL of phosphate buffer (0.2 mM, pH 6.6) and 2.5 mL of potassium ferricyanide. The reaction mixture was vortexed for 10 s and thereafter incubated at 50 °C in the water bath for 20 min. Thereafter, 2.5 mL of 10% trichloroacetic acid (TCA) was added to the reaction mixture, and then vortexed for 10 s, 2.5 mL of the solution was then pipetted out into beakers and mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ was added and absorbance was measured at 700 nm in a spectrophotometer (Lambda 25, Perkin Elmer, Singapore).

3.7 Statistical analysis

All assays were performed in triplicates, and the obtained data were presented as means ± standard deviation. Statistical analysis was performed by testing significant differences ($p < 0.05$) between treatments using multivariate analysis of variance (MANOVA), and Duncan's multiple range test was used to separate means where differences existed. Principal Component Analysis (PCA) was applied to extract the components that explained the variability in the edible insect flours antioxidant and functional properties. All quantitative data were analysed using SPSS 27.0 (2005) (SPSS Inc., Chicago, IL, USA).

3.8 Results and discussion

3.8.1 Proximate composition of edible insect flours

The proximate composition of edible insect flours (*G. belina*, *H. illucens* & *M. subhyalinus*) is depicted in Table 3.1. Protein is the dominant nutrient in all three (3) edible insect flours, followed by crude fat. The protein content was significantly ($p < 0.05$) different between all the edible insect flours, and it ranged from 34.90–52.74%. This is superior to other protein sources, such as beef, eggs, milk, and soybeans, where protein constitutes approximately 30 and 45% of dry matter (Churchward-Venne *et al.*, 2017). The protein content of *H. illucens* (34.90%) was significantly lower ($p < 0.05$) compared to *M. subhyalinus* (52.74%).The findings agreed with the results reported by Bußler *et al*. (2016) on *H. illucens* (34.70%). In a literature review study conducted by Meyer-Rochow *et al*. (2021) the protein content of the *Macrotermes* species ranged from 20.4–39.7%. Moreover, Kwiri *et al.* (2014) reported the protein content of *G. belina* to be (55.41%). These values are higher than the result obtained in this study of the same insect flour. The differences in protein content can be attributed to differences in the edible insect flour, level of individual development, sex, feed type, climate, and geographical location. In this way, the edible insect flours are diversified nutritionally. The edible insects reported in this study may offer an affordable source of protein, especially for low-income communities and be used as ingredients in flour form to minimise the aversion towards consuming insects (Montowska *et al.*, 2019; Anaduaka *et al.*, 2021)

The ash content of *G. belina* (11.38%), *H. illucens* (7.46%) and *M. subhyalinus* (6.38%) was higher than the values reported for *M. nigeriensis* (3.24%) by Omotoso (2015). However, the values were comparable to those *of Macrotermes bellicosus* (11.83%) reported by Adepoju & Omotayo (2014). Torruco-Uco *et al*. (2018) also reported the ash of *Sphenarium purpurascens*) to be 2.31–3%, the values are much lower than the values reported in this study. Nyakeri *et al*. (2017) reported *H. illucens* to contain 14.61% ash which is higher than the value of the similar species in this study which had 7.46% ash content. Considerable levels of ash indicate that the samples are a good source of minerals. The variation among the ash contents of samples may be driven by the difference in location, diet, and season in which the insects are reared and harvested (Clarkson *et al.*, 2018). Therefore, the addition of edible insect flour in processed food products has the potential to enhance the mineral content of food, especially where food fortification is essential. The considerable good ash content of the edible insect flours signifies good mineral composition that the edible insect flours might contain (Ghosh *et al.*, 2017).

As shown in Table 3.1, the moisture of the three edible insect flours ranged from 5.77–6.59% and no significant differences ($p > 0.05$) were observed amongst all the edible insect flours. Siulapwa *et al*. (2012) reported the moisture content of *G. belina* to be 9.1%, which is higher than the value reported in this study. Moreover, Anaduaka *et al*. (2021) also reported high moisture values for *Zonocerus variegatus* and *Oryctes rhinoceros* larva (*O. rhinoceros* larva) to be 11.85 – 26.17%, respectively. The low moisture values obtained in this study suggest that it likely results in low water activity and, therefore, can potentially extend the shelf-life of insect flours.

As illustrated in Table 3.1 the crude fat content in *G. belina*, *H. illucens*, and *M. subhyalinus* was 13.91, 27.92 and 6.35%, respectively. *H. illucens* (27.92%) results were higher than those reported by Payne *et al*. (2016) of the similar species (14%). Ganguly *et al*. (2013) reported the fat of Oxya chinensis to be 2.2%, which is lower than the results obtained in this study. Moreover, Melo *et al*. (2011) reported *S. purpurascens* to be 5.75%, which is comparable to *M. subhyalinus.* However, Sogbesan & Ugwumba (2008) reported the fat of *M. subhylanus* to be 10.6–22.2%, which is higher than the values of the similar species in this study. Fat is a major source of fuel in the body, and it is essential in the cell structures as well as in supplying some oil-soluble vitamins, such as vitamins A, D, E andK.

As the primary source of fibre and calories for humans, carbohydrates are essential components of proper nutrition (Rodríguez-Miranda *et al.*, 2019). The three (3) edible insect flours (*G. belina*, *H. illucens* &*M. subhyalinus)* showed no significant difference ($p > 0.05$) in their carbohydrate content and ranged from 22.33–28.10%, respectively. The observed carbohydrate content is low in comparison with those reported by Mishyna *et al.* (2019)for *Schistocerca gregaria* (*S. gregaria*) and *Apis mellifera* flours which contained 47.2 and 54.10% carbohydrates, respectively.

Energy is primarily derived from carbohydrates, proteins, and fats in food, and because edible insects are high in these macromolecules, they have a high energy content (Ganguly *et al.*, 2013). As shown in Table 3.1 the energy values obtained for the edible insect flours ranged from 379.91–485.58 KJ. No significant differences ($p > 0.05$) were observed for *G. belina* and *M. subhyalinus*. However, *H. illucens* was significantly different ($p < 0.05$) from the other two edible insect flours. The results reported in this study are similar to those reported by Montowska *et al.* (2019) on edible insect flours of 486–524 kcal/100 g. Siulapwa *et al*. (2012) reported *G. belina* energy values of (385 kcal/100 g), which is in the same range as *G. belina* energy value reported in this study.

Table 3.1 Proximate composition of three edible insect flours.

Edible insects	Crude Protein (%)	Ash (%)	Moisture (%)	Crude Fat (%)	Carbohydrates (%)	Energy (KJ)
G. belina	46.70 ± 0.82 ^{b}	11.38 ± 2.20 ^{b}	5.68 ± 0.25 ^{a}	14.04 ± 0.12 ^{b}	22.10 ± 1.45 ^{a}	399.38 ± 6.03 ^{a}
H. illucens	34.90 ± 0.47 ^{a}	7.50 ± 1.65 ^{a}	5.76 ± 0.01 ^{ab}	27.93 ± 6.13 ^{c}	23.66 ± 7.84 ^{a}	485.58 ± 26.69 ^{b}
M. subhyalinus	52.74 ± 1.47 ^{c}	6.41 ± 0.07 ^{a}	6.40 ± 0.06 ^{b}	6.36 ± 0.05 ^{a}	27.27 ± 1.19 ^{a}	379.91 ± 1.06 ^{a}

Values are mean±standard deviation. Means within a column followed by the same superscript are not significantly ($p > 0.05$) different.

3.8.2 Physicochemical properties

3.8.2.1 Colour attributes of edible insect flours

The colour attributes of edible insect flours measured were lightness (L*), greenness (−a*), redness (+a*), blueness (−b*), and yellowness (+b*). Lightness is the luminous intensity of colour measured on a scale of 0 to 100, with 0 indicating black and 100 indicating white [50]. Colour is a crucial factor influencing the acceptance of edible insects (Lucas-González *et al.*, 2019). The descriptive colour determination of the three (3) edible insect flours *G. belina*, *H. illucens* and *M. subhylanus* is shown in Table 3.2. There was a significant difference ($p < 0.05$) in the lightness of the edible insect flours, with *G. belina* (57.95) being the lighter in colour. No significant difference ($p > 0.05$) was observed in the redness of the three edible insect flours; however, *M. subhyalinus* (5.72) was redder compared to *G. belina* (3.92) and *H. illucens* (4.46), respectively, as depicted in Figure 3.1.

Table 3.2 Physicochemical properties of three (3) edible insect flours.

Edible Insects	L*	a*	b*	Bulk Density (g/mL)	pH
G. belina	57.95 ± 0.31^{c}	3.92 ± 1.49^{a}	20.02 ± 1.97^{b}	0.65 ± 0.01^{b}	6.12 ± 0.03^{a}
H. illucens	53.69 ± 0.54^{b}	4.46 ± 0.36^{a}	13.08 ± 2.68^{a}	0.51 ± 0.01^{a}	8.93 ± 0.05^{b}
M. subhyalinus	43.52 ± 0.56^{a}	5.72 ± 3.90^{a}	12.00 ± 2.70^{a}	0.64 ± 0.00^{b}	6.14 ± 0.02^{a}

Values are mean   standard deviation. Means within a column followed by the same superscript are not significantly ($p > 0.05$) different.

Figure 3.1 Ground edible insect flour of three (3) different species. **(A)** *G. belina*; **(B)** *M. subhyalinus*; and **(C)** *H. illucens*

3.8.2.2 Bulk density

Among other vital properties of powder products, bulk density (BD) has significant economic and functional importance, for example, in reducing packaging costs (Piornos *et al.*, 2015). It is determined by particle density, internal porosity, and particle arrangement in the container (Sharma *et al.*, 2012). Table 3.2 represents the bulk density of the three edible insect flours (*G. belina*, *H. illucens* and *M. subhylanus*). The bulk density of the edible insect flours varied from 0.51–0.64 g/mL and no significant difference ($p > 0.05$) was observed. Akpossan *et al*. (2015) reported higher BD for *Imbrasia oyemensis* (*I. oyemensis*) to (1.1 g/mL), while in a study by (Ekpo *et al.*, 2008) on *Imbrasia belina* (*I. belina*) the BD (0.65 g/mL) was comparable to that found in the present study. An apparent correlation exists between the bulk density and the protein content. Thus, the edible insect flours all had a low BD due to high protein content. Low BD of the flours is advantageous when storability and transportation are considered since the products could be easily transported and distributed (Gupta *et al.*, 2019). Low BD flours also find application in the preparation of complementary foods and among the traditional techniques.

3.8.2.3 Water activity and pH of edible insect flours

Water activity is a measure of how efficiently the water present can take part in a chemical (physical) reaction or the water available enough for microbial growth to occur in a food product (Tapia *et al.*, 2008; Sandulachi and Tatarov, 2012). Generally, food deterioration due to microbial growth (yeast and moulds to pathogens) occurs at a range of 0.6 to 1.0 (Tapia *et al.*, 2008). The water activity of the three edible insect flours *G. belina*, *H. illucens*, and *M. subhyalinus* is depicted in Figure 3.2. The A_w of the edible insect flours ranged from *M. subhyalinus* (0.35 ± 0.26), *G. belina* (0.45 ± 0.01), to *H. illucens* (0.53 ±0.01), and there were no significant differences ($p > 0.05$) within the different edible insect flours. This implies that the edible insect flours are not susceptible to microbial growth. However, some enzymatic reactions, such as browning, transpire at the range of 0.3 to 1.0 and increase rapidly at 0.6 to 0.8. In this study, *M. subhyalinus* had the lowest A_w; therefore, it might be susceptible to enzymatic reactions rapidly compared to the other two edible insect flours.

In addition, pH in food contributes to reducing the growth of microorganisms, thereby ensuring food safety. The pH of *H. illucens* (8.93) had a significant difference ($p < 0.05$) between the pH of *G. belina* (6.12) and *M. subhyalinus* (6.14), while there was no statistical difference ($p > 0.05$) between the pH of *G. belina* and *M. subhyalinus* (Table 3.2). Lucas-González *et al*. (2019) reported similar results for *Acheta domesticus* flour (6.31–6.48). The pH of these edible insect flours provides essential information since it determines which type of food matrix, they can be added into without affecting their technological behaviour. Thus, potential food ingredients with pH values close to neutrality, such as those obtained in this study, will be better suited for application to neutral food matrices, such as meat replacers and baked products.

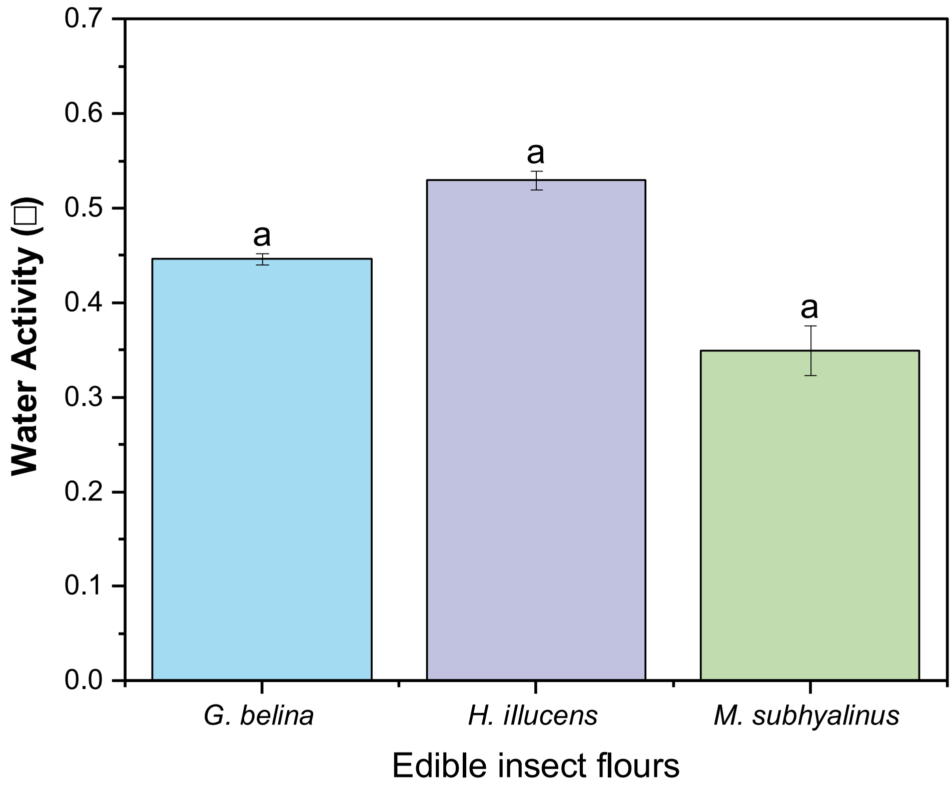

Figure 3.2 Water activity of three (3) edible insect flours. Values are mean ± standard deviation, means with different superscripts are significantly different ($p < 0.05$)

3.8.3 Technofunctinal properties

3.8.3.1 Water binding capacity and oil binding capacity

Water binding capacity (WBC) and oil binding capacity (OBC) are critical features of food ingredients in food processing and applications. They are related to the ability to take up and retain water and oil, respectively, which directly affect the texture and the flavour of the products, especially in the meat and bakery (Wang *et al.*, 2021). There are several intrinsic factors affecting the water-binding properties of food flours with relatively high protein. These include amino acid composition, protein conformation, and surface polarity/hydrophobicity (Akpossan *et al.*, 2015). Table 3.3 depicts the water binding capacity of the edible insect flours. Higher WBC was notable for *M. subhylanus* (1.46 g/g); however, there was no significant difference ($p > 0.05$) between this edible insect flour and that of *G. belina* (1.30 g/g). While the lower WBC value was observed for *H. illucens* flour (1.11 g/g), Zielinska *et al*. (2018) reported higher WBC of *Schistocerca gregaria* (*S. gregaria*) (2.18 g/g). Similarly, Lucas-González *et al*. (Lucas-González *et al.*, 2019) reported the WBC of *Acheta domesticus* flour to be (3.82 g/g). However, the WBC of *M. subhyalinus* (1.46 g/g) was higher than that reported for *T. molitor* (0.4 g/g). The apparent difference in the WBC could be due to the higher protein content in the *M. subhylanus*, which contains more hydrophilic groups to bind to water molecules. The WBC of the edible insect flours is comparable to plant-based flours, such as wheat and rice, which were reported to have WBC from 1.4–1.9 g/g (Chandra, 2013). This information is crucial for the application of these flours in the food industry. The significant difference in water holding capacity between the edible insect flours might be an indication of the different applications they might have in food. This is the first study to report on the WBC of edible insects, such as *G. belina* and *M. subhyalinus*, to our knowledge.

The OBC is shown in Table 3.3. No significant difference was found ($p > 0.05$) between *H. illucens* (1.35 g/g) and *M. subhyalinus* (1.48 g/g), and the lowest value was obtained for *G. belina* (0.89 g/g). These values are lower than those reported for *Gryllidae sp.* (2.02 g/g), *G. sigillatus* (2.82 g/g) and *A. domesticus (*3.37–3.52 g/g) (Torruco-Uco *et al.*, 2018). Assielou *et al*. (2015) reported the OBC of *O. owariensis* larvae flour to be 265.90% (2.65 g/g), which is higher than the OBC in this study. The OBC refers to the ability of the proteins in flour to physically bind to fat through capillary action, which is of great importance because fat is a flavour retainer and increases our ability to taste food. Akubor & Eze (2012) illustrated that OBC has proven useful in the formulation of bakery products and sausages, and this shows that the studied flours *(M. subhyalinus*, *H. illucens*, and *G. belina*), since they are low in OBC are, therefore, low flavour retainers and therefore may be useful in food systems that do not require high WBC/OBC values.

Table 3.3 Techno-functional properties of three edible insect flours.

Edible insects	WBC (g/g)	OBC (g/g)	EC (%)	ES (%)	FC (%)	FS (%)
G. belina	1.30 ± 0.12 ^{ab}	0.89 ± 0.12 ^{a}	41.76 ± 2.84 ^{a}	33.75 ± 2.29 ^{a}	5.81 ± 3.69 ^{a}	95.32 ± 2.37 ^{a}
H. illucens	0.11 ± 0.02 ^{a}	1.35 ± 0.09 ^{b}	67.33 ± 8.49 ^{b}	42.45 ± 5.07 ^{b}	5.69 ± 1.41 ^{a}	97.38 ± 1.70 ^{a}
M. subhyalinus	1.46 ± 0.06 ^{b}	1.48 ± 0.07 ^{b}	45.44± 4.28 ^{a}	32.80 ± 0.47 ^{a}	4.71 ± 2.46 ^{a}	97.51 ± 1.22 ^{a}

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly ($p > 0.05$) different. WBC: water-binding capacity, OBC: oil biding capacity, EC: emulsion capacity, ES: emulsion stability, FS: foam stability, and FC: foam capacity.

3.8.3.2 Emulsion stability and emulsion capacity

Proteins are surface-active agents that can form and stabilise the emulsion by creating electrostatic repulsion on the oil droplet surface. Generally, the emulsifying activity of proteins is affected by their molecular weight, hydrophobicity, conformation stability, surface charge, and physicochemical properties, such as pH, ionic strength, and temperature (Jantzen da Silva Lucas *et al.*, 2020). The results obtained for emulsion capacity (EC) and emulsion stability (ES) of the edible insect flours are presented in Table 3.3. The emulsion capacity of *G. belina*, *M. subhyalinus*, and *H. illucens* were 41.76, 45.44, and 67.33%, respectively. The results for EC in this study are higher than those reported by Mishyna *et al*. (2019).for *S. gregaria* (39.5%) and *A. mellifera* (20.8%) insect flours. The protein emulsification properties are known to be influenced by their surface hydrophobicity, which affects the protein's ability to adsorb to the oil side of the interface. Higher emulsion capacities are usually associated with a greater disintegration (Bußler *et al.*, 2016). . *M. subhyalinus* had the highest EC (61.69%), which agrees with the macronutrient composition reported in Table 3.1.

In this study, the ES of *G. belina* (33.75%) and *M. subhyalinus* (32.80%) were not significantly different ($p > 0.05$). The results are lower than those reported by Akpossan *et al*. (2015) on *Imbrasia oyemensis* (84.76%). ES of *H. illucens* (42.45%) was comparable to that of the larva of *Cirina (*45.36%) reported by Omotoso. Adebowale *at al*. (2005) reported adequate emulsification but poor stability in African cricket (*Gryllidae sp.*) flour. Food manufacturers have a growing demand for sustainable and secure protein sources. Currently, the most widely used emulsifiers are casein and whey (Hall *et al.*, 2017). The studied species show similar or higher emulsion activity than plant seeds rich in protein for example chickpea which has 61.14 ± 0.61 ES and 94.19 ± 1.64 EA (Zielinska *et al.*, 2018). Therefore, It is also possible to use them as an alternative protein emulsifier in existing foods or in new food formulations.

3.8.3.3 Foam capacity and foam stability

Foams are colloidal systems that consist of a continuous aqueous phase and a dispersed gas phase (Hall *et al.*, 2017). Foam formation is governed by the transportation, penetration, and reorganisation of molecules at the air-water interface. To exhibit good foaming properties, a protein must be capable of migrating rapidly to the air-water interface, unfolding, and rearranging at the interface. Table 3.3 displays the FC and FS of the edible insect flours. The FC was higher for *G. belina* (5.81%); however, no significant differences ($p > 0.05$) were observed amongst all three (3) edible insect flours. The FC values reported by Torruco-Uco *et al*. (2018) for *Gryllidae sp*. (6%) were comparable to the reported values in this study.

Zielinska *et al*. (2018) reported FC of *G. sigillatus* (41%) while Assielou *et al*.(2015) reported *Oryctes owariensis* (*O. owariensis*) larvae to have FC of (17.87%), which is also higher than the values reported in this study. This study shows that the low FC can be related to highly ordered globular proteins that resist surface denaturation (Akpossan *et al.*, 2015; Ndiritu *et al.*, 2017).

There were no significant differences ($p > 0.05$) in FS of the edible insect flours in this study. However, the results obtained were higher than those reported by (Ekpo *et al.*, 2008) on *imbrasia belina* larvae flour (1.4–5.1%), whereas Omotoso (2006) reported *Cirina forda* larva FS to be 3.00%, which is much lower than the FS reported in this study. There was a notable significant difference between the FC and FS values of the edible insect flours, and these results indicate that the proteins and other components of the edible insect flours have a greater ability to form a strong and cohesive film around air bubbles and greater resistance of air diffusion from the bubbles (Gravel & Doyen, 2020).

Presently, research is focused on finding alternatives to eggs, which are commonly used as a foaming agent in food products (Hall *et al.*, 2017). The data presented in this study showed that the three (3) edible insect flours (*G. belina. H. illucens*& *M. subhyalinus*) exhibited excellent foaming properties; hence, they can be a suitable foaming agent and has potential for such food applications.

3.8.4 Antioxidant properties

3.8.4.1 DPPH-RS of edible insect flours

The DPPH radical-scavenging (DPPH-RS) assay is a widely used method for evaluating the ability of food matrices to scavenge free radicals generated from the DPPH reagent, which undergo SET mechanism (Liang & Kitts, 2014). DPPH is a stable free radical that shows maximum absorbance at 517 nm in ethanol and changes from purple to yellow in the presence of antioxidants. When a DPPH radical encounters an electron-donating substrate, such as an antioxidant, the radical is scavenged (Ben Khaled *et al.*, 2014). As illustrated in Figure 3.3, the insect flours differed significantly ($p < 0.05$) from one another, with *M. subhyalinus* (55.57%) exhibiting the highest radical scavenging activity followed by *G. belina* (37.44%) and *H. illucens* (3.63%), respectively. In a study reported by Navarro del Hierro *et al*. (2020) *T. molitor* and *A. domesticus* extracts, the DPPH-RS was 57 and 72%, respectively, and the values for *T. molitor* are comparable to those of *M. subhyalinus* from this study. Nabil *et al*. (2020) also reported on *Moroccan cladode* flour, and the radical scavenging activity was between 7.18 and 72.37%, which is in line with the radical scavenging activity reported in this study. The results, therefore, suggest that the edible insect flours could be scavenging agents and imply that they could react with free radicals. This study supports the observation of Mshayisa &

van-Wyk (2021), who proposed that edible insects can be used as novel functional components in food compositions.

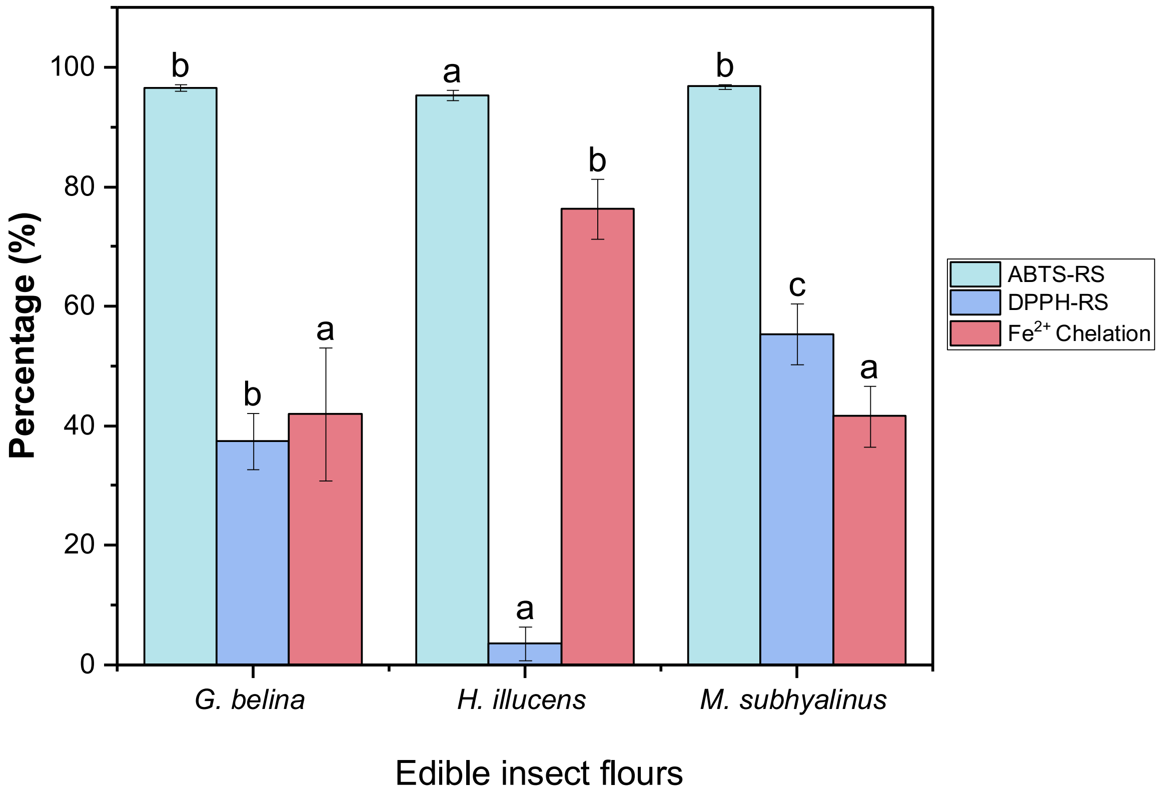

Figure 3.3 Scavenging effect of DPPH-RS, ABTS-RS and Fe2+ chelating activity of edible insect flours. Values are mean ± standard deviation; means with different superscripts are significantly different ($p < 0.05$)

3.8.4.2 ABTS-RS of edible insect flours

The $ABTS^+$ radical scavenging activity was determined to assess the antioxidant potential of *H. illucens*, *G. belina* and *M. subhyalinus*. As depicted in Figure 3.3, no significant differences ($p > 0.05$) were observed between *M. subhyalinus* (96.81%) and *G. belina* (96.61%). However, a significant difference ($p < 0.05$) was observed between the two edible insect flours compared to *H. illucens* (95.32%). It was also observed that *H. illucens* showed lower DPPH-RS as compared to ABTS-RS. The difference in scavenging patterns of ABTS-RS and DPPH-RS could be responsible for these observations. ABTS is more accessible to hydrophilic peptides, while hydrophobic peptides can interact easily with peroxyl radicals, such as DPPH (Chalamaiah *et al.*, 2012). Most importantly, to our knowledge, this is the first study to establish the antioxidant indices of these three (3) edible insect flours. This study's findings have implications for the utilization of edible insect flours as functional components in food.

3.8.4.3 Metal chelation of edible insect flours

The chelation of Fe^{2+} was used to determine the ability of edible insect flours in metal-chelating activity. Ferrozine quantitatively forms complexes with Fe^{2+} ions in the presence of chelating agents, the development of complexes is slowed in the presence of chelating substances disrupted, resulting in the decrease in colour formation (Ben Khaled *et al.*, 2014). As shown in Figure 3.3, all edible insects had a high ability to chelate Fe^{2+}. In this study, the highest chelating ability activity was observed in *H. illucens* (76.30%). Moreover, there were no significant differences ($p > 0.05$) in *G. belina* (42.00%) and *M. subhyalinus* (41.61%). Ferrous ion (Fe^{2+}) is the most potent pro-oxidant among metal ions. This ion can interact with hydrogen peroxide in a Fenton reaction to produce the reactive oxygen species and hydroxyl free radical (OH), leading to the initiation and/or acceleration of lipid oxidation in food (Khantaphant *et al.*, 2011). Therefore, the ability of these edible insect flours to chelate Fe^{2+} suggests they can reduce or avoid the free radical formation. To the best of our knowledge, this is the first study to empirically investigate the Fe^{2+} chelation of edible insect flours, such as *G. belina* and *M. subhyalinus.* The results of this study are vital since they indicate that edible insect flours possess considerable meatal chelating activity, which is critical in antioxidant activity since it reduces the concentration of transition metals that catalyse lipid oxidation.

3.8.4.4 Reducing power of edible insect flours

Reducing power is a useful indicator of food component antioxidant activity. In this test, the ferric chloride/ferric cyanide complex is reduced to ferrous form (Fe^{2+}) in the presence of antioxidants, allowing the Fe^{2+} concentration to be measured spectrophotometrically by measuring the Prussian blue colour produced at 700 nm (Nooshkam *et al.*, 2019). The reducing power assay is often used to evaluate the ability of antioxidants to donate an electron to the free radical (Khantaphant & Benjakul, 2008). In this study, the ability of edible insect flours to reduce Fe^{3+} to Fe^{2+} was investigated, and the results are depicted in Figure 3.4. A significant difference ($p < 0.05$) was observed between all the edible insect flours. *H. illucens* (0.61) had the highest RP, while *G. belina* (0.26) had the lowest RP. As articulated by Zielińska & Pankiewicz (2020), due to their high protein nature, edible insects are, therefore, potential sources of bioactive proteins that could also possess antioxidant activity. In addition, due to the high reducing power, the obtained results suggest that *H. illucens* soluble proteins contain amino acids or peptides that act as electron donors and can react with free radicals to transform them into stable compounds. This is an important aspect in possessing antioxidant activity in the body of human (Zhou *et al.*, 2022).

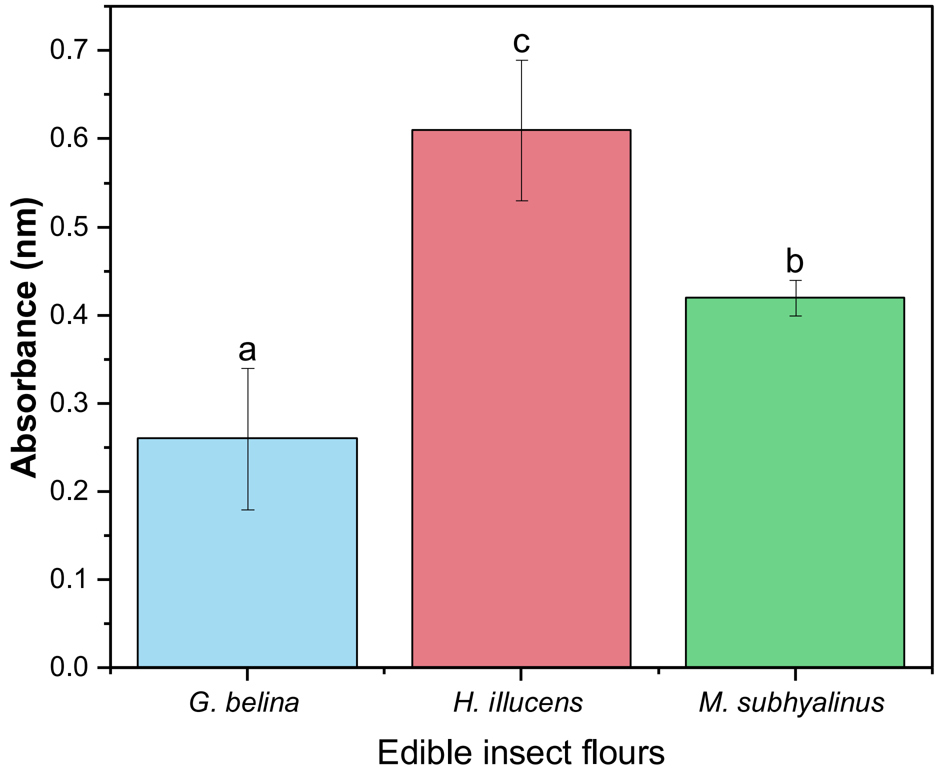

Figure 3.4 Reducing power activity of edible insect flours. Values are mean ± standard deviation; means with different superscripts are significantly different ($p < 0.05$)

3.9 Principal component analysis

Principal component analysis (PCA) was performed to understand the inter-relationships among the measured techno-functional properties and antioxidant activity indices and the similarities and differences among the edible insect samples. The suitability of data reduction by PCA was established by several factors, such as the high correlations between the variables (correlation matrix) and the significant ($p \leq 0.05$) Bartlett's test, as well as the Kaiser–Meyer–Olkin measure (0.68), which was significantly higher than the recommended minimum of 0.6. The PCA results were displayed using score and loading plots (Figure 3.5). To determine the relative contributions of the principal components in overall total variability, only the eigenvalues greater than one were considered. Thus, the first three (3) principal components (PC1, PC2 and PC3) were found to be significant and explained 87.99% variability in the data set (Table S1). Component 1 accounted for 51.39% of the total variability, and represented EC (0.960), Fe Chelation (0.949), ES (0.897) and DPPH-RS (−0.897) while FS (0.837), FC (−0.080), ABTS-RS (−0.531) and WBC (0.515) contributed to PC2, with a total variability contribution of 24.71%. The PC3 accounted for 11.89% of the total variability due to OBC (0.745), FC (0.504), ABTS-RS (0.442) and RP (0.247), respectively, as shown in Table S1 (supplementary materials). The edible insects were clearly distributed into three clusters (figure 5). M. *subhyalinus* can be separated from *H. illucens* based on the DPPH-RS, WBC, and foam stability. In Figure 3.5, *H. illucens* were grouped in close proximity with values of component 1, whereas *M. subhyalinus* and *G. belina* are diametrically opposed in PC2 (meaning they are on the negative and opposite sides). PCA showed that *M. subhyalinus* and *G. belina* located on the opposite sides of PC2, the FS, OBC and FC were to be majorly responsible for the difference in the edible insect flours. This was due to the high FC and OBC exhibited by *M. subhyalinus* samples, while *G. belina* exhibited the lowest OBC. Therefore, PCA could be helpful to provide valuable information on the classification and discrimination of edible insect flours and on relationships between antioxidant indices and techno-functional properties

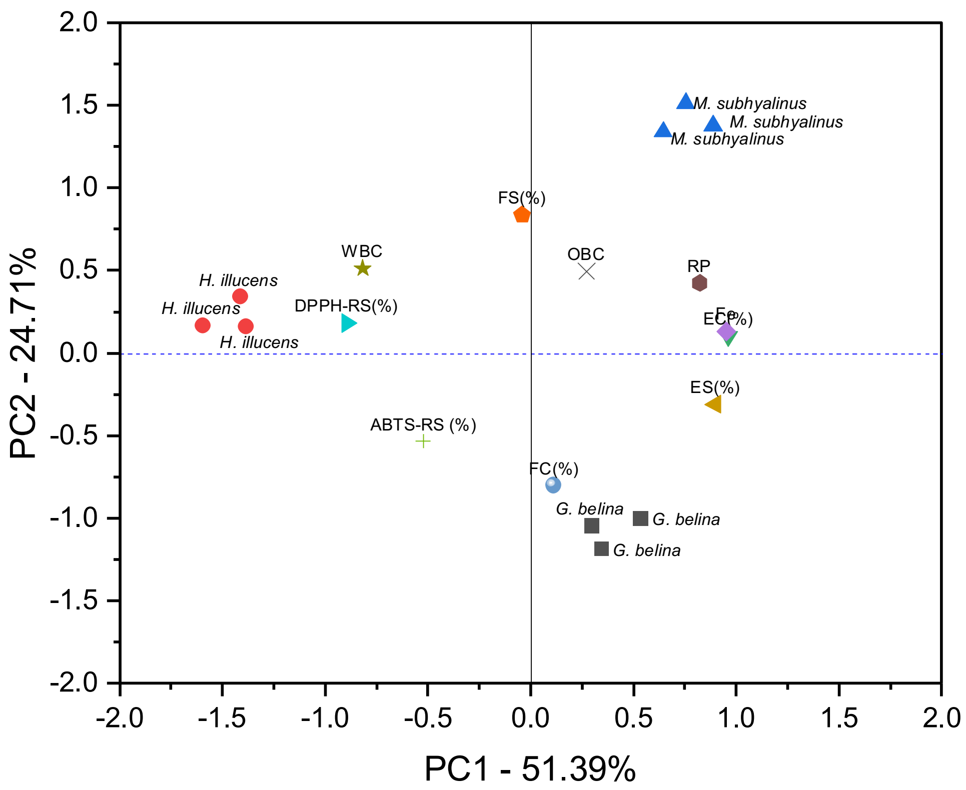

Figure 3.5 Principal components analysis plot for techno-functional properties and antioxidant indices of edible insect flours

3.10 Conclusion

This study was undertaken to establish the potential for edible insect flours as a source of nutrients, as well as their techno-functional and antioxidants properties. The studied edible insect flour species were rich in protein and fat, which are essential nutrients required for the human diet. The results obtained for the physicochemical properties make the flours valuable to the food industry as potential fortifiers, such as *G. belina*, which was yellower and redder in colour since this characteristic is of importance in instances where a noticeable colour change to the product is not desired. *M. subhyalinus* exhibited good water binding capacity, and the flour was generally found to have superior techno-functional properties among the studied species. This makes it useful for producing foods such as sausages and bakery products. The studied edible insects have unique techno-functional properties that can be exploited to provide functional ingredients. Future studies on the shelf life, and rheological and structural properties of the edible insect flours are essential prior to incorporation in food product formulations.

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CHAPTER4: EFFECT OF PROXIMATE COMPOSITION, THE PHYSICOCHEMICAL AND SENSORIAL PROFILE OF BISCUIT ENRICHED WITH EDIBLE INSECT (*GONIMBRASIA BELINA*, *HERMETIA ILLUCENS* AND *MACROTERMES SUBHYALINUS*) FLOURS

Abstract

The growing demand for proteins has prompted the food industry to search for alternatives to conventional protein sources and incorporate them into high-value-added products. This study aimed to develop novel biscuits by supplementing insect flour *G. belina* (*Mashonzha*), *H. illucens* (Black soldier fly larvae) and *M. subhyalinus* (*Madzhulu*) into whole wheat flour at different proportions 0, 5, 10, 15, and 20%, respectively. The insect-wheat composite flours and biscuits' nutritional, physicochemical, and sensorial properties were established. No significant differences were observed in the moisture of the Mashonzha and Madzhulu composite flours ($p > 0.05$), while BSFL-20 composite flour had the lowest moisture. The protein content of the composite flour was higher with the increasing insect flour substitution for all edible insects and ranged from 17.03 – 21.52%. In contrast, the fat of the composite flour samples decreased with increasing flour concentration. A significant difference ($p < 0.05$) was observed in the colour difference (ΔE) of the wheat flour in comparison to the edible insect composite flours. The protein content of biscuits enriched with *G. belina* and *M. subhyalinus* flours increased significantly ($p < 0.05$) compared to the 100% wheat flour biscuits. The fat content of the biscuit samples was higher for MW-20 and BSFL-20. However, the MZ-5 biscuit had the highest fat and ranged from 15.89 to 18.74%. The L^* and b^* coordinates were significantly lower as the concentration increased, indicating the biscuits' darkening. The increase in insect flour concentration did not influence the texture significantly ($p > 0.05$). The spread ratio for the samples MW-10 and BSFL-10 was not significantly higher than the other biscuits. The sensory evaluation confirmed that the edible insect flours could be successfully included in baked goods to provide excellent sensory properties and increased acceptance.

Industrial relevance: Given the increasing world population and the limited water and land resources, finding sustainable ways to produce food is of the utmost importance. They are particularly interesting considering edible insects' high protein content and low environmental impact. The present study demonstrated that edible insect flours could be successfully used to enhance the protein content of baked goods. In addition, the current research demonstrates how edible insects can provide a novel source of innovative ingredients for biscuits in response to consumers' growing awareness of inventive baked goods.

Keywords: Novel biscuits, composite flour, physicochemical properties, sensory evaluation, black soldier fly, *Mashonzha*, *Madzhulu*.

4.1 Introduction

In recent years, promoting non-animal-based protein sources has introduced enthusiasm for plant- and insect-based protein sources in the food product development sphere (Zielińska *et al.*, 2018). Due to their high nutritional value, edible insects have attracted the attention of researchers and the food industry for their potential use in the enhanced food nutrition (Belluco *et al.*, 2013; Ferri *et al.*, 2019). In many parts of the world, insects are a traditional food source and are relished by consumers, while in other countries, they are used as a delicacy. They are particularly rich in protein, calcium, iron, and zinc compared to frequently consumed animals such as chicken, beef, and pigs (Ghosh *et al.*, 2016, 2017).

H. illucens (black soldier fly), *G. belina* (*Mashonzha*), and *M. subhyalinus* (*Madzhulu*) are among edible insect species gaining traction as protein alternatives; the latter two are indigenous to parts of South Africa, where they are vital to food security, rural livelihoods, and poverty reduction (Vanqa *et al.*, 2022). At the same time, *H. illucens* is commercially produced by Agriprotein in South Africa and has been recently proposed by the European Food Safety Authority (EFSA) as an insect species with the greatest potential to be used in food and feed (EFSA Scientific Committee, 2015; Osimani *et al.*, 2018; Vanqa *et al.*, 2022).

Despite the benefits of insect nutrition, consumer perception is a barrier to the wider adoption of insect-derived foods (Zielińska & Pankiewicz, 2020). Several negative associations are associated with insects in Western countries, such as dirtiness, poverty, and disease (Looy *et al.*, 2014; Dobermann *et al.*, 2017; Gere *et al.*, 2017). However, a report by González et al. (2019) demonstrated that some consumers are willing to eat them in a less visible form in modified products indistinguishable from familiar ones. Sensory aspects of food are significant for consumer acceptance; therefore, developing insect-based foods with minimal negative sensory characteristics would be a good idea (Mishyna *et al.*, 2020).

For example, if incorporated in bakery goods such as biscuits, cakes, pastries, cookies, and crackers, edible insects are more likely to be accepted by consumers. They can be used as a vehicle for food fortification. Biscuit is one of the confectionery products representing one of the most significant kinds of snack foods worldwide which individuals widely consume in all age groups (Ogunlakin *et al.*, 2018). They can also serve as a source of essential nutrients (Awobusuyi *et al.*, 2020). Currently, many commercial food products are enriched with protein derived from other cereal grains such as rye, oats, or legumes (Al-Attabi *et al.*, 2017). Likewise, insects are richer in protein than beans (23.5%), lentils (26.7%), or soybean (41.1%)

(Zielińska *et al.*, 2015). In lieu of the aforementioned statement, using insect flour in biscuits may be a way to fight malnutrition in underdeveloped countries, as long as they like the taste.

Despite the mentioned advantages, only a few applications of insect flours in foods have been reported up to now. A very recent proposal has been the inclusion of insect flours from mealworm larvae and silkworm pupae to replace 10% lean pork in emulsion sausages, which increased the cooking yield and hardness of emulsion sausages, confirming the possible application of those insect flours as a novel protein ingredient (Kim *et al.*, 2016). In addition, González *et al.*(2019) characterized the dough behaviour, technological properties, and chemical composition of bread prepared with flour from three different insects (*H.illucens*, *Tenebrio molitor* &*Acheta domestica*). It was concluded that when the bread was prepared using *A. domestica*, it had technological features similar to wheat bread but nutritionally improved in terms of protein and fibre content.

Hlongwane et al. (2021) has written widely on entomophagy among several South African cultures. However, there is a paucity of information with regard to enrichment or fortification of biscuits with edible insects. Although the practice is more prevalent among rural populations, prejudice against entomophagy still exists within some population groups. Incorporating insects into popular value-added baked products, such as biscuits, could promote their utilization as a nutritious food source.

Therefore, the aim of this study was to examine the effects of incorporating three different insect flours into biscuits (*Madzhulu, Mashonzha* & black soldier fly) at varying concentrations on their composition, physicochemical properties, and sensory acceptability.

4.2 Materials and methods

4.2.1 Preparation of composite flours and biscuit

Edible insect flours were prepared following the method described by Vanqa *et al.* 2022. Edible insect flours (*G. belina*, *H. illucens* & *M. subhyalinus*) were each mixed with wheat flour in five (5) formulations with varying insect flour concentrations (0%, 5%, 10%, 15% and 20%,) respectively, to prepare composite flours. The novel edible insect biscuits were prepared according to the product formulation in Table 4.1. To prepare the insect biscuits, all ingredients were mixed and kneaded by at maximum speed in an electric mixer (Kenwood, model KM240 series, United Kingdom) for 10 min. Once the dough had settled for 20 minutes, it was stretched to a height of 5 mm with the help of a rolling pin with rings on both sides with 30 mm diameter. The biscuits were baked in a multi-deck oven (Macadams, South Africa, Johannesburg, South Africa) set at 180°C for 20 min. After baking, the biscuits were cooled for 30 min immediately and packaged in Low-density polyethylene Ziplockags (40 μm) of size 250mm x360mm/100's. The samples were sealed and kept at room temperature (21$°C$) until they were used for chemical analysis and sensory evaluation. Biscuits produced following the same procedure with wheat flour (100%) were used as controls.

Table 4.1 Biscuit formulation for different edible insect flours.

Samples	Wheat flour (g)	Edible insect flour (g)	Sugar (g)	Butter (g)	Egg (g)
WF	100	0	130.0	240.0	94.0
MW-5	95	5	130.0	240.0	94.0
MW-10	90	10	130.0	240.0	94.0
MW-15	85	15	130.0	240.0	94.0
MW-20	80	20	130.0	240.0	94.0
BSFL-5	95	5	130.0	240.0	94.0
BSFL-10	90	10	130.0	240.0	94.0
BSFL-15	85	15	130.0	240.0	94.0
BSFL-20	80	20	130.0	240.0	94.0
MZ-5	95	5	130.0	240.0	94.0
MZ-10	90	10	130.0	240.0	94.0
MZ-15	85	15	130.0	240.0	94.0
MZ-20	80	20	130.0	240.0	94.0

WF = 100% wheat flour (Control), MW =*G. belina* flour, BSFL = *H. illucens*, MZ*: M. subhyalinus*

4.3 Chemical composition analysis

The chemical compositions, i.e. moisture (925.10), crude fat (932.06), and ash content (923.03) of fresh biscuits (as prepared on the day of baking) were determined according to the methods of the Association of Official Analytical Chemists International (AOAC, 2015): the total protein content was determined by the Kjeldahl procedure (920.87), with nitrogen to a protein conversion factor of 5.60 as suggested by (Janssen *et al.*, 2017); dietary fibre (soluble, insoluble & total dietary fibre) by the enzymatic gravimetric method (991.43). Moisture (%) was calculated by drying the sample in a vacuum oven at 105°C for three hours. The dried sample was placed into a desiccator, cooled, and re-weighed. Crude fat was calculated by drying fats after extraction in a Soxhlet assembly using petroleum ether. The ash percentage was calculated by combusting the samples in a silica crucible placed in a muffle furnace at $550°C$. The total carbohydrate content was calculated by subtracting the sum of moisture, protein, fat, and ash percentages from 100%. The energy content of the biscuits was calculated according to the method of Farzana & Mohajan (2015) using equation 4.1:

Energy $(\frac{Kcal}{100g})$ = 4 (% Carbohydrate + % Protein) + (9 × %fat) *Equation 4.1*

4.4 Physicochemical analysis

4.5 Determination of colour attributes

The colour attributes of the biscuit was determined according to the method (Krystyjan *et al.*, 2015). The measurement of the upper surface colour was carried out with the use of Konica MINOLTA CM-3500d equipment (Konica Minolta Inc., Tokyo, Japan), with reference to illuminant D65 and a visual angle of 10. The results were expressed using the CIELab system. The following parameters were determined: L* (L*= 0 black, L* = 100 white), a* - share of the green colour (a* < 0) or red (a* > 0), b*- share of blue (b* < 0) or yellow (b* > 0). The change in colour (ΔE) was calculated, whereas the indices 0 and s indicate measured values of wheat flour, wheat-insect flour composites and biscuit samples, respectively the measurements were carried out on the day of baking.

ΔE= $\sqrt{(L_0 - L_s)^2 + (a_0 - a_s)^2 + (b_0 - b_s)^2}$ *Equation 4.2*

4.6 Determination of water activity

The water activity (Aw) of edible insect flours was measured using the method described by (Vanqa *et al.*, 2022) with minor modifications. Salt humidity standards of 53, 75 and 90% relative humidity were used to calibrate the measurement cell. A sample (5 g) of the insect flour was transferred into a sample dish and placed inside the (AW SPRINT TH500, Novasina analyser, Zurich, Switzerland), and the cell measuring protection filter was immediately closed. The reading was observed after a period of 60 to 80 s.

4.7 Determination of textural properties

The textural properties of the biscuit were determined by the method described by (Suriya *et al.* (2017). The hardness of baked biscuits was measured in terms of breaking strength using a Texture Analyzer (Instron 3340 Series Single Column Table Frames, Grove City, United States of America). The analyser was set to return to the start cycle at a speed of 1.5 mm/s and 15 mm. This experiment simulates the hardness assessment by a consumer holding the biscuit in their hands and breaking by bending. The peak force from the subsequent curve was measured as the breaking strength of the biscuit.

4.8 Determination of physical properties

The physical properties of control and biscuits enriched with edible insect flours were analysed according to the method described by Suriya *et al.* The weight of the biscuit was determined using the analytical balance (ELB3000, Shimadzu, Japan) and the biscuit dimensions for the thickness (the distance between top and bottom surface of biscuits) and width (distance across the biscuit) were determined using Vernier calliper. The spread ratio of the baked cookies was determined by the ratio of width and thickness of the biscuits.

4.9 Microbiological analysis

Microbiological analysis was done using standard ISO food microbiology methods. To obtain the necessary amount of sample, individuals of a single category and species had to be pooled. Samples were homogenized and analysed for Salmonellae (ISO 6579) and *Listeria monocytogenes* (ISO 11290; both parameters in 25 g). Several bacterial counts were also determined, i.e., *Escherichia coli* (ISO 10167), as well as yeasts and moulds count (ISO 21527-1, ISO 21527-2). In addition, homogenized samples were also spread onto blood agar plates for species identification. Once colonies appeared, these were further analysed using common biochemical methods. Coliforms (*E. coli*) were diagnosed via lactose fermentation and gas formation.

4.10 Sensory analysis

An acceptability study was conducted among fifty (50) untrained panellists from the Department of Food Science and Technology, Cape Peninsula University of Technology. Evaluations were made in a sensory room consisting of fourteen (14) individually separated booths under ambient temperature (28 – 30$°C$) and white light. Polystyrene-sealed samples coded with three-digit numbers were served randomly per edible insect sample, each at an interval of 30 min. Panellists were instructed to rinse their mouths between samples. The evaluation of the biscuits among the panellist was based on the following parameters: appearance, colour, aroma, taste, texture, and overall liking on a 5-point hedonic scale, with one (1) representing the least score (dislike very much) and five (5) the highest score (like very much). The study was approved by the faculty of the applied sciences ethics committee, and the participants gave consent to participate in the sensory evaluation of the novel insect biscuits.

4.11 Statistical analysis

Statistical analyses were conducted using the software SPSS 28.0 (2005) (SPSS Inc., Chicago, IL, USA). All measurements were made in triplicate for each sample unless stated otherwise. Results are expressed as the calculated means and standard deviations. Statistical analysis was performed by testing significant differences ($p < 0.05$) between treatments using multivariate analysis of variance (MANOVA), and Duncan's multiple range test was used to separate means where differences existed.

4.12 Results and discussion

4.12.1 Proximate composition *of G. belina*-wheat composite flours and biscuits

The proximate composition of *G. belina*-wheat (MW) composite flours and biscuits is shown in Table 4.2. It can be observed that carbohydrates and protein were the major components in the composite flours and biscuit samples. The moisture content of *G. belina*-wheat composite flours was not significantly different ($p > 0.05$) from one another and, in comparison, to the control sample. The protein content of the *G. belina*-composite flour sample increased with an increasing percentage of the *G. belina* flour substitution and ranged from 13.67 to 21.10%. No significant differences ($p < 0.05$) were observed in the energy values of the Mashonzha-composite flours compared to the control sample and ranged from 360.69 to 367.03 KJ and 368.19 KJ, respectively. The protein content of the biscuits enriched with *G. belina* was significantly different ($p < 0.05$) from the control, ranging from 15.04 to 19.21%.

Table 4.2 Nutritional composition of wheat- *G. belina* composite flour sample and biscuits enriched with *G. belina* flour.

Sample	Moisture(%)	Ash(%)	Protein (%)	Fat (%)	Carbohydrates(%)	Energy (KJ)
			***G-belina*-wheat composite flour**			
WF	7.71 ± 0.27^{a}	1.72 ± 0.53^{a}	16.64 ± 2.81^{bc}	1.18 ± 0.21^{a}	72.75 ± 3.42^{cd}	368.19 ± 3.61^{abc}
MW-5	7.79 ± 0.16^{a}	3.16 ± 0.32^{bc}	13.67 ± 0.08^{a}	0.90 ± 0.06^{a}	74.47 ± 0.16^{d}	360.69 ± 1.10^{a}
MW-10	8.73 ± 0.48^{a}	2.94 ± 0.22^{b}	15.69 ± 0.29^{ab}	2.01 ± 0.24^{b}	70.64 ± 0.58^{bc}	363.38 ± 2.28^{a}
MW-15	7.51 ± 0.12^{a}	3.63 ± 0.36^{c}	18.53 ± 0.31^{c}	2.32 ± 0.58^{b}	68.01 ± 0.73^{ab}	367.03 ± 4.43^{ab}
MW-20	6.92 ± 2.18^{a}	4.41 ± 0.15^{d}	21.10 ± 0.76^{d}	1.82 ± 0.14^{b}	65.75 ± 2.58^{a}	363.78 ± 8.72^{a}
			Biscuit enriched with *G. belina*			
WF	2.66 ± 1.03^{a}	7.21± 0.11^{b}	11.75 ± 2.17^{a}	12.86 ± 0.49^{ab}	65.52 ± 2.67^{d}	425.00 ± 4.00^{a}
MW-5	7.32 ± 2.39^{b}	8.78 ± 0.21^{c}	15.04 ± 2.45^{ab}	14.99 ± 1.73^{b}	53.88 ± 2.92^{ab}	410.67 ± 17.01^{a}
MW-10	4.32 ± 3.33^{ab}	9.47 ± 0.10^{d}	15.13 ± 2.81^{ab}	13.77 ± 0.26^{ab}	57.31 ± 1.56^{bc}	413.67± 11.93^{a}
MW-15	3.30 ± 1.07^{a}	6.62 ± 0.07^{a}	17.71± 0.22^{bc}	10.97± 3.61^{a}	61.40 ± 3.86^{cd}	415.33 ± 19.09^{a}
MW-20	3.33 ± 1.42^{a}	9.38 ± 0.58^{d}	19.91± 2.71^{c}	15.89 ± 1.40^{b}	51.50 ± 3.32^{a}	428.67 ± 3.21^{a}

Means in columns followed by different letters are significantly different ($p < 0.05$). Proximate values are expressed in g/100 g WF: Wheat flour MW: *G. belina*

MW-5- 5g/100g *G. belina* containing flour mixture-based biscuit or composite flour
MW-10- 10g/100g *G. belina* containing flour mixture-based biscuit or composite flour
MW-15- 15g/100g *G. belina* containing flour mixture-based biscuit or composite flour
MW-20- 20g/100g *G. belina* containing flour mixture-based biscuit or composite flour

This suggests that the increased protein content of enriched biscuits is due to the higher protein content of *G. belina* flour. In a study by Vanqa *et al.* (2022), the protein composition of Mashonzha flour was reported to be 46.72%. The protein content in supplemented biscuits was significantly higher than that of the control (WF) ($p < 0.05$). Therefore, it has been found that all the biscuits formulated with *G. belina* insect flour at these concentrations reported in this study can be classified as high in protein according to the R146 regulation (Health, 1972) revised in 2010. The composition of these edible insects' biscuits differs significantly from the results reported by Zielińska & Pankiewicz (2020) due to the different insect species used and the fact that the biscuits were prepared with composition flours ratios. In addition, to the best of our knowledge, this is the first study to report on adding *G. belina* flour at various concentrations to enhance the nutritional properties of biscuits.

The moisture content of the *G. belina*-composite flour samples and of the biscuit were significantly not different in comparison to the wheat flour and ranged from 6.92-8.73% and 2.66- 7.32, respectively. The ash content measures the total amount of minerals present within a food. It is the residue remaining after destroying all the combustible organic matter. The addition of the *G. belina* flour increased the ash content significantly ($p < 0.05$), and the composite flours ranged from 2.94 to 4.41, with MW-20 having the highest ash content. The biscuits showed a significantly ($p < 0.05$) higher ash composition compared to the control. As noted by Vanqa *et al*. (2022), the considerable good ash content of the edible insect flours signifies good mineral composition and thus enriches the biscuits with edible insect flours that bestow or impart nutritional benefits.

4.12.2 Proximate Composition of *H. illucens*- wheat composite flours and biscuits

The proximate composition of biscuits produced with Wheat-*H. illucens* flours is reported in Table 4.3. A linear correlation was observed in the fat and the amount of added of *H. illucens*, with the highest and intermediate value detected in biscuits produced with 20% insect flour, respectively, thus encouraging the use of *H. illucens* for the production of enriched leavened baked goods. The WF (control) differed significantly ($p < 0.05$) in terms of moisture in comparison to composite flour samples which contained *H. illucens*; the moisture of the WF was 7.71%, while that of the Wheat-*H. illucens* composite flours ranged from 6.74% to 7.54%. The energy content of Wheat-*H. illucens* composite flour substitution ranged from 363.35 to 394.96KJ. Energy density was positively related to palatability. If the energy density was high, the palatability was also high. Palatability is related to good taste, increasing appetite and food consumption. As a rule, energy-dense foods are palatable but not satiating, whereas foods with low energy density are more satiating but less palatable. Low-energy-density foods typically contain the most water and the least fat (Dewi *et al.*, 2020).

The moisture content of the *H. illucens* enriched biscuits ranged from 3.68 – 5.37%, significantly higher ($p < 0.05$) than that of the control biscuits (2.66%). This trend can be acceptable as the moisture levels in freshly baked cookies are usually less than 5% (Suriya *et al.*, 2017). The results obtained in this study agree with those reported by González *et al*. (2019) on bread enriched with *H. illucens,* which had moisture levels of 2.03. No significant difference ($p > 0.05$) was observed between the control sample and the moisture of the biscuits enriched with edible insect flour. However, BSFL-15 biscuit had a slightly higher moisture content Table 4.3. This was attributed to the low water-binding capacity of the *H. illucens* protein (Vanqa *et al.*, 2022). However, most non-wheat proteins have been observed to increase the water absorption of dough due to their high water-binding capacity (Al-Attabi *et al.*, 2017). In contrast, González *et al*. (2019) reputed that substituting wheat flour with cricket powder resulted in a decreased water absorption of dough. Perhaps the hydrophilic groups of the *H. illucens* protein are less engaging, resulting in reduced water absorption. The assessment of the nutrient content of both products clearly showed that the substitution of wheat flour with black soldier fly flour substantially increased the nutritional content.

Table 4.3 Nutritional composition of wheat- *H. illucens* composite flour sample and biscuits enriched with *H. illucens* flour.

Sample	**Moisture(%)**	**Ash(%)**	**Protein (%)**	**Fat (%)**	**Carbohydrates(%)**	**Energy (%)**
			Wheat- *H. illucens* composite flours			
WF	7.71 ± 0.27^{c}	1.72 ± 0.53^{a}	16.64 ± 2.81^{a}	1.18 ± 0.21^{a}	72.75 ± 3.42^{b}	368.19 ± 3.61^{abc}
BSFL-5	7.54 ± 0.37^{bc}	3.81 ± 0.08^{ab}	14.14 ± 1.78^{a}	1.75 ± 0.84^{a}	72.75 ± 1.22^{b}	363.35 ± 3.41^{a}
BSFL-10	7.20 ± 0.19^{abc}	3.08 ± 0.27^{ab}	15.40 ± 1.62^{a}	4.30 ± 0.71^{b}	70.02 ± 1.10^{b}	380.40 ± 3.55^{c}
BSFL-15	6.91 ± 0.57^{ab}	6.22 ± 3.74^{b}	15.27 ± 1.15^{a}	5.96 ± 0.44^{c}	65.64 ± 4.97^{a}	377.27 ± 16.00^{bc}
BSFL-20	6.74 ± 0.10^{a}	3.40 ± 0.14^{ab}	17.03 ± 0.59^{a}	7.11 ± 0.42^{d}	65.72 ± 0.79^{a}	394.96 ± 2.97^{d}
			Biscuit enriched with *H. illucens*			
WF	2.66 ± 1.03^{a}	7.21 ± 0.11^{b}	11.75 ± 2.17^{a}	12.86 ± 0.49^{a}	65.52 ± 2.67^{a}	425.00 ± 4.00^{a}
BSFL-5	3.68 ± 2.55^{a}	3.61 ± 0.25^{a}	15.26 ± 0.65^{a}	15.85 ± 0.51^{b}	61.60 ± 2.80^{a}	450.00 ± 11.35^{bc}
BSFL-10	5.00 ± 2.42^{a}	6.99 ± 0.16^{b}	14.45 ± 2.52^{a}	16.01 ± 0.43^{b}	57.56 ± 4.57^{a}	432.00 ± 10.53^{abc}
BSFL-15	5.37 ± 2.95^{a}	9.82 ± 0.26^{c}	14.10 ± 1.44^{a}	16.61 ± 0.64^{b}	54.10 ± 3.03^{a}	422.33 ± 10.02^{ab}
BSFL-20	3.94 ± 2.12^{a}	10.66 ± 0.37^{d}	12.13 ± 9.25^{a}	18.74 ± 0.29^{c}	54.52 ± 11.04^{a}	435.67 ± 9.24^{abc}

Means in columns followed by different letters are significantly different ($p < 0.05$). Proximate values are expressed in g/100 g WF: Wheat flour BSFL: *H. illucens*

BSFL-5- 5g/100g *H. illucens* containing flour mixture-based biscuit or composite flour
BSFL-10- 10g/100g *H. illucens* containing flour mixture-based biscuit or composite flour
BSFL- 15g/100g *H. illucens* containing flour mixture-based biscuit or composite flour
BSFL- 20g/100g *H. illucens* containing flour mixture-based biscuit or composite flour

4.12.3 Proximate composition of *M. subhyalinus*-wheat composite flours and biscuits

The approximate compositions of Wheat- *M. subhyalinus* composite flours (MZ) and biscuits are reported in Table 4.3. the moisture content of the composite flours ranged from 6.83-7.76%, and no significant differences ($p > 0.05$) were observed compared to the control sample. The protein content increased with increasing concentration of the insect flour, with MZ-20 obtaining the highest protein content at 21.52 ± 0.22 and differed significantly ($p < 0.05$) from the wheat flour (control) at 16.64 ± 2.81. The protein content of *M. subhyalinus* was determined to be 52.74% (Vanqa *et al.*, 2022). With the addition of *M. subhyalinus* flour, the nutritional value of the biscuits was altered. The protein content of the biscuits with the addition of *M. subhyalinus* flour ranged from 17.38% to 20.63%, while that of the control sample was 11.75%. This effect was expected because *M. subhyalinus* flour contains the highest protein among all ingredients in the recipe; hence its content causes a proportional increase in protein content in the end product. Similar results were observed by Biró et al. (2020). The protein cricket-enriched biscuits when compared with the control sample without the addition of edible insects. A similar pattern was observed in baby biscuits containing wood grasshopper flour (Dewi *et al.*, 2020). In turn, the fat content of the biscuits was higher and ranged from 15.04% to 16.60% as compared to the composite flour, 0.94 % to 2.21%. This may be due to the butter added to the formulation at a similar level for MZ (5, 10 and 15%) in all biscuits ($p > 0.05$). The carbohydrate content of the biscuits decreased with increasing concentration. Although the nutritional compositions of a product are essential, moisture content impacts preservation, packaging, and transport convenience significantly (Kaur *et al.*, 2017). No significant difference was observed ($p > 0.05$) in the moisture content of the biscuits. The results obtained in this study are in line this those reported by Koffi *et al.* (2013) for *M. subhyalinus* enriched biscuit, which contained 21.66 ± 0.40 protein for the highest concentration (25%).

Table 4.4 Nutritional composition of wheat- *M. subhyalinus* composite flour sample and biscuits enriched with *M. subhyalinus* flour.

Sample	Moisture (%)	Ash(%)	Protein (%)	Fat (%)	Carbohydrates (%)	Energy (%)
			Wheat-*M. subhyalinus* composite flours			
WF	7.71 ± 0.27^{a}	1.72 ± 0.53^{a}	16.64 ± 2.81^{ab}	1.18 ± 0.21^{ab}	72.75 ± 3.42^{b}	368.19 ± 3.61^{a}
MZ-5	6.83 ± 0.35^{a}	2.61 ± 0.30^{ab}	15.03 ± 0.26^{a}	0.94 ± 0.03^{a}	74.59 ± 0.32^{b}	366.91 ± 2.22^{a}
MZ-10	7.74 ± 0.41^{a}	2.58 ± 0.30^{ab}	16.94 ± 1.10^{ab}	1.73 ± 0.62^{bc}	71.01 ± 1.12^{b}	367.37 ± 5.24^{ab}
MZ-15	7.29 ± 0.70^{a}	5.59 ± 3.25^{b}	18.84 ± 0.28^{b}	2.21 ± 0.49^{c}	66.07 ± 4.21^{a}	359.53 ± 12.98^{a}
MZ-20	7.76 ± 1.07^{a}	2.80 ± 1.31^{ab}	21.52 ± 0.22^{c}	1.83 ± 0.15^{bc}	66.10 ± 1.56^{a}	366.92 ± 4.41^{ab}
			Biscuit enriched with *M. subhyalinus* flour			
WF	2.66 ± 0.22^{a}	7.21 ± 0.11^{b}	11.75 ± 2.17^{a}	12.86 ± 0.49^{a}	65.52 ± 2.67^{b}	425.00 ± 4.00^{a}
MZ-5	2.86 ± 0.25^{a}	3.19 ± 0.17^{a}	17.38 ± 0.99^{c}	16.60 ± 7.87^{a}	59.98 ± 8.95^{ab}	458.67 ± 38.08^{c}
MZ-10	6.13 ± 0.17^{b}	6.78 ± 0.23^{b}	14.63 ± 0.52^{b}	15.22 ± 0.49^{a}	57.23 ± 0.54^{a}	424.33 ± 4.16^{ab}
MZ-15	7.90 ± 0.21^{b}	2.84 ± 1.01^{a}	18.36 ± 1.52^{cd}	15.90 ± 0.80^{a}	54.99 ± 1.81^{a}	436.67 ± 6.51^{abc}
MZ-20	6.13 ± 0.23^{b}	3.25 ± 1.64^{a}	20.63 ± 0.73^{d}	15.04 ± 1.63^{a}	54.95 ± 1.47^{a}	437.33 ± 9.29^{abc}

Means in columns followed by different letters are significantly different ($p < 0.05$). Proximate values are expressed in g/100 g WF: Wheat flour MZ: *M. subhyalinus*

BSFL-5- 5g/100g *M. subhyalinus* containing flour mixture-based biscuit or composite flour
BSFL-10- 10g/100g *M. subhyalinus* containing flour mixture-based biscuit or composite flour
BSFL- 15g/100g *M. subhyalinus* containing flour mixture-based biscuit or composite flour
BSFL- 20g/100g *M. subhyalinus* containing flour mixture-based biscuit or composite flour

4.13 Physicochemical properties

4.13.1 Colour attributes

Colour attributes is an important quality characteristic of foods and can significantly influence sensory perception and consumer acceptability of any food sample. Table 4.5 shows the colour attributes of wheat-edible insect composite sample and biscuit enriched with edible insect flour. The lightness of the composite flours decreased significantly ($p < 0.05$) with an increase in edible insect flour addition, while the colour attributes of redness and yellowness did not exhibit any statistical differences ($p > 0.05$). Biscuits generally showed substantially lower L*, indicating that they were darker in colour than the corresponding composite flours. The lower L* values indicate that Maillard browning resulting from reactions between the amino group of proteins and the carbonyl group of sugars within the biscuits due to the heat applied during baking. Higher b* values were found in the control samples compared to the enriched biscuits, indicating the yellowness of the whole wheat biscuits; however, they did not differ significantly ($p > 0.05$). The red colour indicated by the positive a* value was statistically significant in all biscuits enriched with edible insects, respectively. Biscuits enriched with *H. illucens* insect flour exhibited the highest redness (BSFL-5 = 10.64 and BSFL-15 = 10.14). Such results were expected since edible insect flours are darker than the wheat flour used in these biscuits. Hence supplementation with edible insect flours will give products a darker Colour. Usually, the colour of a baked product is directly dependent on the colours of the raw materials used. A similar trend was observed in the composite flours and biscuits enriched with *H. illucens* and *M. subhyalinus* edible insects. Researchers observed similar Colour-darkening results for muffins enriched with mealworm and cricket powder (Pauter *et al.*, 2018), cookies enriched with mealworm powder (Min *et al.*, 2016), bread supplemented with insect flour and pasta enriched with cricket powder (da Rosa Machado & Thys, 2019; Çabuk & Yılmaz, 2020). The highest colour difference in the biscuit samples was seen in samples MW20, BSFL20 and MZ20, which contained the most significant amount of edible insect flour, which was not statically different ($p > 0.05$) from the other concentrations. There was a significant difference ($p < 0.05$) in the colour difference as the increase in concentration increased. Similarly, results were observed by Zielińska & Pankiewicz (2020) on shortcake biscuits enriched *with Tenebrio molitor* Flour. Pauter *et al.* (2018) suggested that consumers tend to see darker bakery products as healthier and containing more fibre or whole grains. Therefore, this colour change may increase consumer interest in this biscuit type.

Table 4.5 The colour measurement parameters of composite flour and biscuit enriched with edible insect flour samples.

Sample	Composite flours				Insect flour-enriched biscuits			
	L*	a*	b*	ΔE	L*	a*	b*	ΔE
				Biscuit enriched with *G. belina*				
WF	93.03 ± 0.15^{e}	1.15 ± 0.43^{a}	9.58 ± 0.58^{a}	0.00 ± 0.00^{a}	79.70± 0.22^{e}	5.97± 0.55^{a}	31.27± 1.09^{b}	0.00 ± 0.00^{a}
MW-5	90.80 ± 0.19^{d}	1.09 ± 0.42^{a}	9.54 ± 0.67^{a}	2.30 ± 0.20^{b}	69.86± 0.20^{d}	6.83± 0.35^{ab}	31.02± 0.75^{b}	9.90 ± 0.21^{b}
MW-10	87.50 ± 0.14^{c}	1.48 ± 0.32^{a}	9.46 ± 0.12^{a}	5.60 ± 0.20^{e}	65.59± 0.26^{c}	7.43± 0.69^{b}	29.19± 0.82^{ab}	14.36 ± 0.31^{b}
MW-15	86.29 ± 0.05^{b}	1.41 ± 0.29^{a}	9.74 ± 0.24^{a}	6.8 0 ± 0.10^{f}	59.68± 0.16^{a}	7.56± 0.64^{b}	29.81± 2.01^{ab}	20.21 ± 0.17^{b}
MW-20	84.61 ± 0.08^{a}	1.46 ± 0.25^{a}	10.09 ± 0.45^{a}	8.50 ± 0.10 ^{gh}	60.50± 0.18^{b}	6.98± 0.89^{ab}	27.79± 1.20^{a}	19.57 ± 0.23^{b}
				Biscuit enriched with *H. illucens*				
BSFL-5	90.06 ± 0.15^{d}	1.64 ± 0.21^{a}	8.13 ± 0.57^{a}	3.40 ± 0.40^{c}	62.45± 0.35^{a}	10.64± 0.29^{b}	31.31± 0.54^{b}	17.73 ± 0.63^{b}
BSFL-10	87.17 ± 0.15^{c}	0.89 ± 0.72^{a}	8.45 ± 0.43^{b}	6.00 ± 0.20^{e}	62.71± 0.03^{a}	10.14± 1.42^{b}	30.35± 1.75^{ab}	17.61 ± 0.33^{b}
BSFL-15	84.91 ±0.13^{b}	0.96 ± 0.13^{a}	8.34 ± 1.17^{b}	8.30 ± 0.20^{g}	64.84± 1.64^{b}	6.44± 0.78^{a}	29.33± 1.21^{ab}	15.05 ± 1.52^{b}
BSFL-20	81.87 ± 1.17^{a}	1.47 ± 0.42^{a}	8.19 ± 0.56^{a}	11.30 ± 1.10^{i}	62.75± 0.23^{a}	6.01± 0.38^{a}	28.35± 1.07^{a}	17.22 ± 0.11^{b}
				Biscuit enriched with *M. subhyalinus*				
MZ-5	89.23± 0.08^{d}	1.68 ± 0.08^{ab}	8.71 ± 0.66^{ab}	4.00 ± 0.10^{d}	59.41 ± 0.25^{c}	8.54 ± 0.72^{c}	30.16 ± 0.54	20.49 ± 0.32^{b}
MZ-10	86.22 ±0.10^{c}	1.81± 0.30^{b}	8.47 ± 0.45^{a}	6.90 ± 0.20^{f}	67.97 ± 0.17^{d}	7.46 ± 0.38^{b}	29.52 ± 1.51^{ab}	12.01 ± 0.38^{b}
MZ-15	84.17 ± 0.08^{b}	1.92 ± 0.33^{b}	9.00 ± 0.47^{ab}	8.90 ± 0.10^{h}	54.14 ± 0.21^{b}	9.17 ± 0.37^{c}	27.68 ± 0.21^{b}	37.94 ± 20.47^{c}
MZ-20	81.53 ± 0.17^{a}	1.98 ± 0.42^{b}	9.49 ± 0.41^{b}	11.50 ± 0.20^{i}	45.74 ± 0.23^{a}	8.65 ± 0.54^{c}	24.31 ± 1.50^{a}	34.79 ± 0.10^{c}

Values are mean □standard deviation. Means within a column followed by the same superscript are not significantly ($p > 0.05$) different. L*= Lightness, a*=Redness, b*=Yellowness

Figure 4.1 Biscuit obtained by replacing (5,10,15 and 20%) wheat flour with different insect flours **(A)** *G. belina*, **(B)** *M. subhyalinus*, **(C)** *H. illucens*

4.13.2 Water activity

The water activity of Mashonzha-composite flour and biscuit is shown in Figure 4.2. A significant difference ($p < 0.05$) was seen in the control sample and the composite flours; however, there were no significant differences ($p > 0.05$) observed between MW-5 and MW-10, similarly for MW-15 and MW-20 with MW-20 composite flour obtaining the highest Aw (0.56). The A_w for the biscuit samples ranged from 0.68 to 0.70 a significant difference ($p < 0.05$) was observed for MW-5 while MW-10 obtained the highest Aw. Low moisture content and water activity is essential for the storage of flour to prevent the growth of microorganisms, fermentation and caking (Eriksson, 2014). Moisture uptake during storage may increase water activity and lead to changes in certain chemical and organoleptic properties. The results of this study contribute to the body of knowledge on the effect of insect flours on the water activity of composite flours and enriched biscuits.

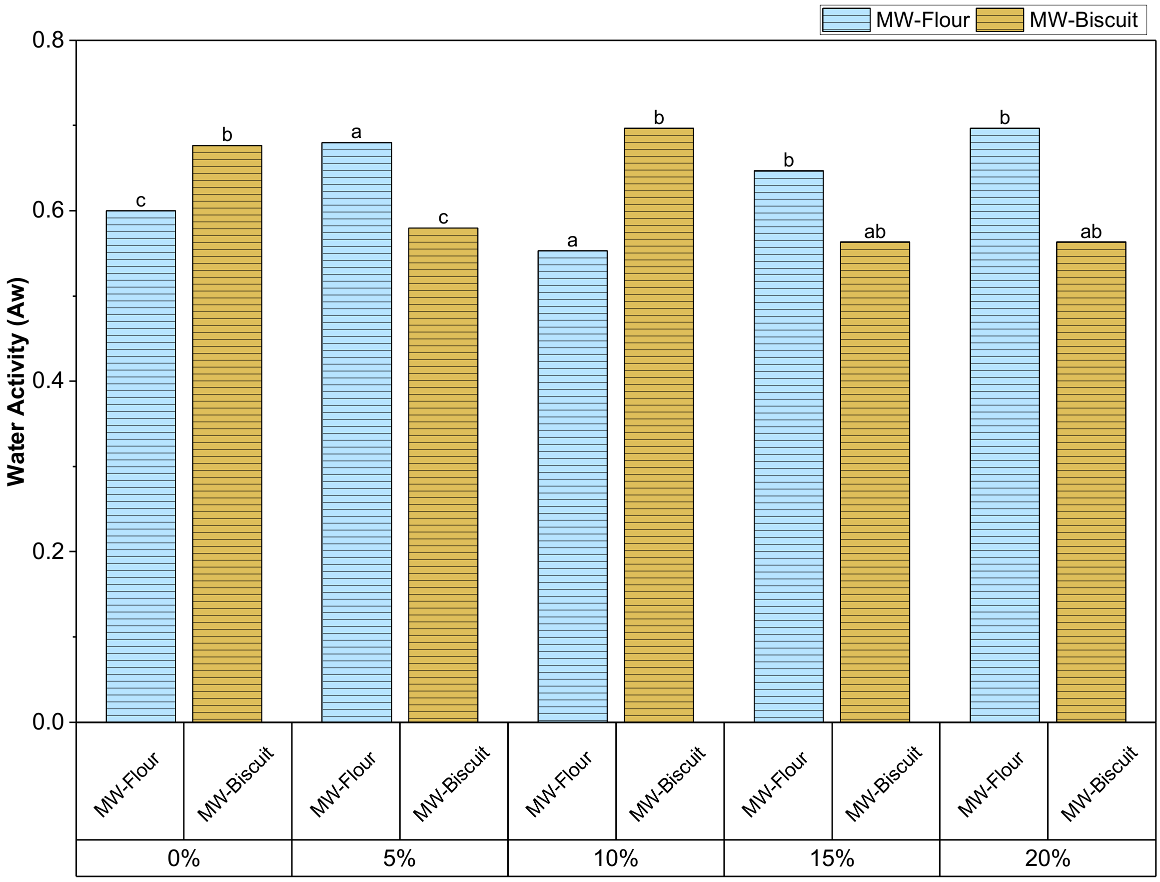

Figure 4.2 Water activity of Wheat- *M. subhyalinus* composite flours and biscuits

The data on the water activity of composite flours and biscuit samples of Black soldier fly is shown in Figure 4.3. With respect to A_w, results demonstrate a significant ($p < 0.05$) difference in the control compared to the BSFL-composite flours. No significant difference was observed in the water activity of the biscuit; however, the control sample differed significantly ($p < 0.05$). Compared to the composite flours, the biscuits water activity increase, and this increase in aw might have been due to the possible change in relative humidity of the environment. Water activity is one critical factor that causes deterioration, i.e., enzymatic changes, non-enzymatic browning, lipid deterioration, microbial profiling, and overall stability of foods(Temba *et al.*, 2017).

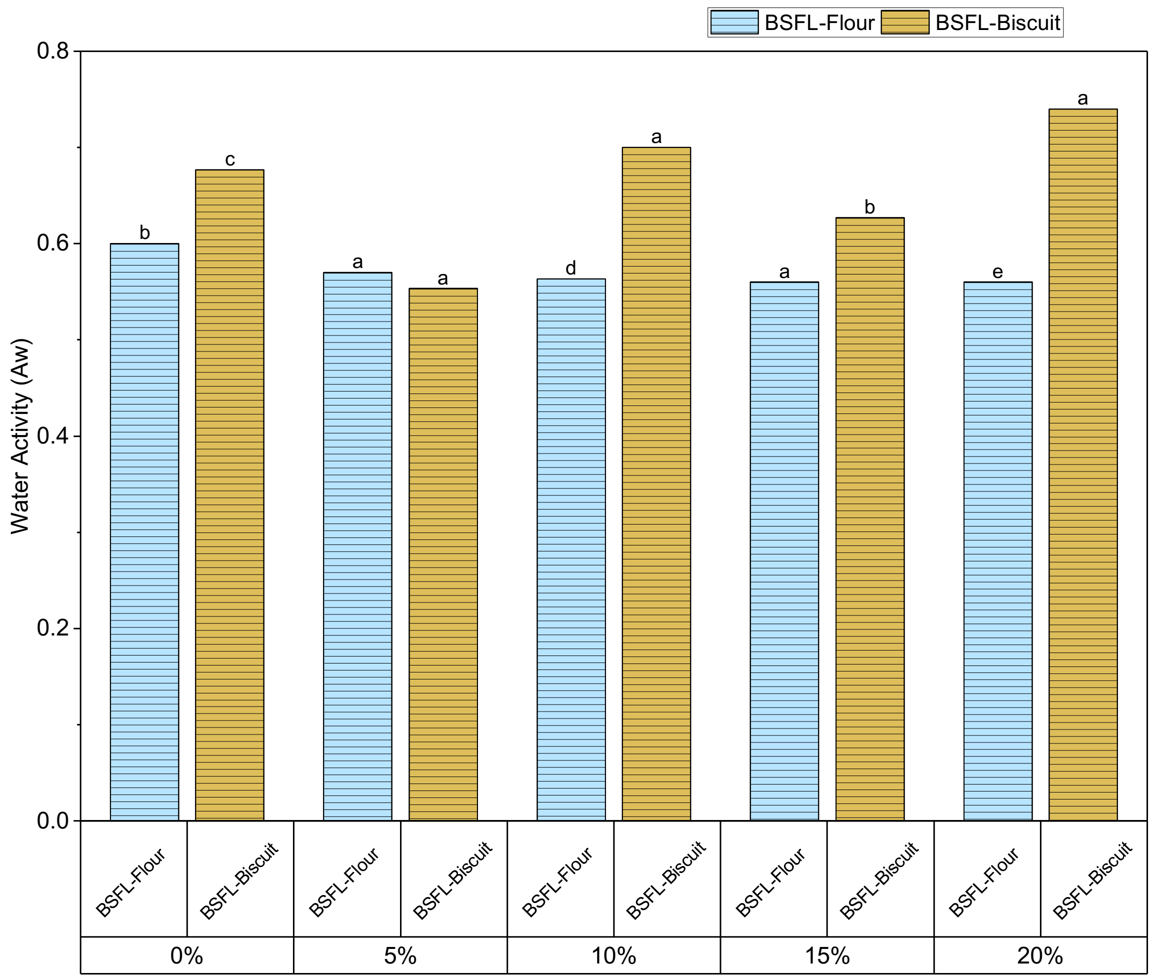

Figure 4.3 Water activity of wheat- *H. illucens* composite flours and biscuits.

The water activity of Wheat-*M. subhyalinus* composite flour and biscuit is depicted in Figure 4.4. A significant difference ($p < 0.05$) was observed in the water activity of the control sample and that of the Madzhulu-composite flour. The water activity of the biscuits ranged from 0.44 to 0.67 and decreased with increasing concentration of *M. subhyalinus* flour substitution and differed significantly ($p < 0.05$) in comparison to the control (0.68). It is evident that there was a decrease in water activity when the flours were subjected to baking which may be an advantage for the biscuits to be less suspectable to microbial growth.

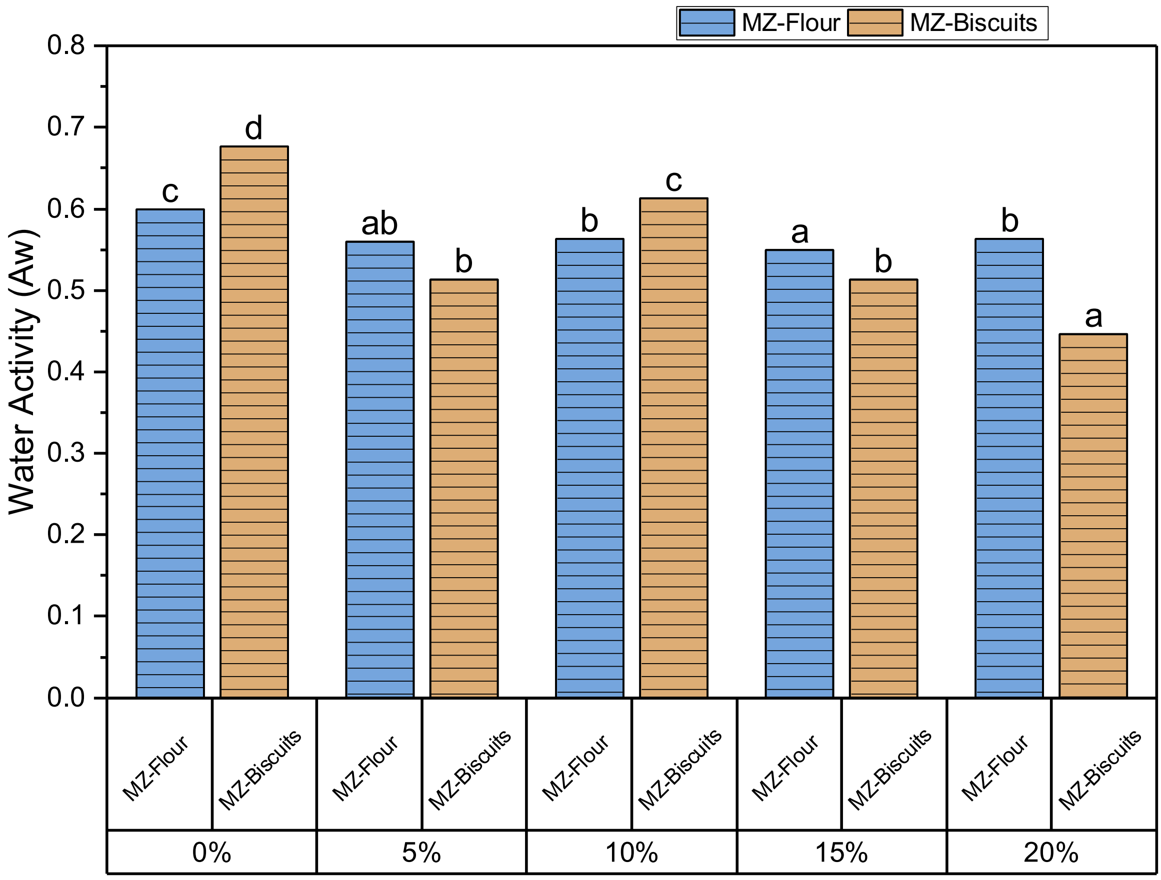

Figure 4.4 Water activity of wheat- *M. subhyalinus* composite flours and biscuits.

4.14 Physical characteristics

The geometrical properties, hardness of the biscuits, and biscuit weight are represented in Table 4.6. Hardness is one of the most important criteria for judging biscuit quality in terms of its textural characteristics (Jan *et al.*, 2016). Moreover, among the quality characteristics of bakery products, texture has the ability to determine the duration of storage It is the most important aspect of biscuit texture (Ahmadi *et al.*, 2022). According to the results obtained for hardness, the added amounts of insect flour do not significantly influence the hardness of the biscuit sample. The hardness (maximum force value of the resistance of the pressed probe) of the control sample (WF) was 38.48 ± 13N. In the case of the edible insect flours at various concentrations (5, 10,15 and 20%), the values ranged from 23.31 ±.79 to 50.02 ± 10.82 N, respectively. Similar results were observed by (Biró *et al.*, 2020). Similarly, González *et al.* (2019) reported that the textural hardness of bread products rose with the addition of bug powder, although rice-flour cakes tended to have a declining tendency Indriani *et al.*, (2020).

The biscuit enriched with *M. subhyalinus* flour exhibited a significant ($p < 0.05$) increase in diameter and thickness. While the opposite was observed for *H. illucens* and *M. subhyalinus* flours with no significant ($p > 0.05$) difference as compared with that of the WF biscuit. Similar results were observed for the enrichment of *Zophobas atratus* powder slightly decreased the diameter and thickness of cookies with no significant difference compared to the control sample (Sriprablom *et al.*, 2022). This behaviour might be due to the role of gluten in the structure of these products, something which is not present in the insect flours (Akande *et al.*, 2020). High spread ratio has been linked to high gluten strength of the wheat and/or high lipid distribution within the dough mix (Akande *et al.*, 2020). Therefore, as shown in Table 4.6 the speed ratio of the biscuit sample decrease with increasing edible insect flour concentration slight variation in the physical properties of the biscuit samples reflects the compositional differences among the enrichments.

Table 4.6 Physical qualities of biscuits enriched with (*G. belina*, *H. illucens* and *M. subhyalinus*) flours.

Sample	Hardness (N)	Weight (g)	Diameter (mm)	Thickness(mm)	Spread ratio
			Biscuit enriched with Mashonzha		
WF	38.48 ± 13.16[a]	14.26 ± 2.21[a]	38.37 ± 0.78[a]	3.58 ± 0.15[a]	10.73 ± 0.30[a]
MW-5	23.31 ± 2.79[a]	16.67 ± 0.73[a]	42.17 ± 1.00[b]	3.89 ± 0.39[ab]	10.90 ± 0.94[ab]
MW-10	28.18 ± 7.16[a]	15.01 ± 2.04[a]	42.57 ± 3.30[b]	3.47± 0.28[a]	12.33 ± 1.60[b]
MW-15	27.52 ± 5.81[a]	15.39 ± 1.58[a]	45.80 ± 0.87[b]	4.10 ± 0.10[b]	11.17 ± 0.35[ab]
MW-20	27.50 ± 5.55[a]	13.88 ± 0.90[a]	44.57 ± 2.31[b]	4.31 ± 0.25[b]	10.36 ± 0.90[ab]
			Biscuit enriched with black soldier fly larvae		
WF	38.48 ± 13.16[a]	14.26 ± 2.21[a]	38.37 ± 0.78[a]	3.58 ± 0.15[a]	10.73 ± 0.30[a]
BSF-L5	37.31 ± 3.40[b]	12.58 ± 0.37[a]	41.44 ± 1.07[b]	3.31 ± 0.12[a]	12.53 ± 0.52[b]
BSFL-10	24.83 ± 9.66[ab]	14.61 ± 0.75[ab]	47.10 ± 0.62[d]	3.47 ± 0.06[a]	13.59 ± 0.33[b]
BSFL-15	24.97 ± 2.36[ab]	15.22 ± 1.18[b]	45.43 ± 1.25[d]	3.40 ± 0.20[a]	13.40 ± 1.00[b]
BSFL-20	21.42 ± 1.84[a]	13.52 ± 0.35[ab]	43.31 ± 1.25[c]	3.42 ± 0.16[a]	12.69 ± 0.87[b]
			Biscuit enriched with Madzhulu		
WF	38.48 ± 13.16[a]	14.26 ± 2.21[a]	38.37 ± 0.78[a]	3.58 ± 0.15[a]	10.73 ± 0.30[a]
MZ-5	50.02 ± 10.82[a]	14.26 ± 2.21[ab]	46.40± 1.35[c]	3.64 ± 0.23[a]	12.78 ± 0.51[b]
MZ-10	34.81 ± 6.49[a]	14.71 ± 0.90[b]	45.10 ± 2.23[c]	3.64 ± 0.41[a]	12.48 ± 1.21[b]
MZ-15	45.21 ± 1.18[a]	11.59 ± 0.22[a]	42.74 ± 0.64[b]	3.61 ± 0.01[a]	11.85 ± 0.16[ab]
MZ-20	43.40 ± 6.55[a]	12.20 ± 1.15[ab]	47.30 ± 0.44[c]	4.35 ± 0.17[b]	10.89 ± 0.51[a]

Values are means standard deviation. Means within a column followed by the same superscript are not significantly ($p > 0.05$) different, MW: *G. belina* flour, BSFL: H*. illucens* flour, MZ: *M. subhyalinus* flour

4.15 Microbial analysis

Microbial safety is one of the major concerns in insects utilization in food products (Megido *et al.*, 2017). As reported, general insects can harbour *Salmonella spp.*, Campylobacter, and *Staphylococcus spp.* Which can fluke foodborne and waterborne pathogens and chemical hazards (Belluco *et al.*, 2013), thus raising concerns about the utilization of edible insects for food. The results of the microbial quality assessment of the samples showed they are safe and within standard microbial safety requirements depicted in Table 4.7. The samples showed low values for yeast and mould yeasts, moulds, Madzhulu showed significantly low values for mould compared to the literature. Grabowski and Klein (2017) showed powdered Bombyx mori containing more yeasts and mould and Klunder *et al*. (2012) found up to 2.2–2.8 cfu/g in total bacteria count in cooked and stir-fried insects. The remarkable microbiological characteristics of the products can be attributed to hygienic insects and the overall preparation of the samples.

Table 4.7 Microbial quality of edible insect flours (*G. belina*, *H. illucens* and *M. subhyalinus*).

Sample	**TPC (cfu/g)**	**S. aureus (cfu/g)**	***E. coli* (cfu/g)**	***Yeast* (cfu/g)**	***Mould* (cfu/g)**	***L. monocytogenes* (per 25 g)**
Mashonzha	0.00	No growth	No growth	No growth	No growth	Negative
black soldier fly	0.00	No growth	No growth	No growth	No growth	Negative
Madzhulu	>1500	No growth	25e	No growth	50e	Negative

Data presented are microbial content of edible flours CFU-count forming units, TPC: total plate count. S.aureus = *Staphylococcus aureus*, E.coli= *Escherichia coli*, L. monocytogenes = *Listeria monocytogenes*

4.16 Sensory properties

4.16.1 Sensory evaluation of *H. illucens*

Sensory properties are important criteria accompanying the consumption of edible insects, and the organoleptic properties, such as the taste, and flavour of insects, are very diverse. The effect of *H. illucens* flour incorporation on the organoleptic characteristics in presented in Figure 4.4A. The organoleptic evaluation indicated that BSFL addition significantly affected ($p < 0.05$) all the attributes which were tested. A significant difference ($p < 0.05$) was observed in the appearance of the control sample and that of BSFL-10, BSFL-15 and BSFL-20 with consumers preferring more BSFL-5. A similar trend was observed in the colour of the biscuit with BSFL-5 not being significantly different ($p > 0.05$) from the control sample. The taste was also a determining factor for the acceptance of biscuits enriched edible insect flour, the control differed significantly ($p < 0.05$) in comparison to the edible insect-enriched biscuits. The derived taste of the biscuits could also have been a contributing factor and could be attributed to the release of occurring at the surface of the insect organism, which is mainly responsible for taste and flavour (Awobusuyi *et al.*, 2020). The 100% wheat biscuits (control) had a smoother surface than those containing the wheat–insect meal. Biscuits supplemented with the wheat–insect meal were uneven at the surface. However, this observation did not seem to have compromised the product quality or acceptability, as the ratings were similar. In addition, the biscuit containing 5% and 10% insect flour were more acceptable than those with higher concentrations of insects (BSFL-15 and BSFL-20). These results agree with those reported by Kinyuru *et al.*(2009) who developed wheat buns enriched with edible termites. The authors found that wheat buns were more acceptable at a 5% concentration of termites compared to 20%. In a similar study, the sensory analysis showed that the incorporation of termites in biscuits was acceptable up to 25% substitution (Kinyuru *et al.*, 2009). Previous authors have also reported the high acceptability of sorghum-based biscuits (Koffi *et al.*, 2013) These are promising findings especially where a lack of acceptance has often limited the idea of using insects as a food source.

4.16.2 Sensory evaluation of *G. belina*

The sensory properties of biscuits enriched with *M. subhyalinus* edible insect flour are shown in Figure 4.4B as a likeness score, in comparison with the wheat biscuits. Statistically, no significant differences ($p > 0.05$) were perceived by the panellist for the texture of the insect-enriched biscuits and the control sample (100% wheat flour) which correlates with the results obtained in Table 4.6 of the biscuit's hardness, there were no significant differences ($p > 0.05$) observed within the different concentrations and the control sample. Significant differences in aroma were observed between the MW-15, MW-20, and the control samples, with the latter receiving higher aroma scores (4.53 ± 0.80). MW-15 and MW-20 were perceived poorly (or disliked) in terms of the taste attribute and were rated 2.47 ± 1.28 and 2.41 ± 1.50, respectively (Figure 4 B). It can be observed that with the increase in the Mashonzha flour, the appearance, colour, aroma, and texture scores of biscuits decreased. In terms of appearance, no significant differences ($p > 0.05$) between MW-5, MW-10, and the control (WF) were perceived by the panellist. Moreover, there were no significant differences ($p < 0.05$) between the control and MW-5 in terms of the overall acceptability of the biscuits. This implies that increasing the Mashonzha concentration to above 10% will decrease the acceptability of the biscuits. Therefore, the enrichment of biscuits with Mashonzha flour of up to 5% produced protein-enriched biscuits with moderately desirable overall acceptability. The incorporation of 5% cricket powder into wheat pasta was studied by Duda et al. (2019) these authors demonstrated how introducing insect proteins into pasta influenced cooking time, colour, texture and flavour, the latter being the most distinctive. Overall, the sensory evaluation showed that the fortified wheat pasta met consumer expectations, displaying no significant difference compared to whole-wheat pasta. In a study conducted by de Olivera, (2017), bread enriched with 10% Cinereous cockroach (Nauphoeta cinerea) was still globally appreciated based on sensory evaluation results, even if the panel ultimately preferred bread without the insect ingredient. The results of our research are in line with the literature data, which show that edible insect powders are useful in the production of bakery goods. Their use improves nutritional value and affects the product's properties, including sensory attributes. The quality of the enriched products depends, inter alia, on the insect species, method of powder production and the bakery product recipe; therefore, the level of the enrichment must not be too high and must be determined in studies.

4.16.3 Sensory evaluation of *M. subhyalinus*

To create a viable product, high nutritional value and health-promoting properties must be accompanied by sensory attractiveness (Pauter *et al.,* 2018). Taste, smell, and appearance are some of the most important factors influencing a consumer's purchase decision. Data presented in Figure 4C shows that the control had the highest appearance rating compared to all the enriched biscuits. A significant difference ($p < 0.05$) was observed in the control sample and MZ-15 and MZ-20, with MZ-5 and MZ-10 being more preferable in appearance. A similar trend was observed in the colour of the biscuits enriched with edible insect flour and the control sample. Enrichment at 5% and 10% levels kept the sensory parameters close to the control sample in terms of colour. According to the correspondence analysis (Figure 4C), the assessors associated different attributes with each sample. The control sample was considered preferable in terms of taste and aroma. MZ-5 was the most liked insect-enriched biscuit, which is reflected in this analysis, as Just-about-right colour, taste and pleasant aroma attributes are close to the control sample. Granular texture, too dark in colour, and insect odour appear along with samples MZ-10, MZ-15 and MZ-20, which were least preferred compared to the control sample and MZ-5. The results observed in this study are similar to those observed by Biró *et al.* (2020). The control sample earned the highest overall acceptability regarding biscuits supplemented with edible insect flours, and the panel favoured the 5% insect-containing biscuit among the enriched goods.

Additionally, there were no discernible variations across the texture-liking variables. In another study by de Oliveira *et al.*, (2017) and Osimani *et al.*(2018), the colour of the sample with 5% *Acheta domesticus* earned the highest ratings among the products' colours as in our study. The addition of various amounts of edible insect flour had no effect on the odour or texture preference, as evidenced by the lack of discernible changes in the three samples' hardness levels.

Comparing our study to the international literature, this is the first research which used Mashonzha and Madzhulu flour as a base for insect-enriched biscuits. These flours have better nutritional characteristics in terms of high protein. Our results support the evidence that pairing insects with these flours are a viable option for developing novel baked goods that could gain consumers' acceptance.

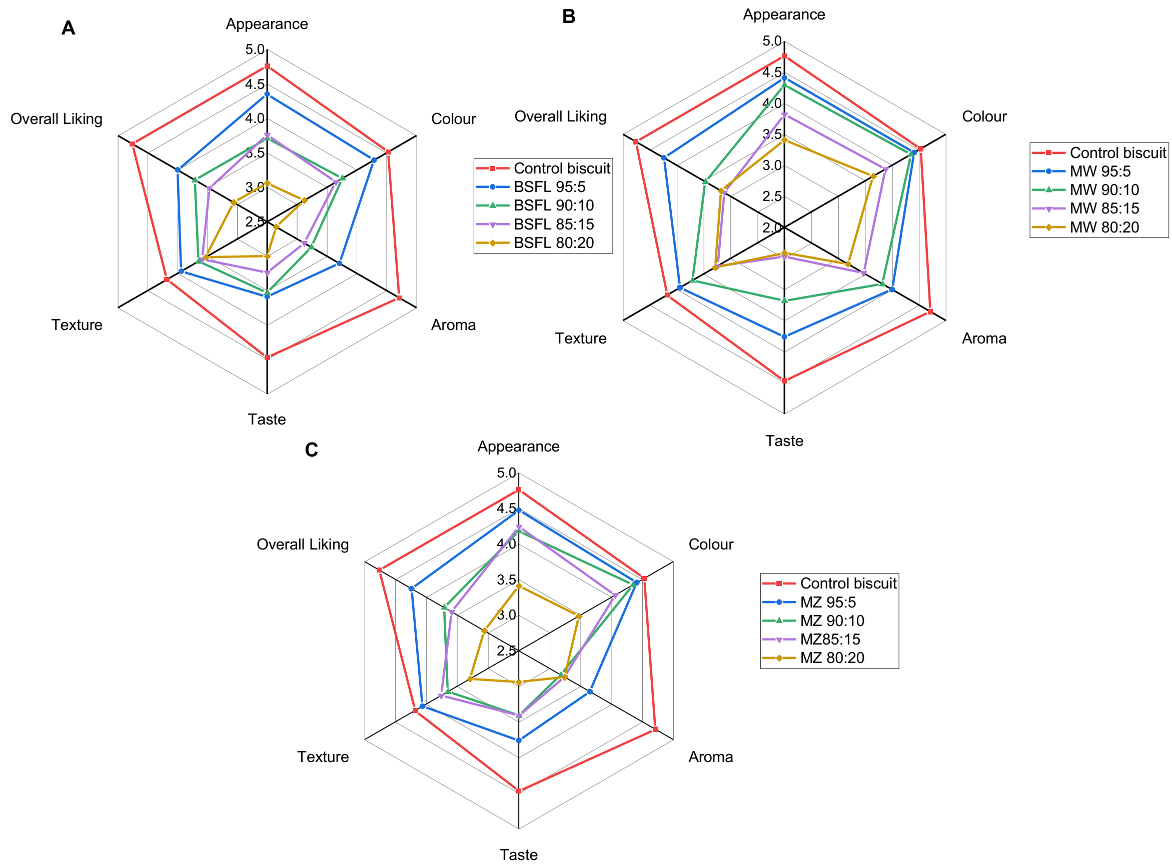

Figure 4.5 Spider web chart of the sensory properties of biscuits enriched with edible insect flours **(A)** *H. illucens* **(B)** *G. belina* **(C)** *M. subhyalinus*

4.17 Conclusion

This work evaluated the impact of *Gonimbrasia belina*, *Hermetia illucens* and *Macrotermes subhyalinus* flour fortification on nutritional values, textural characteristics, and physical attributes. The study's findings showed that adding edible insect flour to wheat flour at a rate of 20% (w/w) caused significant nutritional changes, including higher protein and fat and lower carbohydrate content. To sustainably supply the expected growth in world population, *G. belina*, *H, illucens* and, *M. subhyalinus* flour may prove useful in reformulating traditional products, such as biscuits. Nevertheless, a cultural barrier still prevents people from eating insects. Additionally, from a technological standpoint, there are some issues with their impact on the composition of food products, their colour, or flavour, among other features. In conclusion, this study is essential since it offers fundamental information that can be used as a guide when creating new baked goods with substitute protein sources. Further studies should be conducted to optimise the biscuit formulations, focusing on final product shelf life and sensorial properties.

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CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Recently there has been an increase in the use of edible insects as alternative protein sources to animal-derived ones in response to the imminent scarcity of food. The aim of this study, therefore, was to determine the proximate, techno-functional, and antioxidant properties of three edible insect flours (*Macrotermes. subhyalinus, Gonimbrasia. belina* & *Hermetia. illucens*) with the view of incorporating the edible insect flours in the production of snack goods such as biscuit and determine the effect on the sensorial profile of biscuits.

The first objective was to determine the proximate (Ash, moisture, crude fat, crude protein, carbohydrates, and energy) properties of the edible insect (*M. subhyalinus*, *G. belina* & *H. illucens*) flours. The results from this study revealed that *M. subhyalinus, G. belina,* and *H. illucens* are rich sources of macro food nutrients. Their application in food products as novel functional ingredients should be encouraged. Therefore, the hypothesis that edible insect flours from insects *M. subhyalinus*, *G. belina* and *H. illucens* will provide alternative protein sources was accepted.

The second objective was to evaluate the physicochemical properties (Colour attributes, water activity, bulk density, and pH) of *M. subhyalinus*, *G. belina* and *H. illucens* flours. The edible insect flours revealed that they could be acceptable, considering that these properties play a major role in the product's acceptability and shelf life.

The third objective of was to evaluate the techno-functional properties ((water binding capacities and stability), emulsion capacity and emulsion stability, oil binding capacity and foam capacity and foam stability) of the edible insect flours (*M. subhyalinus*, *G. belina* and *H. illucens*). The edible insect flours showed high OBC, foam capacity/stability and, moderate WBC making them beneficial in food applications where such properties may be required. Double check your objective again and check if they correspond with what you did. What about the microbial quality?

The fourth objective of chapter one was to determine the antioxidant properties (DPPH-RS, RP, ABTS-RS and Fe chelation). The assessment of the antioxidant capacity of food products is of importance since it provides a wide variety of information on factors such as oxidation resistance, the quantitative contribution of antioxidants, or the antioxidant effects that can occur in the body at the time of consumption. In this study the edible insect flours were efficient antioxidants, radical scavengers and Fe^{2+} ion chelators. This suggests that edible insect flours may be a suitable candidate to produce food supplements and topical products with antidiabetic and cosmeceutical properties.

Chapter four was undertaken to identify the effect of wheat-insect composite flour concentration (5%, 10%, 15% and 20%) on the nutritional and sensorial profile of the biscuits.

The results revealed that the enrichment of wheat flour with edible insect flour improved the nutritional value of the products. Therefore, the hypothesis was accepted that the inclusion of edible insect flours would not adversely affect the biscuits' nutritional properties. There was a progressive increase in biscuits' protein and ash contents as the flour concentration increased. In turn, the fat content and energy value did not differ significantly. The acceptability of the biscuit enriched with 5% edible insect flours was acceptable compared to the control.

The following conclusions can therefore be drawn from this study:

1. *M. subhyalinus*, *G. belina* and *H. illucens* flours can be used to develop tailor-made food products which are high in protein.
2. The physicochemical and techno-functional properties *of M. subhyalinus*, *G. belina* and *H. illucens* flours make them valuable to the food industry as potential novel food ingredients and fat binders, amongst other functions.
3. The antioxidant properties of the edible insects' flours further demonstrate their potential for application as novel functional ingredients.
4. The addition of the edible insect flours does affect the overall nutritional profile of the biscuits and fortification of biscuits with edible insect flower at concentration exceeding 20% is not positively perceived by consumers. In general, biscuits containing edible insects were accepted overall but the sensory panel.
5. What about the microbial quality

5.2 recommendations

The following recommendations are suggested for further research:

1. In the future, more studies need to be conducted on the product's storage and shelf stability and the availability of nutrients in vivo.
2. From a nutritional point of view, further studies on protein digestibility and utilization, the bioavailability of essential mineral and trace elements, as well as other factors such as pH, the concentration of enhancers, and inhibitors (dietary fibre and polyphenols) would be crucial, particularly for edible insect-based food products.
3. It is also recommended that the analysis of nutrient components such as vitamins and fatty acids is another area of research that is beneficial in facilitating nutritional knowledge prior to using flours for developing human food.
4. Studies investigating the thermal, structural, techno-functional, and rheological properties of proteins extracted from edible insects from the South African context are recommended to exploit their use as value-added compounds in food products.

APPENDIX A: DATA COLLECTION APPROVAL

Cape Peninsula University of Technology

Statement of Permission

Data/Sample collection permission is required for this study.

Reference no.	215062965/05/2021
Surname & name	Vanqa, N.
Student Number	215062965
Degree	Master of Food Science and Technology
Title	The impact of composition and techno-functional properties of edible insect (*Encosternum delegorguei, Gonimbrasia belina and Hermetia illucens*) flours on the nutritional and sensorial profile of biscuits
Supervisor(s)	Mr V. Mshayisa
FRC Signature	
Date	2021 May 31

APPENDIX B: ETHICAL APPROVAL LETTER

Cape Peninsula University of Technology

Statement of Permission

Data/Sample collection permission is required for this study.

Reference no.	215062965/05/2021
Surname & name	Vanqa, N.
Student Number	215062965
Degree	Master of Food Science and Technology
Title	The impact of composition and techno-functional properties of edible insect (*Encosternum delegorguei, Gonimbrasia belina and Hermetia illucens*) flours on the nutritional and sensorial profile of biscuits
Supervisor(s)	Mr V. Mshayisa
FRC Signature	
Date	2021 May 31

APPENDIX C: SENSORY EVALUATION CONSENT FORM

Cape Peninsula
University of Technology

Department of Food Technology
P. O. Box 1906
Bellville 7535

INFORMED CONSENT FOR EDIBLE INSECT RESEARCH

Hello

We are scientists from the Cape Peninsula University of Technology. We are researching to find new food use for edible insect flour. No value will be added to any products except for the consumers who endorse them. Hence, we are approaching you to be part of this study. We realize you need to make an informed decision whether or not to be part of this study, hence we have provided below further details with regard to the research to assist in your decision process.

Title of Research Project:
The impact of composition and techno-functional properties of edible insect flours (*Macrotermes subhyalinus, Gonimbrasia belina* and *Hermetia illucens*) on the nutritional and sensorial profile of biscuits

Investigator:

Nthabeleng Vanqa	Tel:	0818963455
Vusi Mshayisa	Tel:	0219594386
Dr Moses Basitere	Tel :	0214603170

Purpose of the Research:
In 2050, the world population is proximately expected to be more than 9 billion people, resulting in an additional need for food and feed output. However, there is a limited amount of agricultural land and this is due to global warming. Climate change and the environmental destruction from industrial development also negatively affect food production. Considering the increasing resource shortage, conventional protein sources will not be enough for the global human population thus the introduction of novel, resource-efficient and sustainable sources of protein on the market and implementation of it in some form into a balanced healthy diet for consumers might be the way to tackle the issue and several foods have been proposed as alternatives, with insects receiving the most attention.

The purpose of this study is to determine the effect of the use of edible insect flours on the nutritional quality and acceptability of biscuits. This study will be of great interest to the agri-food industries working to promote edible insect flours and fight against the shortage of conventional sources of protein.

Description of the Research:
This is an invitation to participate in the sensory study. The procedure to be adopted in the study as well as the terminologies on the score form will be explained to the panellists before tasting sessions. You will receive five biscuit samples four of which were produced with edible insect flour. You will be required to test them and rate your preference (on a simple questionnaire) for each based on appearance, crust colour, crumb colour, taste, aroma, texture and overall acceptability. Each tasting session will last for 15 - 30 minutes depending on the individual.

Potential Harm, Injuries, Discomforts or Inconvenience:
The three edible insect flours are staples for thousands of Africans; their consumption does not pose any hazard to human health. Therefore, this study has no known harm associated with tasting the edible insect flours products.

Potential Benefits:
You will not benefit directly from participating in this study.

Confidentiality:
Confidentiality will be respected and no information that discloses the identity of the participant will be released or published.

Participation:
Participation in this research is voluntary. If you choose to participate in this study you may withdraw at any time.

Contact
If you have any questions about this study, please contact:

Nthabeleng Vanqa	Tel:	0818963455
Vusi Mshayisa	Tel:	0219594386

Consent:
By signing this form, I agree that:

1. The study was explained to me and all my questions were answered.
2. I have the right to participate and the right to stop at any time.
3. I have been told that my personal information will be kept confidential
4. There is no likely harm from tasting biscuit to which edible insect flour had been added.

I hereby consent to participate in this study:

....

Name of Participant	**Signature & Date**
....	....
Name of Researcher	**Signature & Date**

APPENDIX D: SENSORY EVALUATION FORM

Biscuit fortified with edible insect flour
PRODUCT EVALUATION FORM

Instruction: **You are provided with 5 samples of biscuits fortified with edible insect flours. Please take a sip of water before you start tasting and in between tasting the different samples. Please rate each sample on its own merit based on the given attributes. Do not compare the samples**

Name of product: **Edible biscuit** **Code**: ....

	Dislike very much (1)	**Dislike moderately (2)**	**Neither Like nor Dislike (3)**	**Like moderately (4)**	**Like very much (5)**
Appearance					
Colour					
Aroma					
Taste					
Texture					
Overall acceptability					

Comments:

....

....

Biscuit fortified with edible insect flour
PRODUCT EVALUATION FORM

Instruction: **You are provided with 5 samples of biscuits fortified with edible insect flours. Please take a sip of water before you start tasting and in between tasting the different samples. Please rate each sample on its own merit based on the given attributes. Do not compare the samples**

Name of product: **Edible insect biscuit** **Code**: ....

	Dislike very much (1)	**Dislike moderately (2)**	**Neither Like nor Dislike (3)**	**Like moderately (4)**	**Like very much (5)**
Appearance					
Colour					
Aroma					
Taste					
Texture					
Overall acceptability					

Comments:

....

....

Biscuit fortified with edible insect flour
PRODUCT EVALUATION FORM

Instruction: **You are provided with 5 samples of biscuits fortified with edible insect flours. Please take a sip of water before you start tasting and in between tasting the different samples. Please rate each sample on its own merit based on the given attributes. Do not compare the samples**

Name of product: **Edible insect biscuit** **Code**: ....

	Dislike very much (1)	**Dislike moderately (2)**	**Neither Like nor Dislike (3)**	**Like moderately (4)**	**Like very much (5)**
Appearance					
Colour					
Aroma					
Taste					
Texture					
Overall acceptability					

Comments:

....

....

Biscuit fortified with edible insect flour
PRODUCT EVALUATION FORM

Instruction: **You are provided with 5 samples of biscuits fortified with edible insect flours. Please take a sip of water before you start tasting and in between tasting the different samples. Please rate each sample on its own merit based on the given attributes. Do not compare the samples**

Name of product: **Edible insect biscuit** **Code**: ....

	Dislike very much (1)	**Dislike moderately (2)**	**Neither Like nor Dislike (3)**	**Like moderately (4)**	**Like very much (5)**
Appearance					
Colour					
Aroma					
Taste					
Texture					
Overall acceptability					

Comments:

....

....

Biscuit fortified with edible insect flour
PRODUCT EVALUATION FORM

Instruction: **You are provided with 5 samples of biscuits fortified with edible insect flours. Please take a sip of water before you start tasting and in between tasting the different samples. Please rate each sample on its own merit based on the given attributes. Do not compare the samples**

Name of product: **Edible insect biscuit** **Code**: ....

	Dislike very much (1)	**Dislike moderately (2)**	**Neither Like nor Dislike (3)**	**Like moderately (4)**	**Like very much (5)**
Appearance					
Colour					
Aroma					
Taste					
Texture					
Overall acceptability					

Comments:

....

....

We would like to obtain information about you. Kindly complete this brief questionnaire appropriately:

1. What is your Gender: Female ☐ Male ☐

2. What is your race? Black ☐ Coloured ☐ White ☐ Indian ☐ Other ☐

3. Are you a student or staff? Student ☐ Staff ☐

4. If you are a student, are you an international student? Yes ☐ No ☐

5. What is your age group?

☐ Less than 20
☐ 20-29
☐ 30-39
☐ 40 & above

APPENDIX E: PUBLISHED MANUSCRIPT FIRST PAGE

foods

MDPI

Article

Proximate, Physicochemical, Techno-Functional and Antioxidant Properties of Three Edible Insect (*Gonimbrasia belina*, *Hermetia illucens* and *Macrotermes subhylanus*) Flours

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Abstract: In this study, edible insect flours from *Gonimbrasia belina* (Mashonzha), *Hermetia illucens* (black soldier fly larvae) and *Macrotermes subhylanus* (Madzhulu) were prepared and assessed in terms of proximal, physicochemical, techno-functional and antioxidant properties. The crude protein of the edible insect flours varied between 34.90–52.74%. The crude fat of the insect flours differed significantly ($p < 0.05$), with *H. illucens* (27.93%) having the highest crude fat. *G. belina* was lighter (L*) and yellower (+b*) compared to *H. illucens* and *M. subhylanus*, and there was no significant difference ($p > 0.05$) in the redness (+a*) of the edible insect flours. There were no significant differences ($p > 0.05$) in foam capacity and foam stability of all three edible insect flours. Moreover, the antioxidant activity against the DPPH radical was low for *H. illucens* (3.63%), with *M. subhylanus* (55.37%) exhibiting the highest DPPH radical. Principal component analysis (PCA) was applied to the techno-functional properties and antioxidant indices of the edible insect flours. PC1 accounted for 51.39% of the total variability, while component 2 accounted for 24.71%. In terms of PC1, the FS, OBC and FC were responsible for the major differences in the edible insect flours. The findings revealed that edible insect flours are a good source of antioxidants and can be used as an alternative protein source and a potential novel food additive due to their techno-functional qualities.

Keywords: edible insect flours; *G. belina*; *H. illucens*; *M. subhylanus*; nutritional properties; techno-functional properties; antioxidant activity; metal chelation; Mashonzha; Madzhulu; black soldier fly

check for updates

Citation: Vanqa, N.; Mshayisa, V.V.; Basitere, M. Proximate, Physicochemical, Techno-Functional and Antioxidant Properties of Three Edible Insect (*Gonimbrasia belina*, *Hermetia illucens* and *Macrotermes subhylanus*) Flours. *Foods* **2022**, *11*, 976. https://doi.org/10.3390/foods11070976

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1. Introduction

As vast as the challenge is to feed 9 billion people by 2050, increasing food availability is insufficient due to the increasingly limited resources, such as agriculturally cultivable land [1]. This, without a doubt, calls for innovative, alternative ways of ensuring that adequate, quality, safe and nutritious foods are available and accessible to all people at all times [2]. As early as 1975, Meyer-Rochow [3] argued and proposed that edible insects could play a role in alleviating food security and combating protein deficiency in some underdeveloped countries. Over the last two decades, there has been a renewed interest on edible insects for human consumption globally [4–6]. The FAO report titled "*Edible insects: Future prospects for food and feed*" [6] and other scientific literature seems to have re-invigorated the earlier call made by Meyer-Rochow in 1975. This is because, compared to conventional protein sources, edible insects have an excellent feed conversion ratio; a source of protein, fat and minerals, and this characteristic is particularly valuable given that future protein consumption is expected to increase with a declining food supply [7–10].

Entomophagy, the practice of consuming insects, has been practised worldwide for centuries, yet it has only recently gained momentum in Western cultures [11]. Insects