# THE INFLUENCE OF MARULA SEEDCAKE, MOPANE WORM AND MEALWORM ON BREAD QUALITY

By

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## DECLARATION BY CANDIDATE

"I hereby declare that the dissertation submitted for the degree Master of Applied Sciences: Food Technology, at the Tshwane University of Technology, is my original work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged using a comprehensive list of references."

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Signature:……………………………….

2024

## DEDICATION

I dedicate this dissertation to my Lord Jesus Christ who is the author and finisher of every decent work. He made me grow in knowledge and allowed me to bear fruits by blessing the work of my hands. I overflow with thanksgiving.

I dedicate this dissertation in honour of my dad Pieter Morudu and my mom, the late Sarah Morudu, who have been my source of inspiration and planted the seed of learning in me. They gave me unconditional moral, spiritual, emotional and financial support to achieve my goals.

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## ABSTRACT

The food industry faces a significant challenge with the surging demand for protein that minimizes environmental impact. Projections by the United Nations indicate that the global population, currently at 8.2 billion, could reach 8.6 billion by 2030. This growth is expected to intensify pressures on land use, greenhouse gas emissions, energy consumption, and food security. In this context, Africa's rich biodiversity and indigenous knowledge, particularly regarding wild flora and insects, offer promising solutions. The practice of entomophagy, or insect consumption, is increasingly recognized as a viable method to meet the rising protein needs associated with population growth, as insects have traditionally formed part of many African tribes' diets. The composition, safety, and functional properties of flour made from marula (*Sclerocarya birrea*) seed cake, mopane worm (*Tenebrio molitor*), and mealworm (*Imbrasia belina*) were evaluated, this assessment included their potential for breadmaking. The total viable count (TVC) was highest in mopane worm flour at $5.10\times10^{5}$ cfu/mL, followed by marula seed cake flour at $1.75\times10^{3}$ cfu/mL and mealworm flour at $2.70\times10^{2}$ cfu/mL. These counts exceed safety standards; in comparison to white wheat flour with a TVC of less than 25 cfu/mL. The total fungal count was also highest in mopane worm flour at $6.9\times10^{3}$ cfu/mL, followed by mealworm flour at $2.70 \times 10^{2}$ cfu/mL. Marula seed cake flour and white wheat flour both had TFC values <25 cfu/mL. No mycotoxins were detected on any of the flour samples.

The analysis of these flours revealed important nutritional values: ash content was measured at mopane worm (0.40%), mealworm (0.31%), and marula seed cake (0.18%); moisture levels were mopane worm (7.60%), mealworm (4.27%), and marula seed cake (3.79%); fat content was recorded at mopane worm (13.6%), mealworm (10.5%), and marula seed cake (6.7%); while protein was high for mopane worm (60.6%), mealworm (49.7%), and marula seed cake (38.0%). Dietary fiber was highest in marula seed cake at 15.1%, compared to mealworm 6.6% and mopane worm 3.9%. This suggests that incorporating these alternative flours into baked bread could enhance their protein content. The essential amino acid profile of white wheat was significantly lower than that of mealworms, mopane worms, and marula seed cake, revealing its limitations as a protein source. Amino acids such as isoleucine (0.42% vs 2.26% in mealworms), leucine (0.86% vs 3.95%), and lysine (0.28% vs 3.42%) were

notably deficient in wheat, with lysine being the first limiting amino acid. Other essential amino acids, including methionine (0.24%), phenylalanine (0.6%), and threonine (0.35%), showed lower levels compared to the alternative sources. The amino acid composition of white wheat was inadequate, particularly in essential amino acids like lysine and leucine, making it a less optimal choice for dietary protein needs. The total polyphenol index (TPI) varied among the flours, with white wheat flour showing a high value in water extract (441), while acetone extracts had lower values for all samples except for mopane worm which had a TPI of 770 indicating its superior antioxidant activity compared to others. In terms of baking performance, different percentages of alternative flours were incorporated into bread doughs (2.5%, 5%, 10%, 15%, and 20%). The rheological properties were characterized using Mixolab analysis; mopane worm flour exhibited the highest torque values across various concentrations, indicating stiffer dough. Baking trials indicated that bread made with white wheat flour had the brightest crust and crumb colours compared to those made with composite flours like mopane worm flour which produced darker colour at higher concentrations (20%). The increased levels of flour substitution led to a significant decrease in the height of all composite breads. The C-Cell analysis highlighted that the slice areas decreased as higher percentages of alternative flours were included. The texture analysis revealed that bread incorporating mealworm flour maintained a lower hardness compared to standard white wheat bread over 7 days. The inclusion of alternative flours, particularly mealworm flour and mopane worm flour, significantly affected the microbial quality of the bread over time. The high microbial counts observed, raise concerns regarding the safety and shelf life of products made with these flours. Although the absence of harmful microorganisms such as *Staphylococcus aureus* and Salmonella spp. in the samples is reassuring, careful monitoring and management of microbial loads are still essential in the processing of these alternative flours. A 15% replacement of wheat flour in bread with mealworm, mopane worm, or marula seed cake flour, resulted in protein contents (%) of 11.8, 12.9 and 10.7 compared to the 8.1 of standard white wheat bread. The estimated cost of ingredients (15% replacement) for the marula seed cake bread is R9.15 per loaf, which is lower than the mopane bread at R11.44 per loaf but higher than the standard bread at R7.68 and it is notably less than the mealworm bread at R30.18 per loaf. Thus, incorporating insects or marula seed cake into a staple item like bread could be a viable option to improve the nutritional status of the South African population.

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| | |
|---|---|
| AAPH | 2,2'-Azobis(2-amidinopropane) dihydrochloride |
| ABTS | Azino ethylbenzothiazoline sulfonic acid |
| AFB | Aflatoxin $B_1$ |
| AOAC | Association of Official Agricultural Chemists |
| AOAC | Association of Official Agricultural Chemists |
| BSE | Bovine spongiform encephalopathy |
| CAGR | Compound annual growth rate |
| CAR | Central African Republic |
| CFU/g | Colony-Forming Units per Gram |
| DAFF | Department of Agriculture, Forestry, and Fisheries |
| DGA | Dietary Guidelines for Americans |
| DM | Dry matter |
| DPPH | Diphenyl-1-picrylhydrazyl |
| DRBC | Dichloran Rose Bengal Chloramphenicol Agar |
| DRC | Democratic Republic of the Congo |
| DS | Dietary supplements |
| EAA | Essential Amino Acids |
| EAA | Essential Amino Acids |
| EDTA | Ethylenediaminetetraacetic Acid |
| ELISA | Enzyme-Linked Immunosorbent Assays |
| ENA | European Nucleotide Archive |
| EU | European Union |
| FAO | Food and Agriculture Organisation |
| FAO | Food and Agriculture Organization |
| FRAP | Ferric Reducing Antioxidant Power |
| GAE | Gallic acid equivalent |
| GHI | Global Hunger Index |
| GMP | Good Manufacturing Practices |
| GRFC | Global Report on Food Crises |
| HPLC | High-Performance Liquid Chromatography |
| INSFEED | Insect feed for poultry and fish production in sub-Saharan Africa |

| | |
|---|---|
| MALDI-TOF | Matrix-Assisted Laser Desorption/Ionization Time-of-Flight |
| MPW | Mopane worm |
| MPWF | Mopane Worm Flour |
| MSC | Marula seed cake |
| MSCF | Marula Seed Cake Flour |
| MWF | Mealworm |
| MWF | Mealworm Flour |
| MYP | Mannitol Egg Yolk Polymyxin |
| NDP | National Development Plan |
| NHANES | National Health and Nutrition Examination Survey |
| NLEA | Nutrition and Labelling Education Act |
| NTFP | Non-Timber Forest Products |
| OECD | Organisation for Economic Co-operation and Development |
| ORAC | Oxygen Radical Absorbance Capacity |
| PCR | Polymerase Chain Reaction |
| SABS | South African Bureau of Standards |
| SAGL | South African Grain Laboratory |
| TE | Trolox Equivalents |
| TEAC | Trolox Equivalent Antioxidant Capacity |
| TFC | Total Fungal Count |
| TPI | Total Polyphenol Index |
| TUT | Tshwane University of Technology |
| TVC | Total Viable Count |
| UAE | United Arab Emirates |
| USDA | US Department of Agriculture's |
| WFC | World Food Conference |
| WFP | World Food Programme |
| WHO | World Health Organisation |
| WHO | World Health Organization |
| WWF | White Wheat Flour |
| WWF | Whole Wheat Flour |

# CHAPTER 1

# INTRODUCTION

## 1.1 BACKGROUND AND MOTIVATION

The world's population is expected to exceed 9 billion by 2050, reaching 10.4 billion by 2100, leading to a continuous increase in food demand. Although eradicating hunger and food insecurity and ensuring sustainable agriculture are key aspects of the 2030 Agenda for Sustainable Development, recent data suggest that the global number of undernourished people has been increasing since 2015, particularly in Africa (FAO, 2019). Malnutrition has a significant negative effect not only on human development but also on a country's social and economic success, as well as its health condition (WHO, 2018). South Africa is a multicultural and multi-ethnic country. As the population urbanises, a substantial proportion of it transitions from traditional, rural lives to more "Westernised" modern lifestyles (Labadarios *et al.,* 2000; Vorster, 2001, 2010). Simultaneously, dietary pattern changes and nutrient intake during the nutrition transition significantly impact nutritional status and health. Typically, consumption of refined foods (such as white bread and refined maize porridge) rises.

The refining process causes the loss of much of the nutritional value of grain products, leading to an imbalanced diet for a sizeable portion of the population. Although the fortification of maize meal and wheat flour with vitamins and minerals has been mandatory in South Africa since 2003 (South African regulation R504 of 2003), this strategy is only a medium-term solution that does not address all aspects of malnutrition in vulnerable communities (FACS, 2019). The World Health Organization (WHO, 2018) proposes dietary diversification as a long-term strategy to combat malnutrition. The Food and Agriculture Organisation promotes using underutilised traditional foods from local sources, particularly those with high nutritional value and availability (FAO, 2019). Proteins are important dietary components that include both essential and non-essential amino acids. Because the human body cannot synthesise essential amino acids (EAAs), they must be provided from food.

Proteins can be derived from animals, plants, microalgae, and microbes. However, due to their excessive cost, they are rarely used as supplements in Africa. Despite their lower nutritional value compared to animal proteins due to insufficient and/or imbalanced EAA content (Millward, 2017), plant proteins are commonly used as supplements, with positive effects on the environment. African cuisine is based on edible leaves from Asteraceae, Lamiaceae, Asphodelaceae, Amaranthaceae, and Brassicaceae. In Southern Africa, the Fabaceae family (leaves, fruits, seeds, roots, and gums), Euphorbiaceae (roots, leaves, and fruits), and Aizoaceae (roots, leaves, and fruits) are also commonly used edible plant species (Van Wyk, 2011; Akinola *et al*., 2020; Held, 2021). *Sclerocarya birrea*, known as the marula, is famous for its fruits, commercially manufactured spirits (Amarula Cream Liqueur, Distell, South Africa), and seed oil (Komane *et al.,* 2017). Currently, there is a trend toward domesticating fruitbearing indigenous trees, including marula (Van Wyk, 2011) resulting in a high number of underutilised by-products. These by-products have the potential to be converted into useful products, such as protein supplements. Marula seed cake is currently available for R10.00/kg and is used as a protein supplement (47% crude protein) since it contains sulphur amino acids such as methionine, cysteine, homocysteine and taurine (Mashau *et al.,* 2022; Mchunu *et al*., 2024). Insects are another promising source of high-quality proteins. They have the potential to contribute significantly to alleviating global hunger while minimising environmental effects (Patel *et al.,* 2019). Insects being a part of many societies' traditional diets (Van Huis, 2017; Cappelli *et al.,* 2020), human consumption of insects (entomophagy) can be considered one of the most solid solutions to the rising protein demand induced by global population growth. An estimated 2 billion people in Africa, Asia, Central and South America, and Australia consume insects in various forms, not only because they taste good but also because they are a nutritious and low-cost food source (Murefu *et al.,* 2019). The global edible insect market is projected to grow at a compound annual growth rate (CAGR) of approximately 25.2% from 2024 to 2030, predicting that the global edible insect market will reach $1.53 billion by 2024. By 2025, the insect protein market is projected to reach USD 1,336 million, a significant increase from USD 144 million in 2019 (Market Data Forecast, 2024).

Several factors contribute to market growth, including a shift in preferences from animal protein to alternative proteins such as insect protein, due to growing concerns about future sustainability as well as increased public and private support for new insect protein research projects in both developed and developing countries. Insect farming has potential because insects reproduce quickly and are easy to breed

(Market Data Forecast, 2024). Globally, the most commonly consumed insects are those that are readily available, such as beetles (Coleoptera: 31%), caterpillars (Lepidoptera: 18%), bees, wasps, and ants (Hymenoptera: 14%), grasshoppers, locusts, and crickets (Orthoptera: 13%), cicadas, leafhoppers, plant hoppers, scale insects, true bugs (Hemiptera: 10%), termites (Isoptera: 3%), dragonflies (Odonata: 3%), flies (Diptera: 2%), and others. Africa, Asia, and Latin America have the greatest insect-eating rates (Jongema, 2015). Insects can be consumed intact, cooked, or ground into flour. Insects are rich in protein, fatty acids, vitamins, fibre, and minerals. An analysis of over a hundred edible insect species revealed that EAAs account for 46% to 96% of total amino acids (Xiaoming *et al.,* 2010). Although much research has focused on the protein content of insects as a food source, it is important not to overlook their positive fat content, as lipids are a crucial component of insects (Allegretti *et al.,* 2018). These fats can benefit human nutrition by supplying energy and necessary fatty acids (Ramos-Elorduy, 2008). Despite being traditionally ingested in South Africa, edible insects are classified as "food of the poor" and despised by metropolitan groups. However, people often consume and commercially sell numerous bug species. Ghazoul (2011) states that people in the Limpopo province use mopane worms (*Imbrasia belina*), caterpillars of the emperor moth, for subsistence and profit. Baiyegunhi and Oppong (2016a; 2016b) found that 63% of the gathered mopane worms are sold during the production year. Mealworms, the larval form of the *Tenebrio molitor* or darkling beetle, are produced for pet food but are making their way onto more plates around the world (Ooninx & De Boer, 2012; Van Huis, 2017; De Smet *et al.,* 2019). With 33% fat, 51% crude protein, and 43% true protein on a dry matter basis (Finke & Oonincx, 2014; Allegretti *et al.,* 2018), this edible bug is gaining popularity worldwide. For example, in South Africa, the 14F initiative increased yellow mealworm production to industrial capacity to meet future demand for high-protein foods. Crickets and mealworms have already been used to make biscuits, bread, muffins, sweets, soups, smoothies, chips, and spaghetti. These products are already accessible in the United States and Europe.

Because of the increased demand for protein, insects for human consumption have the potential to contribute to food security and help address future animal protein shortages. Incorporating insects into a staple item like bread will make it more accessible and acceptable to a larger portion of the South African population, providing the greatest nutritional value.

Gluten-forming proteins are responsible for wheat flour's distinctness in the manufacturing of leavened baked goods. Other grains contain comparable protein groups, but their gas-holding capacity is limited (Dobraszczyk, 2001). Wheat dough maintains gas due to gluten's viscoelastic properties, resulting in high-quality bread. Although substituting marula seed cake and insect flour for wheat flour will provide nutritional and economic benefits, the bread's quality and shelf life may suffer.

## 1.2 PROBLEM STATEMENT

According to the United Nations report (World Population Prospects 2024), the current global population of 7.6 billion is expected to reach 8.6 million in 2030, increasing the pressure on land use, greenhouse gas emissions, energy consumption and ultimately food security. Malnutrition is a serious public health problem that has been linked to a substantial increase in the risk of mortality and morbidity[1]. In Africa, it is estimated that 31% of the children are underdeveloped and 6% underweight with 9% of infants born with a low birth weight (Masuku-Maseko & Owagga, 2012).

Every year, 3.1 million children under five die due to malnutrition. According to recent reports, malnutrition remains a serious concern facing South Africans, especially children and women in rural areas with about a third of all children in Gauteng and the Free State being stunted as a result of chronic malnutrition. Although protein supplements are readily available, they are costly to the poor communities. In addition, the number of cost-effective protein supplements is limited in the South African market, limiting the number of available fortified products to consumers.

## 1.3 OBJECTIVES

The study aimed to assess the rheological properties of dough and bread characteristics as a function of two factors, flour type and percentage substitution. Regarding the flour types, three types were tested: marula seed cake flour, mopane worm flour and mealworm flour to replace wheat flour in bread and increase its nutritional content. Regarding the percentage of substitution, five levels were tested:

[1] https://www.who.int/quantifying_ehimpacts/publications/eb12/en/. Accessed the 03/07/2024

2.5%, 5.0%, 10%, 15% and 20%.

**The objectives were to:**

- Assess the microbiological safety and proximate composition of marula seedcake and insect flour.
- Assess the impact of varying percentages (2.5%, 5.0%, 10%, 15% and 20%) of marula seedcake flour and insect flours on bread dough rheology.
- Determine how varying percentages of marula seedcake flour and insect flours affect bread baking performance and shelf life.

## 1.4 HYPOTHESES

- Dried insect flours are predicted to include microorganisms.
- It is predicted that doughs containing different ratios of insect or marula seedcake flour will exhibit significantly distinct rheological qualities compared to wheat flour.
- It`s expected that marula and insect flours will enhance bread quality and nutritional value in comparison to normal wheat bread.

## 1.5 FORMAT OF THE STUDY

The study was divided into three separate phases which are represented in Figure 1.1

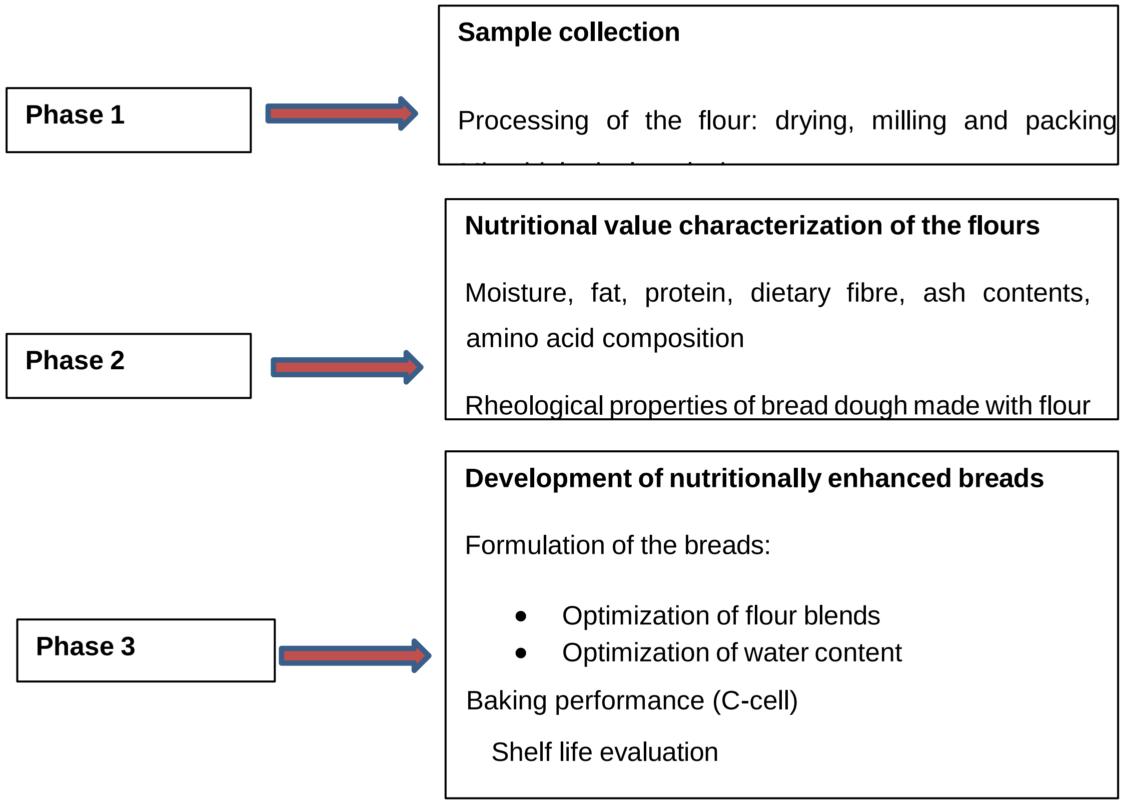


Figure 1.1: Experimental design of the separate phases of the study

The first phase was concerned with the flour and its safety, while the second phase concentrated on the description of the flour to define the physicochemical properties of each type of flour used in this study. Finally, the third part of the project involved optimising the manufacturing of fortified bread by determining the ideal blend based on baking performance and bread shelf life.

The dissertation contains five chapters. The first chapter is a broad introduction that explains the project's background and motivation, as well as the research aim and objectives. Chapter 2 provides a review of relevant literature for the project as well as a current understanding of food security status, insect and indigenous crop consumption, and their potential for use as alternative protein sources in baked goods.

Chapter 3 investigates the safety and physicochemical properties of the flours, while Chapter 4 investigates the dough rheology and baking performance of bread made with flours supplemented with various percentages of alternative flours. Finally, Chapter 5 summarised conclusions and recommendations for future research.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 INTRODUCTION

This literature review gives an overview based on the relevant literature across the scope. The review starts by presenting information on the incidence of food insecurity and the current strategies pursued to achieve food security. The second section discusses the importance, safety, and sources of dietary supplements, while the next section focuses on the usage of South African food plants and edible insects and their potential as nutritional supplements. This section goes into detail about the importance of marula and mopane worms. The concluding section is dedicated to bread and provides an in-depth overview of all elements of bread-making based on relevant literature.

## 2.2 FOOD SECURITY

The majority of the world's population struggles to get enough healthy food to get through the day. The public eye has been focused on food security for decades, with its influence beginning in the 1970s at the inaugural World Food Conference (WFC) held in Rome in 1974. In 1996, there were believed to be over 200 definitions of food security (Smith *et al.,* 1993). The World Food Summit (1996) defines food security as "when all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food that meets their dietary needs and food preferences for an active and healthy life." This demonstrates the varied aspects of food security, which include not only the availability of food but also safety, nutrition, and cultural preferences. The Food and Agriculture Organisation (FAO) defines food insecurity as a scenario in which individuals do not have secure access to adequate, safe, and nutritious food for normal growth. While this definition is brief, it emphasises the importance of having access to adequate and nutritious food for people's health and development. It is now recognised that food security and, consequently, food insecurity is a multi-dimensional phenomenon that, on a larger scale, can be illustrated by one simple question: can we provide enough food to feed the world?

### 2.2.1 Prevalence worldwide

The increasing concern regarding the ability of countries to secure an adequate and suitable supply of foodstuffs for their population can be traced back to 1943, during the Hot Spring Conference of Food and Agriculture. However, food security is a difficult concept to measure since it encompasses various aspects of the food chain ranging from production to consumption. However, to a larger extent, some would point to overpopulation, migration (urbanisation) and unsustainable agricultural practices as factors threatening food security and these factors are clearly described in the literature. The world's population is growing at a rapid pace, as is its food demand. Feeding the world's growing population necessitates an increase in food production and distribution, which will undoubtedly have an impact on already limited resources such as land, water, energy, oceans, and fertilisers (FAO, 2013), as well as climate change in terms of fuel emissions from heavy transportation networks. However, due to a lack of commonality in the level of economic development, particular nations of each continent or region require specific, adapted resource management strategies.

The 2023 report was alarming (O'Neill, 2023). The Global Hunger Index (GHI) score dropped to 18.3 in 2023, a point lower than the GHI score of 19.1 in 2015, when the SDGs were implemented. In recent years, the number of undernourished individuals worldwide has increased, from approximately 572 million in 2017 to approximately 735 million in 2023. According to the European Commission, an estimated 124 million people in 51 countries were experiencing acute hunger. Europe is not immune to the difficulty of feeding its population; according to European Union research, an estimated 8% of Europe's population is experiencing moderate to severe food insecurity. By the end of 2023, Eastern European countries such as Ukraine, Albania, Serbia, and Bulgaria had a larger estimated percentage of their populations suffering from hunger as shown in Table 2.1.

Africa came in second with 19.6%, including some nations where more than 5 million people needed food aid. However, the FAO reported that Asia had more hungry individuals and malnourished children. Nonetheless, other continents, like Latin America and, to a lesser degree, Western Asia, have seen a minor increase in the number of undernourished people. The countries' economies, primarily due to an imbalance in international primary commodity trade and an economic crisis (Caraher

& Coveney, 2016), have primarily contributed to the increase in undernourished people on these continents. In 2018, the number of malnourished individuals continued to increase, and in 2019, a steady frequency of undernourishment was reported. By the end of 2019, experts anticipated that hunger would persist for more than 821.6 million people, while a staggering two billion would experience moderate or severe food insecurity. Approximately 516.5 million people lived in Asia and the Pacific, with Sub-Saharan Africa accounting for 239 million of them. According to the Global Hunger Index (GHI), released in October 2019, Hunger and malnutrition impacted the Central African Republic (CAR) the most, with a GHI rating of 53.6, followed by Yemen (45.9), Chad (44.2), Madagascar (41.5) and Zambia (38.1). It is not unexpected that the Central African Republic ranked first, given its history of instability, sectarian bloodshed, and civil conflict since 2012. Violent conflict and political upheaval have led to extreme poverty and forced migration in nations like Burundi, Congo, Eritrea, Libya, Somalia, and South Sudan, which is linked to food insecurity. Overall, because any GHI score greater than 35 indicates an alarming to extremely alarming hunger condition, it is clear that Africa is the continent with the most countries experiencing food insecurity. Therefore, the recorded rate of hunger reduction and food insecurity since 2000 suggests that by 2030, 129 nations will be unable to achieve low hunger levels, rendering the goal of a hunger-free world by 2030 unattainable.

Table 2.1: Prevalence of undernourishment according to FAO in 2022

| **Continent, region, or country** | **%** |
|---|---|
| World | 10.7 |
| Pakistan | 13 |
| India | 15 |
| Omar | 8 |
| Saudi Arabia | 4 |
| UAE | 4 |
| Egypt | 5 |
| Europe | 2.5 |
| Western Asia | 9.5 |
| Latin America | 7.7 |

### 2.2.2 The African continent

The prevalence of malnutrition in Africa has been steadily increasing for decades and while the most recent data suggest that the curve has flattened in 2019 (FAO 2019), there were an estimated 282 million hungry individuals (20% of the population) in Africa. According to the most recent FAO and UN Economic Commission for Africa report, over 239 million people lived in sub-Saharan Africa, including 17 million in the northern part of Africa. The research also explains the origins of the insecurity, stating that conflict, economic slowdowns, including downturns, and climate change, were the contributing factors to the decline in food security. As seen in Table 2.2, Central Africa has the highest prevalence of undernutrition in Africa (30.3%), followed by the Eastern subregion of the continent (28.6%).

Despite drought and economic downturns in the south, this sub-region has the continent's second-lowest prevalence of undernourishment, at 9.7%, after only the north. Severe or acute insecurity hotspots might classify several African countries.

Conflict frequently accompanied by severe weather conditions and disease outbreaks such as the Ebola virus impacted most of these countries. The conflict has disrupted farming activities, cattle, and crops in numerous sections of the DRC (Democratic Republic of the Congo) and the Central African Republic (CAR). In the Central African Republic (CAR), approximately 10 million people require food assistance, while the Democratic Republic of the Congo (DRC) faces an even more severe situation, with over 27.4 million individuals living under extreme pressure and 13 million requiring urgent food aid. Sudan, particularly South Sudan, is also grappling with starvation; the World Food Programme (WFP) warned in December 2019 of a potential famine due to worsening hunger exacerbated by drought and political strife, affecting an estimated 6 million people. Zimbabwe is experiencing significant food insecurity, with 5.5 million rural residents affected and 3.8 million requiring assistance due to the deteriorating economy and drought from the 2018 to 2019 crop season. Urban areas are not spared, as at least 2.2 million city dwellers face food insecurity. Ethiopia has made strides in reducing chronic hunger, yet high food prices driven by currency devaluation and sporadic violence have left 8.1 million people chronically food insecure, with 5.2 million at risk of famine. In the Lake Chad basin, prolonged conflict has undermined food security across the region, impacting an estimated 3.4 million people in need of immediate assistance.

Table 2.2*:* Prevalence of undernourishment in Africa and its sub-regions in 2023

| **Regions/sub-regions*** | **%** |
|---|---|
| World | 9 |
| **Africa** | **20** |
| Eastern Africa | **28.6** |
| Central Africa | 30.2 |
| Sub-Saharan Africa | 24.1 |
| Western Africa | 15.1 |
| **Southern Africa** | **9.7** |
| Northern Africa** | 7.5 |

### 2.2.3 South Africa

South Africa's constitution is one of the most innovative and young in the world. Section 27(1)(b) of the Constitution states that "everyone has the right to sufficient food and water." Since the introduction of land reform, the issue of food security in South Africa has been a heated concern. Many people and organisations have questioned if such a change will result in a sharp drop in food production, hence jeopardising food security. According to Statistics South Africa (Stats SA), an estimated 25.2% of South Africans have been living in food poverty since 2016. A 2017 poll revealed that 6.8 million South Africans were hungry, affecting 1.7 million households across the country. It is vital to remember that South Africa, like other African countries, depends on agricultural activity to exist and feed itself. Limpopo province with 37.9% has the highest percentage of agricultural households while simultaneously being the poorest region in the country. Additionally, the Eastern Cape and KwaZulu-Natal are predominantly rural, with 33.4% of households depending on agriculture for their livelihood. In these provinces, smallholder farming is the primary source of income and, in many cases, food supply.

### 2.2.4 Solutions

It is now widely understood that to achieve a resilient, stable, and hunger-free world, it is necessary to end conflicts, empower women, nurture and educate children, and for governments and organisations to collaborate to develop rural infrastructure and strengthen social safety nets. Nonetheless, the most crucial option is to close the gap between emergency and development efforts. To achieve this goal, stakeholders have proposed several alternatives, such as production, livelihood aid, and long-term development support.

The United Nations Office for the Coordination of Humanitarian Affairs has addressed environmental concerns, such as the vulnerability and unsustainable use of environmental resources, as well as public understanding of the importance of the ecological system (Randers, 2012). Concerning the rapid increase of environmental damage, corporations consume most of this energy through the generation of greenhouse gases, which are harmful to the environment (Kotler, 1971). Fundamental improvements in industrial processes, along with a holistic approach that redefines how companies operate, can harmonise the interests of all stakeholders, including the environment and society (Bocken *et al*., 2014).

*2.2.4.1 Economic development, commerce and governance*

Although most governments recognise the critical need for adopting programs such as social protection, rural development, and education, many countries around the world, particularly in Africa and Asia, continue to lack comprehensive policies. The South African government established an interministerial national food security and nutrition plan, which is coordinated by the Presidency. This reflects a comprehensive and coordinated approach to addressing food security issues across various government departments. The National Development Plan (NDP) identifies agricultural productivity and rural development as critical priorities for job creation, economic growth, poverty reduction, and food security. This highlights the recognition of the agricultural sector's importance in achieving broader development objectives. Section 27(b) of the South African Constitution emphasises the state's obligation to formulate reasonable legislative efforts and take other measures to achieve the progressive realisation of the right to food. This underscores the government's commitment to ensuring food security for all citizens.

The Department of Agriculture, Forestry, and Fisheries (DAFF) creates agricultural policies and implements assistance programs to support food production and alleviate food insecurity among South Africans. The Food and Nutrition Security Policy is instrumental in aligning with the goals of the National Development Plan and Goal 2 of the United Nations Sustainable Development Goals (SDGs) aim to end hunger, achieve food security, improve nutrition, and promote sustainable agriculture by 2030. Given the broad scope of food security, collecting, processing, and analysing data on food and nutrition security is crucial for understanding the impact of government measures and informing policy decisions. The passage cites the Food and Agriculture Organisation's definition of food security, emphasising the importance of continuous physical, social, and economic access to sufficient, safe, and nutritious food for all individuals.

The term "desert" is intrinsically geographical, emphasising the physical absence of healthy food vendors in low-income communities (Cummins & MacIntyre, 1999). The US Department of Agriculture's (USDA) Food Accessibility Mapper, which identifies census tracts with limited food access, exemplifies this notion (USDA, 2013). Lowincome neighbourhoods and communities have limited geographic access to supermarkets (Apparicio *et al.,* 2007; Block & Kouba, 2006).

### 2.2.5 Food quality and safety

Consumers in developed countries increasingly expect food products of excellent and consistent quality, with a diverse selection available year-round at reasonable rates. There is also growing concern about food safety and the harmful effects of industrial food production on health and the environment. According to Rocourt *et al.* (2003), food contamination is a fundamental problem that sickens millions of people in The Organisation for Economic Co-operation and Development (OECD) countries each year. This emphasises the necessity of ensuring food safety across the entire production and distribution process. The food industry has rapidly internationalised, with retailers and food manufacturers sourcing items from all around the world. Globalisation has turned the food business into an integrated system with intricate relationships, enabling the supply of fresh goods from faraway regions at reasonable rates. Governments, both national and international, are addressing these concerns by enacting new legislation and regulations. Examples include the Codex Alimentarius standards established by the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO), as well as the General Food Law and EU regulations addressing specific issues such as BSE (bovine spongiform encephalopathy) in the European Union. These regulations aim to ensure safe and animal-friendly production practices, restrict pollution, and promote resource efficiency in food production and distribution. They reflect efforts to address concerns related to food safety, environmental sustainability, and animal welfare in the context of a globalised food industry.

## 2.3 DIETARY SUPPLEMENTS

Dietary supplements can be valuable in addressing malnutrition, especially when obtaining sufficient nutrients from common food sources is difficult. Here are some types of supplements that can help alleviate malnutrition: multivitamins and minerals, protein supplements, iron supplements, vitamin D supplements, and fortified foods.

Dietary supplements (DS) fall into three basic groups based on their nutritional role or origin: 1) substances with established nutritional properties, such as vitamins, minerals, amino acids, and fatty acids. 2) Botanical products, including concentrations and extracts. 3) Other compounds with diverse sources and physiological activities, including pyruvate, steroid hormone precursors, and chondroitin sulphate. Recent growth in the DS industry has featured the rapid increase of the botanical product segment, which is comparable in size to the vitamin and mineral market.

### 2.3.1 Importance

Some recent thorough articles have examined a variety of supplements (Antonio & Stout, 2001; Talbott, 2003). Use any dietary supplement that is effective in enhancing performance. Ergogenic aids are prohibited by sports governing organisations for one of two reasons: they endanger the individual's health or they provide an 'unfair' advantage. This poses ethical concerns.

### 2.3.2 Economic value

Various perspectives, including industry revenue, healthcare cost savings, and the potential for improved productivity and well-being, demonstrate the economic value of dietary supplements. The use of dietary supplements can have several positive effects on the economy, contributing to various sectors and fostering economic growth.

### 2.3.3 Nutritional value

Millward's adaptive metabolic demand model explains this apparent discrepancy by proposing that the body adapts to either high or low intakes and adjusts to variations in intake only very slowly (Millward, 2001). High protein intake increases protein breakdown and amino acid oxidation. An athlete consuming a high-protein diet experiences an abrupt decrease in protein intake, leading to a loss of lean tissue until a new equilibrium is reached.

### 2.3.4 Adults

Nutritional deficiencies vary by age, gender, race, or ethnicity, and can affect up to one-third of some population groups. The most common shortages were for vitamin B6, vitamin D, and iron. The DGA advocates eating nutrient-dense foods as part of a balanced eating pattern, as well as fortified foods and dietary supplements in some situations, to attain nutrient adequacy.

### 2.3.5 Children

Dietary supplements are not generally given to children who have access to a wellbalanced diet. For example, some conditions that may require supplements are cancer, chronic infections (e.g. asthma) and malnutrition. In this case, multivitamins and minerals are the most commonly given supplements with high-proteins food products. It is estimated that in the last decades, more children have been prescribed supplements as parents are promoting healthy eating habits to their children in addition to supplements. Thus, the current projections of the children's supplements market indicate an astonishing 3985.4 million US$ by 2034[2]. This is associated with a growing awareness of the health benefits of the supplements and the rapidly growing population of working mothers who see the infant powder formula and dietary food as ready and easy to give to their children. In Africa, food supplements could ease malnutrition in millions of children. Although global health officials wondered whether a food supplement was superior in relieving malnutrition, in 2016, a study in Uganda found that a therapeutic food supplement made of peanut butter, dried skim milk, vitamins, and minerals reduced malnutrition by 33%. One of the upcoming supplements is moringa leaf powder. In South Africa, various programs have been implemented to reduce childhood malnutrition through the use of available indigenous crops like *Moringa oleifera*. In their review, Sokhela *et al.* (2023), reviewed the potential of Moringa leaf powder as a fortificant.

### 2.3.6 Role in reducing food insecurity

The Global Report on Food Crises (GRFC), a collaboration among 16 organisations, presents a thorough assessment of acute food insecurity around the world. According

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[2] https://www.futuremarketinsights.com/reports/childrens-health-supplement-market. Access on 23/10/2024.

[3]https://www.voanews.com/a/food-supplement-ease-malnutrition-children-africa-study/3185592.html. Accessed on 23/10/2024.

to the GRFC, the number of individuals experiencing acute food insecurity hit a new high of 135 million in 2019. This growth shows that the situation has worsened since previous years. Conflicts were highlighted as the leading cause of acute food crises in 2019. However, research emphasizes the increasing importance of extreme weather events and economic shocks as contributors to food insecurity. These factors, combined with conflicts, have resulted in a significant increase in the number of people suffering acute food shortages and famine worldwide, Latin America and the Caribbean have seen the fastest growth in food insecurity, rising from 22.9% in 2014 to 31.7% in 2019. An estimated 2 billion people worldwide experience moderate or severe food insecurity.

### 2.3.7 Risks

Dietary supplements are not required to be approved by the FDA before they are marketed, which means there is often limited evidence regarding their safety and efficacy. Under the Dietary Supplement Health and Education Act of 1994, the FDA can only monitor adverse events after products are on the market. This lack of premarket scrutiny raises concerns about the potential for harmful ingredients or misleading claims. Supplements can interact with prescription medications, leading to adverse effects. For instance, vitamin K can reduce the effectiveness of blood thinners like warfarin, while St. John's wort can interfere with various medications, including antidepressants and birth control pills. Such interactions can pose significant health risks, particularly for individuals on multiple medications (Fairman & Yapp, 2005; Hutter, 2006).

### 2.3.8 Regulations

Individuals may take DS for several reasons, including maintaining nutritional adequacy, protecting tissue structures and functions, lowering the risk of certain diseases and age-related changes, and improving physical performance. The scientific evidence supporting the functions of DS varies depending on the supplement ingredient. Some supplements may have significant scientific support for their claimed benefits, while others may have limited or inconclusive evidence. The FDA regulates claims about health benefits or other positive outcomes on DS labels and advertisements. However, the key regulatory obligations for DS manufacturers and marketers are centered on product safety and the correct labelling of product identification and composition. DS, unlike pharmaceuticals, are classified as food products, which eliminates the need to demonstrate efficacy. Instead, producers and marketers are primarily responsible for ensuring product safety and accurately describing its identity and composition on labels.

## 2.4 SOURCES OF DIETARY SUPPLEMENTS

Over the years, the usage of nutritional dietary supplements (DS) has expanded in all main categories. Some people incorporate DS into their healthy lifestyle, while others use it to reduce risk factors for specific diseases, like heart disease (vitamin E, folic acid, and garlic extracts). Other DS is used for short-term effects such as sleep management and athletic performance enhancement (pyruvate and creatine). Food law controls DS, although several restrictions only apply to them. Thus, DS are subject to food law, but with certain exceptions, they are eligible for Food and Drug Administration (FDA)-authorized health claims under the Nutrition and Labelling Education Act (NLEA). Health claims for folic acid and calcium have already been approved, although with significant restrictions that apply to DS. Athletes at the ancient Greek Olympics popularised high-protein diets, which had a long history in sports nutrition when the DS Act passed in 1994. Research shows that intense training increases protein requirements, and experts often recommend that athletes consume 50–100% more protein than their counterparts (Tarnopolsky, 2001).

### 2.4.1 Fruits

Fruits and vegetables contain a diverse range of antioxidants. The total antioxidant capacity of common fruits and vegetables was evaluated using the Oxygen Radical Absorbance Capacity (ORAC) assay, which used AAPH as a peroxyl radical generator.

According to Ou *et al.* (2002), the antioxidant capacity of kale, blackberries, strawberries, spinach, blues, cranberries, and raspberries was greater than 100 µmol TE/g dry matter (DM). The antioxidant capacity of beets, prunes, plums, red peppers, and Brussels sprouts ranged from 60 to 100 µmol TE/g DM, while garlic, pink grapefruit, onions, cherries, tomato, lettuce, and corn ranged from 20 to 60 µmol TE/g

DM. Fruits and vegetables include potatoes, sweet potatoes, yellow squash, cucumbers, string beans, apples, celery, and bananas (Halliwell *et al*., 1992).

### 2.4.2 Leaves

The presence of antioxidant components in botanicals is widespread. In addition to findings, additional studies have found antioxidant activity in common fruits and vegetables and tea. Antioxidants can also be found in berry and fruit wines, evening primrose meal, buckwheat groats, *Ginkgo biloba*, aromatic herbs, mulberry leaves, the leaves of the little *Vernonia amygdalina* tree, *Perilla frutescens* leaves, and hawthorn organs. They did this by using a wide range of methods. While a wide variety of botanicals have well-documented antioxidant components, demonstrating antioxidant activity may not necessarily require consistent methods for comparing various sources. Developing more of these sources as antioxidant dietary supplements will necessitate consistent analytical techniques for evaluating these supplements (Halliwell *et al., 1992*).

### 2.4.3 Roots

The pea and rice protein supplement features a blend of plant-based proteins, specifically from peas and rice, providing 26 grams of protein per serving. It also includes added vitamins, minerals, and phytonutrients aimed at reducing inflammation and improving gut health. Designed to serve as a nutritious meal replacement or supplement, the Root Reset Protein Powder offers a versatile option for individuals seeking to boost their protein intake and reap the benefits of root-based ingredients. Dietary supplements often use beetroot for its high nitrate content, which can enhance athletic performance, and improve blood flow but also reduce inflammation, lowering blood pressure, and promoting gut health[3]. While not a direct protein source, beetroot powder can be combined with protein powders to create a more balanced supplement that supports endurance and recovery. *Hydrastis canadensis* L. (goldenseal) root extracts, a herbal product in the US, that belongs to the Ranunculaceae family, are widely used as phytomedicines to treat skin infections, urinary tract infections, gastrointestinal diseases and upper respiratory tract infections. These extracts contain

[3] https://www.medicalnewstoday.com/articles/beet-root-powde. Accessed on 28/10/2024.

the isoquinoline alkaloids β-hydrastine and berberine, which are both beneficial to health and effective against germs (Mukherjee *et al.,* 2011).

### 2.4.4 Algae

This diverse group of photosynthetic eukaryotes has been the center of attention for decades due to their good nutritional profile, sustainability advantages and nutraceutical contents. According to Wu *et al*., (2023), algae are an excellent source of nutrients such as protein, vitamins, minerals, essential fatty acids and oils. Cyanobacteria have been commercialised as dietary supplements and have acquired popularity due to their health benefits for adults and children, such as increased energy, improved mood, antioxidant qualities, and anti-cancer effects (Merel *et al.*, 2013)

### 2.4.5 Insects

Dietary supplements derived from insects are gaining popularity as a sustainable and nutritious protein source. Here are some notable sources of protein from insects used in dietary supplements:

Crickets, particularly the species *Acheta domesticus*, are one of the most common sources of insect protein. They contain approximately 60-70% protein by dry weight and provide a complete amino acid profile, making them a valuable protein source for dietary supplements.

Mealworms, the larvae of the darkling beetle (*Tenebrio molitor*), are another rich source of protein, containing around 50-60% protein by dry weight. They also provide essential fatty acids and micronutrients.

Grasshoppers are also a good source of protein, containing about 60-70% protein by dry weight. They are rich in essential amino acids and are often consumed whole or ground into a powder for use in protein supplements.

Insect protein is not only high in protein content but also offers several nutritional advantages:

Complete Protein: Many edible insects provide all nine essential amino acids, making them a complete protein source.

Micronutrients: Insects are rich in vitamins and minerals, including iron, zinc, and B vitamins, which are beneficial for overall health.

Sustainability: Insect farming has a lower environmental impact compared to traditional livestock, requiring less land and water and producing fewer greenhouse gases.

### 2.4.6 Seeds

Seeds are a rich source of plant-based protein that can be used in dietary supplements. Here are some notable examples: Hemp seeds are an excellent source of plant-based protein, providing 10 grams of protein (30 grams). They contain all 20 amino acids, making them a complete protein source. Hemp seeds are also rich in anti-inflammatory omega fatty acids and magnesium.

Pumpkin seeds, also known as pepitas, contain approximately 8 grams of protein per (28 grams). They are a good source of magnesium and tryptophan, which may help with sleep quality. Pumpkin seeds are low in lectins.

Chia seeds provide 4 grams of protein per (28 grams). They are a complete protein source and are also rich in fiber, omega-3 fatty acids, and various micronutrients.

Seeds are a versatile and nutritious source of plant-based protein that can be easily incorporated into a variety of supplements. They provide a range of additional health benefits due to their high content of healthy fats, fiber, and micronutrients. When choosing seed-based protein supplements.

## 2.5 USE OF SOUTH AFRICAN FOODS, PLANTS AND INSECTS AS DIETARY SUPPLEMENTS

### 2.5.1 Introduction

The global food problem is frequently discussed in terms of overpopulation and famine in poor countries, as well as the higher environmental impact of people in affluent countries who require more resources to produce animal protein for consumption. According to Pimentel *et al.*(1975), there is limited energy (fossil fuels) and land resources to meet the increasing demand for food due to rapid population expansion. Livestock farming, including feed crop production, takes up 70% of the world's agricultural area and uses 77 million metric tonnes of protein to produce 58 million metric tonnes of protein for human consumption annually (Steinfeld *et al.,* 2006). While these data pertain to animal protein for human consumption, there is an increasing demand for animal protein as pet food in Westernised societies. The situation could be worse than these data suggest. First, as urbanisation grows, more arable land is lost for food production. Second, climate change will have an impact on global food production. The food system is committed to deliver fundamental agricultural commodities. Agricultural food commodities such as fruits and vegetables play a key role in the economic development of many countries throughout the world. In early 2000, fruit and vegetable production in South Africa (SA) contributed approximately 3% to the GDP (Oleofse, 2013). In 2022, the sector contributed over 2.5% to GDP and was one of the sectors that exhibited a rise in economic output in the third quarter of 2022.

### 2.5.2 Case of *Sclerocarya birrea* (Marula)

#### *2.5.2.1 Origin and Occurrence*

Marula (*Sclerocarya birrea* subsp. caffra), an African savannah Indigenous tree, is particularly well known for its fruits (Hiwilepo-van Hal *et al.,* 2013) Fundamental enhancements in industrial processes, along with a holistic approach that redefines how companies function, can harmonize the interests of all stakeholders, including the environment and society (Bocken *et al.,* 2013). Marula trees are distributed in dry rural areas of Phalaborwa (Limpopo province, South Africa), but also occur in other provinces, including Mpumalanga, Kwa-Zulu Natal, and the Eastern Cape.

The marula tree prefers clay or sandy loam soils and is common in areas receiving 200–370 mm of rainfall annually (Orwa *et al.,* 2009). The fruit of the marula abscises (drops) before ripening, while still green, and then ripens rapidly within 8 days (Van Hal, 2013). The colour then changes from green to yellow, while the aroma develops and the flesh softens. Rural communities highly value the tree's fruits, using them to make juices, jams, conserves, dry fruit rolls, and alcoholic beverages (Nerd & Mizrahi, 1993). The nut is about 2-3 cm long, with one to four cavities containing 1-4 kernels, people sun-dry the kernels, eat them as is, and extract the oil. (Mojeremane & Tshwenyane, 2004).

*2.5.2.2 Proximate Value*

According to Wynberg (2012), Van Hal, (2013) and Komane *et al.* (2015), marula oil has significant rejuvenation properties due to its unsaturated fatty acids, making it a popular ingredient in cosmetic product formulations[4]. The high levels of vitamin C and phenolic compounds contribute to its antioxidant capacity. The seeds of marula are rich in protein and fats, primarily oleic acid.

*2.5.2.3 Products*

People eat marula kernels raw, roasted, and/or use them to extract oil via cold press processes. In addition to traditional cooking, marula nuts are a reliable source of protein and unsaturated fats. Rural households use marula nuts to augment proteindeficient meals (Van Hal, 2013). Another aspect of nuts is their oil content, which is a popular element in cosmetic product compositions (Wynberg, 2012; Van Hal, 2013).

*2.5.2.4 Attitudes of consumers*

The marula tree plays a crucial role in the livelihoods of rural households in eSwatini, contributing significantly to poverty alleviation and economic empowerment (2013 - 2018). According to the socioeconomic survey conducted in the Lubombo region of eSwatini, 53.3% of respondents harvest marula to supplement their household income, with 68.5% considering it a significant source of family income. Half of the respondents earn approximately $36.2 (+/-R500) per season from harvesting marula, providing substantial benefits to many households. Households often use this income to cover essential expenses such as education (books, uniforms, and school fees), groceries, electricity, and medication. Marula contributes not only to economic well-being but also to cultural and spiritual enrichment. The gathering of marula fosters social bonds within communities, highlighting its role beyond mere economic benefits (Secretariat of the Convention on Biological Diversity, 2010; Sunderland *et al*., 2004; Wynberg *et al*., 2002). Various socio-demographic characteristics, including gender, age, unemployment, and educational level, influence people's involvement in marula harvesting. For many individuals facing unemployment or lacking formal education,

[4] https://www.medicalnewstoday.com/articles/marula-oil. Accessed on the 24/11/2024.

marula harvesting provides a means of livelihood (Fisher, 2005). The use of nontimber forest products (NTFPs) like marula represents a sustainable approach to leveraging natural resources for poverty alleviation. By harvesting and selling marula fruits and seeds, local communities can generate income while promoting the conservation of their environment.

Table 2.3: Nutritional comparison of cold press nuts from the marula seed with the extraction of oil from the nut

| **Nutrient component** | **Nuts (cold press)** | **Cake** | **Reference** |
|---|---|---|---|
| **Protein (mg/100 g)** | 39.1 | 47.21 | *Malebana (2017) Mdziniso et al. (2016) Zalsi (2016)* |
| **Ether Extract (mg/100 g)** | 41.1 | 28.96 | *Malebana (2017) & Zalsi (2016)* |
| **Crude fiber (mg/100 g)** | - | 4.79 | *Malebana (2017) & Zalsi (2016)* |
| **Ash (mg/100 g)** | 4.65 | 6.61 | *Malebana (2017) & Zalsi (2016)* |

### 2.5.3 Significance of Entomophagy

Entomophagy, the practice of eating insects, has indeed been a longstanding tradition in many cultures worldwide and has garnered increasing attention in recent years due to its potential contributions to nutrition, food security, and livelihoods (Hlongwane, *et al*., 2020). However, Western countries do not widely accept the intake of insects and continue to underexplore them due to barriers such as fear and disgust associated with eating insects (Yen, 2009). Furthermore, in developed countries, eating insects is often considered primitive, dirty, or food for the poor (Balzan *et al*., 2016). In Latin American countries, such as Mexico, people have been consuming insects as a traditional dish for centuries (Ramos-Elorduy & Moreno, 2002). Entomophagy is prevalent in China due to the long-standing incorporation of certain insect species into traditional cuisine (Chen *et al*. 2009). In Thailand, insects are a popular and culturally significant food source. A study by Hanboonsong *et al.* (2013), shed light on the diversity of edible insect species consumed in Thai cuisine and their nutritional benefits. As far as the African continent is concerned, entomophagy is widespread across various African countries, where insects are a traditional and important protein source (Alamu *et al.,* 2013). In these countries, the customary use of insects as food dates back to the beginning of human history. Various nations in Africa consume over

500 insect species as food (Kelemu *et al.,* 2015). In Africa, people use edible insects as a staple, an emergency food supply during times of food scarcity, or an important delicacy (Agea *et al*., 2008; Niassy *et al*., 2016). Consumption and preference for edible insects vary geographically. For example, some people enjoy eating insects, whether fried, roasted, or raw, while others find them repulsive (Alamu *et al.,* 2015).

In South Africa, edible insects include Lepidopteran caterpillars, termites, grasshoppers, jewel beetles, ants, and stink bugs (Makhado *et al*., 2009). Edible insects are an essential natural resource for vulnerable people, as well as a source of income for traders and harvesters (Van Huis, 2015). The “vhaVenda” people in the Limpopo province of South Africa exclusively consume and regard stink bugs (*Hemiptera*: Tessaratomidae) as a delicacy while specific species in specific locations in various African countries hold traditional value (Teffo *et al*., 2007). In South Africa, edible insects have been a component of the human diet since prehistoric times; Ledger (1971) stated that South Africans ate *Trinervitermes trinervoides* (a termite) and *Apis mellifera unicolour* (a honeybee) in the early 100,000 BCE. In addition, Quin (2013) revealed that the “baPedi” people in South Africa continued to consume edible insects for nutritional benefits in the early 1950s. The vhaVenda, baPedi, and Vatsonga people in the Limpopo region consume edible insects the most in South Africa (Nonaka, 2009). In addition, the vhaVenda, baPedi, and Vatsonga people in the Limpopo region also consume edible insects in Mpumalanga and KwaZulu-Natal (Shackleton, 2002), Northwest, and Gauteng (Teffo, 2007).

Furthermore, edible insects contribute significantly to food security, rural livelihoods, and poverty elimination. Makhado *et al.,* (2014), observed that trading edible insects generates an average income of $202,915 per dealer throughout one season. Edible insects provide seasonal work opportunities for unemployed people in Southern Africa, lowering poverty and promoting human well-being (Makhado *et al*., 2014).

Malnutrition and food scarcity are key difficulties faced by developing countries, the majority of South Africans are food insecure, meaning they lack access to sufficient food to meet their daily nutrient requirements. Edible insects are ingested because they are a nutritious traditional food source that has long been utilised to complement diets in Africa (Niassy *et al*., 2016). In addition, the consumption of insects is good for human well-being, as the nutritional value of edible insects can help promote human health and reduce the vulnerability to malnutrition of children, pregnant women, and older people (Belluco *et al*., 2013).

### 2.5.4 Case of *Gonimbracia belina* (Mopane worm)

*Gonimbrasia belina*, commonly known as the mopane worm, is a species of emperor moth native to southern Africa, particularly thriving in regions with mopane trees (*Colophospermum mopane*) that serve as its primary food source. This caterpillar is not only a vital protein source for local communities but also plays a significant role in the region's economy and culture. First scientifically described by Westwood in 1849, the mopane worm has garnered attention for its nutritional value, containing high levels of protein and essential fatty acids, making it a key component of traditional diets in countries like Zimbabwe and Botswana. However, the species faces threats from overharvesting and habitat destruction, raising concerns about its sustainability and the ecological balance of its native environments. As interest in edible insects grows globally, the mopane worm stands out as a symbol of both culinary heritage and biodiversity conservation efforts in southern Africa.

#### *2.5.4.1 History*

Much of the research on prehistoric diets focused on meat procurement, or the slaughter of large animals. This was a significant socio-cultural aspect of antiquity. However, McGrew (2001) cautions against a distorted understanding of early eating. In terms of animal protein, archaeological evidence from around the world suggests a varied diet that includes fish, birds, lizards, mice, rabbits, turtles, crabs, molluscs, and shellfish “Barker (2009)”. Coprolite tests show that early American Indians (up to 9,500 B.P.) ate termites and predaceous diving beetles, as well as ants, dung beetle larvae, and caterpillars in Mexico (> 5,400 B.P.) (Elias, 2010; Sutton, 1995). Archaeologists are increasingly turning to anthropological studies of ancient forager communities like this to better understand prehistoric existence. According to Sutton (1990), the Kun-San of the Kalahari and the Australian Indigenous people closely mirror such living. It is becoming increasingly obvious that research into these people's ecological anthropology has missed their use of insects for nourishment. The Kun-San consume termites, grasshoppers, caterpillars, and ants (Nonaka, 1996).

### *2.5.4.2 Utilisation*

Insects are considered traditional delicacies in the majority of Sub-Saharan African (SSA) countries, with over 470 species used for human consumption and a handful for animal feed (Kelemu *et al.,* 2015). Governments in SSA countries, however, regard the gathering, trading, and use of insects for food and feed as unofficial and unregulated. The utilisation of *Gonimbrasia belina*, commonly known as the mopane worm, is deeply embedded in the socio-economic fabric of South Africa, particularly in rural communities. This edible caterpillar serves as a vital protein source, especially during its seasonal availability from September to November when it is hand-harvested primarily by women and children from mopane trees (Mufandaedza *et al.,* 2021), (Baiyegunhi *et al*., 2016). The mopane worm industry has significant economic implications, with estimates suggesting that around 1.6 million kilograms are traded annually in South Africa, contributing to both local consumption and export markets (Wikipedia contributors, 2024, Stack, *et al.,* 2003). Sustainable harvesting practices are essential to ensure the continued availability of *G. belina*, as overharvesting and habitat destruction threaten its populations (Wikipedia contributors, 2024). Governance structures surrounding mopane worm harvesting vary across regions, with communal systems often allowing local access while private lands impose restrictions. Thus, the utilisation of the mopane worm not only supports food security but also fosters economic resilience and biodiversity conservation in southern Africa (Gondo *et al*., 2010).

### *2.5.4.3 Mopane worm occurrence*

Elevation, rainfall, temperature, soil depth, and kind influence the distribution of the Mopane tree. Its elevation ranges from 300 to 1,000 metres, and it receives between 400 and 1,000 millimetres of precipitation per year in areas with low to moderate summer rainfall. Although there is still debate about the freezing limits and sensitivity of mopane to frost damage, its southern limit suggests that it does not extend well beyond 5 °C. Low winter temperatures could explain the difference in Mopane distribution between South West Africa and Botswana along the Okavango River system. *Terminalia* sp.'s southern range in Botswana ends about 26 kilometres north of Mahalapye and *Acacia nigrescens* takes over (van Voorthuizen, 1971). Adult mopane moths emerge throughout their range between October and November, as well as again in February and March. The grownups do not consume food. They only live for a few days, and males find females by following chemical attractants (pheromones) emitted by the females. Adult moths don't wander far from where they emerge. After mating, the female lays a single cluster of 50–200 eggs on the twigs or leaves of the host plant. The eggs hatch in about ten days, resulting in small black larvae (caterpillars). These larvae go through five phases of development, each lasting around 5-7 days. Each larval growth stage is referred to as an instar (instar I being the earliest and instar V the most recent). Overall, during instar stages I–III, the caterpillars feed on the leaves of their host plants in groups of 20–200 and leave the colony at stage IV. Mopane worms are the caterpillar's fourth and fifth instar stages. During certain years, the host trees may have millions of caterpillars, a condition called an "outbreak." The fifth instar caterpillar (insumbe in Ndebele) burrows into the dirt at the base of mopane trees and pupates after the larval stages (Tendayi *et al*., 2010).

*2.5.4.4 Proximate Value*

After harvesting, the removal of the stomach contents from the mopane worms, followed by roasting over charcoal or boiling can extend the shelf life by over a year. This makes “Omagungu” available and provides protein in people's diets (Glew *et al.,* 1999). Degutted, dried worms are readily available at Namibian informal markets, national trade fairs, and cultural festivals. Producers used milled worms to create mopane-Omagungu soup premixes. Dreyer and Wehmeyer first published the nutritional composition of Omagungu and Omakunde in 1982, followed by Mamiro *et al.* in 2011. Mopane worms (Omagungu) express a stronger nutritional profile compared to legumes such as cowpeas (Omakunde), particularly in terms of protein (63% (N x 6.25%), ash (7.6%), iron (330 mg/kg), zinc (170 mg/kg), and copper (95 mg/kg) whereas cowpeas content of protein (25% (N x 6.25%), ash (4.3%), iron (18 mg/kg), zinc (28 mg/kg), and copper (14.7 mg/kg). Mopane worms (Omagungu) are thus a prospective protein source for lowering the prevalence of micronutrient deficiencies and protein-deficient diets in disadvantaged countries such as Namibia (Namibia Alliance for Improved Nutrition, 2010). However, certain people (infants and adults) may object to ingesting insects such as mopane worms (Omagungu). Van Huis (2017) outlines many strategies for improving consumer attitudes about eating insects. These include information about the food's safety and nutritional content; transforming the insect into recognisable product forms; emphasising the similarities between edible crustaceans and insects; and simply creating delicious insect products.

*2.5.4.5 Mopane worm derived products*

Researchers identified two principal processing procedures for obtaining mopane worms: boiling and roasting on ambers. The research showed that there was not enough variety in mopane-worm-based products. Transforming mopane worm (Omagungu) into new, tasty, and well-known food forms is crucial for its effective utilisation in addressing malnutrition. In addition, Kembo *et al.* (2022) provided insights into the proximate and mineral composition of the developed mopane-wormcontaining products.

*2.5.4.6 Safety Considerations*

Insects may contain a variety of harmful and spoilage microorganisms, such as Enterobacteriaceae, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, Salmonella, *Clostridium perfringens*, *Clostridium botulinum*, and lactic acid bacteria. These microorganisms can pose health risks if present in copious quantities or under specific conditions (Amadi & Kiin-Kabari, 2016; van der Fels-Klerx *et al*. 2018). The microbial makeup of different commercially marketed edible insect species may vary depending on factors such as insect type and habitat (Park *et al*., 2019).

Understanding these microbial profiles is crucial for assessing the safety of edible insects as food products. As a result, the study aimed to assess the microbiological safety of dried insects, specifically mopane worms, termites, and stink bugs marketed in a South Africa. This assessment was essential given the importance of these dried insect products as food items in the local market and the potential risks associated with microbial contamination.

## 2.6 BREAD AS STAPLE FOOD

### 2.6.1 Introduction

In all its varieties, bread is one of the most popular foods consumed by humans. People typically use wheat flour to make bread. Currently, only wheat and a few other commonly consumed cereal grains, along with certain cereals, pulses, and legumes, can be combined with flour and water to create a sticky dough that can be baked into bread. Genetic manipulation may eventually combine wheat's protein qualities with those of other more conveniently produced and processable seeds, such as providing no crease or a more rounded form, but for the time being, the study deals with a genetically unmodified cereal crop Zohary (1969).

This is one of the oldest manufactured foods, having played a vital role in religion and society since the dawn of agriculture. Bread is another macronutrient-rich staple. Human health requires macronutrients (carbohydrates, protein, and fat) and micronutrients (minerals and vitamins). There are 180 diverse types of baked or steamed bread, ranging from baguettes to whole wheat bread, based on their basic ingredients, culture, and country. Cappelli *et al,* (2020) argue that the quality of the flour source, milling process, and dough qualities distinguish good bread. Researchers constantly seek alternative composite flours due to the common use of wheat-based flours in dough-based items such as bread, pasta, and pastries (Sarawong *et al*., 2014).

Wheat flour combined with rye flour are the most widely investigated composite flour. The gluten-forming proteins and gas-holding capability of wheat flour make it distinctive in the preparation of leavened baked goods (Dobraszcyk, 2001). However, when researching breadmaking, it is critical to assess various features, particularly when the goal is to fortify bread. Rheology, the dough's baking properties, and the bread's quality are among the most important parameters to be considered. However, since the goal is to create a product acceptable to customers, sensory evaluation and shelf life studies are essential too.

### 2.6.2 History and significance in human nutrition

People have been making bread since 10,000 B.C., during the Neolithic period, coinciding with the introduction of agriculture when seeds were the primary ingredient. Some of Egypt's oldest surviving papyri contain bread-making instructions, revealing that the first bread was in a form similar to today's (Karnahoriti, 2016).

Bread is a tasty and nutrient-dense food that forms the basis of our daily diet and is part of the traditional diet, especially among the poor populations. The origins of bread can be traced back to prehistoric times, with evidence suggesting that early humans discovered the process of mixing ground grains with water to create a dough that could be cooked over fire. This simple yet revolutionary technique marked the beginning of bread-making, which quickly spread across cultures and continents (Standage, 2005) The ancient egyptians are credited with developing leavened bread around 6000 BC, likely through their brewing practices that introduced wild yeast into dough mixtures. This innovation allowed for lighter and airier loaves compared to earlier flatbreads. As bread-making techniques evolved, different civilizations adapted their methods and ingredients. The greeks and romans further refined these processes by experimenting with various grains and enhancing flavours through herbs and spices. During the middle ages, bread-making became a skilled profession often performed in monasteries known for producing high-quality breads. The invention of steam-powered roller mills in the 18th century revolutionized flour production by making it more accessible and affordable for the general population. The industrial revolution in the 19th century brought about significant changes in bread-making technology. New machinery improved efficiency, allowing for mass production of bread on an unprecedented scale. However, this shift also led to concerns about the quality of mass-produced bread due to the use of preservatives and artificial ingredients. Despite these advancements, traditional artisan bread-making has seen a resurgence as consumers seek natural ingredients and time-honored techniques that produce unique flavours and textures. Today, while modern technology allows for rapid production, many still appreciate the craftsmanship involved in artisan baking (Parker *et al*., 2018).

### 2.6.3 Principles of bread making

The principles of bread making involve several essential stages that contribute to the development of flavour, texture, and structure in the final product. It begins with ingredient selection, where high-protein flour, yeast, water, and salt are carefully chosen to create the right dough. The mixing and kneading process is crucial for developing gluten, which gives bread its structure. Following this, the dough undergoes fermentation, allowing yeast to ferment sugars and produce carbon dioxide, causing the dough to rise. After the first rise, the dough is shaped and allowed to rise again in a process known as proofing. This second rise enhances the bread's volume and texture. Once proofed, the dough is baked in a preheated oven, where steam is often introduced to create a moist environment that aids in crust formation and oven spring. Finally, proper cooling is essential to set the crumb structure and develop flavour (Demirkesen *et al.,* 2010 ; Mohammed *et al.,* 2010 ; Coda *et al.,* 2011).

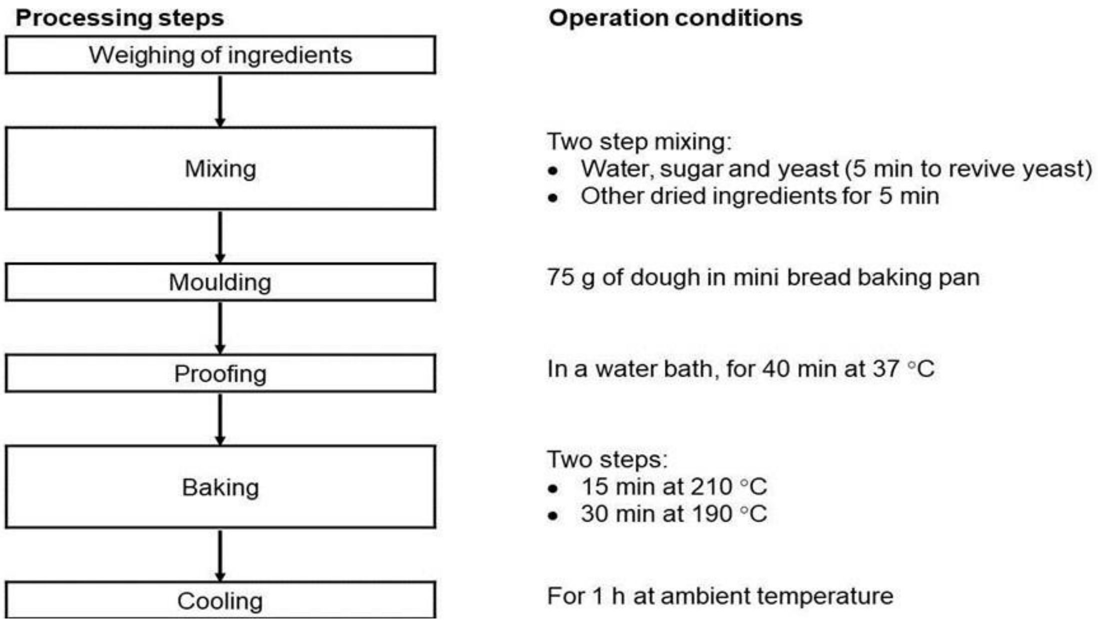


Figure 2.1: Flow chart of the commercial bread-baking process

### 2.6.4 Bread ingredients and functional properties

Bread is a staple food eaten worldwide, and its components contribute to the final product's structure and flavour. Flour is a crucial constituent and functional characteristic of bread, providing structure through gluten formation. Wheat, rye, barley, and rice flour are among the many flour forms. Wheat flour is the most often used flour because it contains gluten (Shewry & Halford, 2002). Water activates the gluten in the flour and helps to hydrate the dough. It helps regulate the dough's consistency and texture. The amount of water used influences the final crumb structure (Gänzle, 2015).

The yeast ferments the sugars in the dough, generating carbon dioxide ($CO_2$) that enables the bread to rise while also contributing to its flavour (Gobbetti *et al.,* 1994). Active dry yeast and quick yeast are two common types used in bread making. Salt plays a vital role by strengthening the gluten structure, reducing yeast activity, and enhancing flavour. Sugar not only feeds the yeast but also sweetens the dough and improves crust color, affecting fermentation and promoting browning during baking (Figoni, 2008). Fats such as butter, oil, and shortening soften the crumb, add moisture, and prolong shelf life. The combination and proportions of these ingredients contribute to the specific characteristics of various types of bread, including texture, flavour, and appearance. During the baking process, the functional properties of each component interact to form the final product. Changing the ratios and types of ingredients can cause variances in the texture and flavour of the bread, allowing for a wide variety of bread types to suit different tastes and cooking traditions (Wandersleben *et al.,* 2018).

### 2.6.5 Understanding dough rheology

Dough rheology is the study of the flow and deformation behaviour of dough, which is used to manufacture a wide range of baked goods, including bread, pastries, pizza and many others. Rheology involves understanding how dough reacts to different stress, strain, and temperature conditions (Bourne, 2002). Proteins, particularly gluten-forming proteins like glutenin and gliadin in wheat flour, have an essential role in dough rheology. Gluten gives the dough flexibility and strength, allowing it to expand and retain gas formed during fermentation (Scanlon *et al.,* 2013). The amount of water in the dough affects its rheological properties; gluten and dough are formed when flour proteins are hydrated (Amjid *et al.,* 2013).

The fermentation process affects the dough's extensibility and elasticity, and several additives including enzymes, emulsifiers, and dough conditioners can modify these rheological properties (Kadam *et al*., 2021). Food manufacturers often use these additives to enhance mixing, fermentation, and overall product quality. The mixing technique affects gluten formation and dough consistency; overmixing can lead to excessive gluten weakening and to an undesirable result (Scanlon *et al.,* 2013).

Combining and proofing the dough at different temperatures affects the fermentation rate, enzyme activity, and rheology of the dough (Rhee *et al*., 1982). Understanding dough rheology is essential for bakers and food scientists to improve production processes and ensure consistent quality in finished baked goods. Rheological tests such as mixolab and extensograph analysis are commonly used to assess dough properties and performance. These tests help to fine-tune dough formulation and processing parameters so that the end goods have the correct texture, volume, and overall quality (Amjid *et al.,* 2013).

### 2.6.6 Bread quality features

Bread quality attributes comprise several characteristics that define the final product's texture, volume, flavour, appearance, and overall appeal. Specific characteristics may vary depending on the type of bread and consumer preferences. A desirable bread often has a golden to deep brown crust, which should be crisp for baguettes and softer for sandwich bread (Heenan *et al.,* 2008). The crumb's (internal) structure should be well-developed, with uniform cell size and texture. The ideal crumb texture is soft and elastic, but this may differ depending on the type of bread. Tasty bread, varying in flavour from moderate to rich, is often associated with a pleasant, yeasty scent. The bread should have an appropriate level of moisture. It should not be too dry or wet (Faridi *et al.,* 2010). The bread should be the correct size and shape, well-risen, and have a consistent volume, size, and shape. Depending on the type of bread, maintain a balanced crust thickness, neither too thick nor too thin. Aesthetically pleasing bread has a well-shaped form and proper scoring. A combination of sensory analysis, instrumental measurements, and consumer choice determines bread quality (Schober *et al.,* 2003).

Baking performance relates to the overall behaviour of dough throughout the baking process and how it affects the quality of the finished baked goods. It is a set of physical, chemical, and biological activities that take place during mixing, fermentation, proving, and baking. Protein quantity and quality influence gluten formation, which is critical to the final product's structure and texture (Hoseney, 1994). Because the mixing process is crucial for gluten development and optimal component distribution, flour's ability to absorb water (water absorption (WA)) affects dough consistency and hydration levels, which influence the final texture of baked items. Overmixing or undermixing might affect the structure of the dough. The fermentation process produces carbon dioxide gas, causing the dough to rise; this time is critical for flavour development and crumb structure (Rosell *et al.,* 2007). Understanding how dough flows and deforms (rheology) is essential for optimising processing conditions and achieving the desired texture. Before baking, the last rise, or proof, permits the dough to expand; controlling the proofing time and temperature is crucial for achieving the desired volume and texture (Pyler & Gorton, 2010). To optimise these factors, a combination of skill, experience, and knowledge of baking science is required. Bakers extensively use dough analysis equipment, such as the Mixolab or Farinograph, and they may modify recipes and processes depending on the properties of certain flours and environmental conditions (Musim Mas, 2023).

### 2.6.7 Aroma

The term "aroma" refers to something's pleasant or distinctive smell. It is usually a complex mixture of different organic chemical compounds (Trifković *et al*. 2016) and is an important aspect of the sensory experience, particularly when it comes to tasting food and beverages. The sense of smell intricately links to the sense of taste, significantly influencing the perception of flavours. Aroma is often considered alongside taste and texture as one of the key elements contributing to the overall enjoyment of a dish. For example, the aroma of freshly baked bread, the complex bouquet of fine wine, or the fragrant spices in a curry all contribute to the sensory experience and can enhance the enjoyment of the meal. Additionally, in the production of food and beverages, one can manipulate or desire specific aromas to create a desired sensory profile (Qian & Reineccius, 2002).

### 2.6.8 Shelf life

Understanding the shelf life of products, particularly food items like bread, is crucial for making informed decisions about consumption and safety. The duration a product can maintain its quality influences factors such as flavour, texture, colour, and safety. Various elements impact bread's shelf life. Ingredients like fats and preservatives can affect the rate of staling and microbial growth, thus extending or shortening the time it remains fresh (Healthline, 2019). Natural antioxidants, such as those found in whole grains, may also play a role in prolonging shelf life by minimizing oxidative processes (Musim Mas, 2023). Regulations, such as those outlined by South Africa's Minister of Health, often dictate guidelines for indicating product quality, particularly through "best quality before date" and "sell-by date" labels. These regulations help ensure consumer safety and prevent the sale of compromised products, especially those intended for vulnerable populations like infants and young children (SADC, 2024). Monitoring parameters such as staling, microbial spoilage, and colour loss over time helps determine a product's shelf life. Techniques like texture analysis and spectroscopy aid in assessing these changes, while microbial risk assessment can identify and mitigate potential foodborne pathogen risks in the bakery chain (Bakery Academy, 2023). Maintaining product uniformity and quality, including colour consistency, is essential for customer satisfaction. Food-grade colourimetric spectrophotometers help bread producers achieve this goal by providing detailed colour data to ensure consistency across batches (Bianchi *et al*., 2024).

## 2.7 CONCLUSION

Food security remains a pressing global issue, particularly in regions like Southern Africa, where access to safe and nutritious food is often compromised due to various factors, including poor harvests and economic instability (UN, 2024). Dietary supplements can play a role in bridging nutrient gaps, especially among food-insecure populations[5]. However, their use tends to be lower in these communities, exacerbating nutritional deficiencies (World Health Organization, 2020). South African foods, including indigenous plants and insects, offer rich sources of essential nutrients and can enhance dietary diversity (van der Merwe *et al.,* 2018). Incorporating these local foods into diets not only supports food security but also promotes sustainable practices and cultural heritage (South African Department of Agriculture, Forestry and Fisheries, 2019). Bread serves as a staple food in many diets, providing a reliable source of carbohydrates and energy. Its widespread consumption underscores the importance of ensuring that staple foods are accessible, affordable, and nutritious for all populations. Addressing food security requires a multifaceted approach that includes promoting local food sources, integrating dietary supplements where necessary, and ensuring that staple foods like bread remain available to support the nutritional needs of vulnerable populations (National Research Council, 2015).

[5] https://www.ifpri.org/blog/world-food-day-2024-the-critical-role-of-healthy-diets-for-realizing-the-right-tofood/. Accessed on the 24/11/2024.

# CHAPTER 3

# MICROBIOLOGICAL SAFETY AND PROXIMATE COMPOSITION OF MARULA SEED CAKE AND INSECT FLOURS

## 3.1 INTRODUCTION

In recent years, there has been a growing interest in exploring alternative sources of protein and nutrition to address global food security challenges. Marula seed cake and insect flours represent two unconventional and promising sources of protein that have gained attention for their potential to supplement or even substitute traditional dietary staples. Understanding their microbiological safety and proximate composition is critical when evaluating their suitability as viable food ingredients. For millennia, ancient civilizations have consumed plants and animals for their primary dietary needs. Their superior food preparation and preservation techniques have aided in the production of the diverse cuisine enjoy today (Albala, 2011). Wheat, maize, rice, and barley contribute more tonnage to the global food supply than any other crop combined, accounting for at least 75% of total cereal grain production (Hung, 2016). Cooking, grinding, and storage knowledge, as well as pulse and cereal technology development, have all contributed to an increase in the availability of healthful basic ingredients.

Marula (*Sclerocarya birrea*) is a fruit-bearing tree native to the southern regions of Africa. Traditionally, people have consumed its fruits for their nutritional and medicinal properties but often discard or use the seeds as animal feed (Coates, 2002). However, recent research has focused on the potential of marula seed cake, a by-product of oil extraction from marula seeds, as a nutrient-rich ingredient for human consumption (Dlamini *et al*., 2012) Insects, on the other hand, have long been a part of human diets in many cultures around the world. Rich in protein, fats, vitamins, and minerals, insects offer a sustainable and environmentally friendly source of nutrition. As such, insect flours derived from various species have emerged as novel food ingredients with the potential to address both nutritional and sustainability concerns.

The safety of marula seed cake and insect flours, despite their nutritional benefits, ensures their suitability for human consumption (Rumpold & Schlüter, 2013).

Microbiological safety evaluations involve examining the presence of harmful microorganisms, such as bacteria and fungi, that could pose health risks if consumed. Proper processing and storage techniques are essential for minimising microbial contamination and ensuring the safety of these products (van Huis *et al*., 2013). The implication of the HACCP system for the production and consumption of larvae is still in the implementing phase as several critical control points should be identified throughout the different stages of the processing with acceptable limits determined (Arevalo *et al*., 2022). Good manufacturing practices (GMPs) are recognised as one of the most significant tools for improving sanitary conditions in food processing, as well as incredibly valuable instruments for ensuring product safety. Mitigating risks by applying GMPs results in a more efficient and structured work environment, which improves the overall production process. (Yüksel & Oglu, 2007). A thorough inspection of flours should involve determining the absence of yeast, mould, and mycotoxins.

Furthermore, since the flour's physicochemical properties (e.g., colour, protein, fat, dietary fibre, carbohydrates, and moisture) influence its quality, these parameters must be understood before making a baked product such as bread (Lin *et al.,* 2019). The aim was to investigate the microbiological safety and proximate composition of marula seed cake and insect flours to evaluate their potential as sustainable and nutritious food ingredients. This can contribute to the growing body of knowledge surrounding alternative food sources and inform future strategies for enhancing food security and sustainability on a global scale, as these characteristics influence the quality of composite bread.

## 3.2 MATERIALS AND METHODS

The studies were conducted at Tshwane University of Technology (TUT), Arcadia campus, Department of Biotechnology and Food Technology in Pretoria, South Africa, and the research procedures were by the university's internal ethics committee's guidelines and standards, adhering to appropriate national regulations and standards set forth by South African authorities, such as the Department of Health or the South African Bureau of Standards (SABS).

### 3.2.1 Collection and source of materials

The collection process involved careful selection and handling of samples to minimise contamination. After collection, the samples were stored in sealed bags in a refrigerator maintained at 4±1 °C. Refrigeration commonly preserves the freshness and quality of food samples while inhibiting microbial growth. Strict hygiene measures were maintained during raw material acquisition, transit, storage, and processing. Commercial wheat flour obtained from Ruto Mills (PTY) Ltd., a local flour miller located in Pretoria, South Africa, was used as a reference for comparison to the composite flours developed in the study. It serves as standard commercial wheat flour. Mopane Worms was acquired at a local street market in Pretoria North, South Africa. Gathered in the field, specifically in the Mpumalanga province, it was cooked in salted water and sun-dried for a few days before transportation to Pretoria (Figure 3.1). Mealworm (Darkling Beetle) was purchased from Scaled Impact Insectivor®, a rearing company located in Johannesburg, South Africa (Figure 3.2). Marula Seed cake was obtained from the MarulaGuys, located in Modimolle, Limpopo, South Africa.

### 3.2.2 Mopane worm

Mopane worms, which are edible caterpillars from the *Gonimbrasia belina* species, are a popular delicacy in various parts of southern Africa, including South Africa. Before their purchase from the open Marabastad street market in Pretoria (South Africa), the Mopane worms were harvested by hand from mopane trees, gutted and cleaned to remove the digestive tract. The cleaned worms were boiled in salted water to ensure their safety for consumption and sun-dried.

The sun-dried worms were transported for further drying at 50 °C in a convectional drier for two days before milling (Foss Homogenizer 2096 from Sweden). The flour was sieved in a sequential manner, starting from the largest mesh size to the smallest, separation of different particle sizes of meshes (0,750 μm, 0,500 μm, 0,250 μm, and 4,7 μm).

Figure 3.1: Sun drying mopane worm

Figure 3.2: Dried mopane worm sold on the street market in Pretoria

### 3.2.3 Mealworm

One kilogram of dried yellow mealworm (darkling beetle) (Figure 3.3) was purchased from the rearing company, Scaled Impact Insectivor® (Johannesburg, South Africa).

Figure 3.3: Mealworms (**iStockphoto**. n.d.)

The worms were mostly fed on wheat bran, brewery and milling waste products as well as off-cut carrots. Before harvest, the larvae were degutted and frozen at -20 °C (overnight). The frozen worms were boiled for 3 min. and then air dried at 200 °C for 2 h, before milling (Foss Homogenizer 2096 from Sweden). The flour was sieved in a sequential manner, starting from the largest mesh size to the smallest, separation of different particle sizes of meshes (0,750 μm, 0,500 μm, 0,250 μm, and 4,7 μm).

### 3.2.4 Marula seed cake

Cold-pressed marula seed cake was sourced from MarulaGuys (Modimolle, Limpopo, South Africa). The cold-pressing process, which mechanically extracts the oil, is environmentally friendly and retains the nutrients of the seeds, positioning marula seed cake as a potentially valuable ingredient for food products. The large shell pieces were removed by hand and the smaller pieces and flour were sieved through various mesh sizes, ranging from 4.7 μm to 750 μm, with the shells removed. The dried marula seed cake underwent air drying at 50 °C in a conventional dryer for two days before milling using a Foss Homogenizer (Sweden).

### 3.2.5 Chemical consumables

Organic solvents (methanol, ethanol, lactic acid) were purchased from Sigma Aldrich (Aston Manor, Johannesburg, South Africa) and were analytical reagent (AR) grade.

For the microbial count assays, culture media and related reagents were purchased from Merck (Johannesburg, Gauteng, South Africa).

## 3.3 MICROBIOLOGICAL QUALITY OF INSECTS AND MARULA SEED CAKE FLOUR

Total Viable Count (TVC) and the enumeration of total fungal count are critical microbiological methods for assessing the microbial quality of food products, including insect-based foods and marula seed cakes. According to the AOAC (2002), (Association of Official Analytical Chemists), these methods are essential for ensuring food safety and quality.

### 3.3.1 Total viable count

Microbial analyses for composite flours were determined according to AOAC (2002). This was done by placing a 10 g composite flour sample into a sterile homogenising container and then adding 90 mL sterile buffered peptone water. The mixture was then homogenised for 2 min, followed by three serial dilutions, by mixing 1 mL of the sample with 9 mL of sterile peptone water. Enumeration of Total viable count (TVC) was done according to (AOAC 2002: 990.12), and pre-prepared sterile Plate Count Agar (PCA, Biolab, Johannesburg, South Africa) was used for culturing and enumerating bacterial colonies. Inoculating the sample onto the PCA plates was done aseptically by pouring 1 mL of the sample from each dilution onto a separate Petri dish, sterile processed cooled (50 °C) 15 mL PCA into the sterile Petri dish mixed and left to set. Incubation was done at 37 °C for 24-48 hours. The number of bacterial colonies was counted and expressed as colony-forming units per millilitre (cfu/mL) of the sample and colonies in the range of 30 to 300 were to be counted. The cfu/mL was counted and calculated using Equation 3.1 for all plate counts.

Microorganisms on selective media are utilized to isolate specific types of organisms by allowing certain microorganisms to grow while inhibiting others. This method can be time consuming but is fundamental for identifying viable organisms (*Bacillus cereus*, Coliform, *Escherichia coli* etc).

……….Equation 3.1

$$\text{cfu/mL} = \frac{\text{Number of cfus}}{\text{Volume plated (mL)}} \text{ x Total dilution factor}$$

Where:

cfu/mL is the number of colony-forming units per millilitre of the original sample

The number of cfu is the total number of colonies counted on the plate

Volume plated (mL) is the volume of the diluted sample spread onto the agar plate

The total dilution factor is the overall dilution of the original sample

### 3.3.2 Total fungal count

The extent of yeast and moulds on the selected flours was determined using the ISO 21527-1:2008 standard. 0.1 mL of each dilution was placed on Dichloran Rose Bengal Chloramphenicol Agar (DRBC, Biolab, Johannesburg, South Africa) using a spread plate approach. After 5 days of incubation at 25 °C, yeast and moulds were counted and calculated.

### 3.3.3 Bacillus cereus

ISO 7932:2004 specified a horizontal method for the enumeration of viable Bacillus cereus using the colony-count technique at 30 °C. This method involved the homogenization of samples, serial dilutions, and plating on selective media, such as Mannitol Egg Yolk Polymyxin (MYP) agar. After 18-24 hours of incubation, colonies were identified based on their characteristic color and halo formation on MYP agar, and the counts were calculated.

### 3.3.4 Coliforms

The detection of coliforms was performed using the ISO 4831:2006 method. A volume of 1 mL from each dilution was placed on MacConkey agar (MacConkey agar, Biolab, Johannesburg, South Africa) using a spread plate approach. These methods typically involved multiple-tube fermentation techniques or membrane filtration.

### 3.3.5 Escherichia coli

The identification of Escherichia coli was conducted using ISO 7251:2005. A volume of 1 mL from each dilution was placed on MacConkey agar (MacConkey agar, Biolab, Johannesburg, South Africa) using a spread plate approach. The procedure included incubation at 37 °C for 24 hours, followed by confirmation tests such as indole production.

### 3.3.6 Enterobacteriaceae

The Enterobacteriaceae family was identified using ISO 21528-1:2004. A volume of 1 mL from each dilution was placed on Violet Red Bile Glucose Agar (Violet Red Bile Glucose Agar, Biolab, Johannesburg, South Africa) using a spread plate approach. The procedure included incubation at 37 °C for 24 hours and the performance of biochemical tests to confirm the presence of Enterobacteriaceae.

### 3.3.7 Salmonella

Salmonella was identified by ISO 6579:2017. A volume of 1 mL from each dilution was placed on Hektoen enteric agar (Hektoen enteric agar, Biolab, Johannesburg, South Africa). This method included preenrichment in a non-selective broth, followed by selective enrichment in specific media.

### 3.3.8 Staphylococcus aureus

The detection of Staphylococcus aureus was guided by ISO 6888-1:1999. A volume of 1 mL from each dilution was placed on Baird Parker agar (Baird Parker agar, Biolab, Johannesburg, South Africa). This standard involved inoculating samples onto selective media, incubating at 35 °C for 24-48 hours, and confirming colonies through biochemical tests such as coagulase testing.

## 3.4 MYCOTOXINS

Samples were ground or homogenized to create a representative test portion. The uneven distribution of mycotoxins in food matrices necessitated thorough sample preparation for accurate analysis. Appropriate solvents and extraction techniques were used to extract mycotoxins from the prepared samples. After milling, thorough mixing of the samples was performed to ensure that all portions were uniformly represented, which was critical for reliable analysis. Organic solvents were employed to extract mycotoxins from the sample matrix, following a conventional approach used in the initial extraction phase.Solid-Phase Extraction (SPE) was utilized to purify the extracts by removing interfering substances that could compromise analytical accuracy. This technique selectively retained mycotoxins while allowing other components to pass through.The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction method was particularly beneficial for complex matrices like bread. This method combined extraction with a clean-up step using acetonitrile and salts, facilitating efficient purification.High-Performance Liquid Chromatography (HPLC) was used to separate and quantify mycotoxins after extraction, providing high-resolution separation of compounds (Sulyok et al., 2006). Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) was employed as a highly sensitive and specific method that allowed for the simultaneous detection of multiple mycotoxins in a single analysis, making it a preferred choice for comprehensive mycotoxin profiling. The SAGL method was validated for 13 regulated mycotoxins, including aflatoxins, deoxynivalenol, fumonisins, and zearalenone. Validation ensured that the techniques employed met regulatory standards and provided reliable results (Meyer et al., 2019).

## 3.5 NUTRITIONAL VALUE OF FLOUR

The Nutritional value of each type of flour was determined as detailed below.

### 3.5.1 Moisture content

The moisture content of the different flour samples was determined using the Kern Moisture Balance Analyzer (Kern MLB_N, Kern, Germany). In this case, 4± 0.1 g of flour of each sample was spread evenly on an aluminium sample dish and dried at 120 °C until a constant weight was reached. The moisture content of the flour was expressed in percentage per sample.

……….Equation 3.2

$$\text{Moisture content (\%)} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \text{ x } 100$$

Initial Weight: The weight of the sample before drying (including moisture).

Final Weight: The weight of the sample after drying (after moisture has been removed)

### 3.5.2 Protein

The Dumas combustion analysis technique was used to determine crude protein. This was performed according to the Association of Official Analytical Chemists (AOAC) method using a Leco TruMacTN combustion nitrogen/protein determinator (Leco Corp., St. Joseph, MI, USA). Two grams (n=3) of flour were weighted into ceramic crucibles and placed into the autosampler. Each crucible was loaded automatically into the furnace at 1050°C. Combustion at elevated temperature in pure oxygen sets nitrogen-free, which is measured by thermal conductivity detection. (Sciences & Corporation, 2018) and the protein content was calculated by multiplying the determined nitrogen content by a nitrogen-to-protein conversion factor of 6.25. The protein content was then expressed as a protein / 100 g sample as is.

The use of EDTA standards for calibration in analytical chemistry, particularly in the context of elemental analysis, involves utilizing certified reference materials (CRMs) to ensure accuracy and reliability in measurements. LECO Corporation, a leading provider of analytical instruments, offers calibration samples that contain EDTA (Ethylenediaminetetraacetic acid) with well-defined elemental compositions.

……….Equation 3.3

$$\text{Protein Content (\%)} = \text{Nitrogen Content (\%)} \times \text{Protein Conversion Factor}$$

Nitrogen Content (%): This is determined through chemical analysis, such as the Kjeldahl method, where the amount of nitrogen in a sample is measured.

Protein Conversion Factor: This is a factor that converts nitrogen content to protein content. The most commonly used factor is 6.25, based on the assumption that protein contains 16% nitrogen (1/0.16=6.251/0.16 =

6.251/0.16=6.25).

### 3.5.3 Ash

The ash content of the different samples was determined using the AACC method 08-01 -01. The flour samples were weighed into ceramic crucibles (Infusil, USA) and placed into the ashing furnace (Carbolite AAF 1100, Carbolite Gero Ltd, United Kingdom), which was preheated at 550±2 °C maximum operating temperature.

The ashing was carried out until the residue was white (± 24 hours). Thereafter, the residual ash was placed in a desiccator (210 mm, Labotech USA) for 1 h to cool down and weighed. The ash content was expressed as a percentage of the residue weight over the sample weight and calculated using the following equation:

……….Equation 3.4

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \text{ x } 100$$

### 3.5.4 Fat

The fat content was determined according to the AACC method 30-25.01, using a Buchi E–812/816 HE extraction unit (Büchi Labortechnik AG, Flawil, Switzerland) and hexane (Sigma Aldrich, South Africa) as a solvent. One hundred millilitre of hexane was transferred into each beaker of the extraction unit and a temperature-stable paper thimble (1.7 mm width, 35 mm ø, Buchi, Switzerland) containing the flour (35 g) was inserted into the beakers with the solvent. The extraction temperature was raised 96 °C and was kept constant for 20 min. Thereafter, the hexane was removed by evaporation in an oven at 105 ± 1 °C for 30 min. The resulting oils in the beakers were cooled in a glass desiccator (210 mm, Labotech, USA) for 1 h, weighed and the oil yield was measured and expressed in percentage (%) calculated using the following equation:

……….Equation 3.5

$$\text{Oil yield (\%)} = \frac{(\text{Mtotal} - \text{Mbeaker})}{\text{Msample}} \text{ x } 100$$

### 3.5.5 Dietary fibre

The dietary fibre content was determined at the South African Grain Laboratory (SAGL) using in-house methods, The Method 021 (AOAC 985.29) and Method 007 (Southgate Method) employed an enzymatic-gravimetric technique to ascertain the total dietary fibre content in food samples. It starts with breaking down the sample with enzymes to get rid of the non-fibrous parts. Then, the residue was filtered, washed, dried, and weighed to separate and measure the dietary fibre fraction. The Southgate method is well-established and commonly used in dietary fibre analysis.

……….Equation 3.6

$\text{Dietary Fiber Content } (\%) = \text{Weight of Sample} + \text{Weight of Residue} -$
$\text{Weight of Protein x } 100$ and Ash ×100

Where:

Weight of Residue: The weight of the insoluble material left after enzymatic digestion and filtration.

Weight of Protein and Ash: The combined weight of protein and ash in the residue, is determined by subtracting their respective contributions from the total residue.

Weight of Sample: The initial weight of the food sample used for the analysis.

### 3.5.6 Amino acids

The amino acids analysis was performed at the South African Grain Laboratories using the in-house method 028 (ISO/IEC 17025:2005). The analysis was done in triplicate using a Waters AccQ-Tag method using the Waters AccQ-Tag kit and a Waters UPLC with Empower Software (Waters, Millipore Corp., Milford, MA, USA). All Amino Acid Standards H-Class were purchased from Pierce (Prod no: 20088) and L-Norvaline (Sigma Aldrich, Johannesburg, South Africa) was used as an internal standard.

### 3.5.7 Total phenolic compound

Prior to the extraction of the secondary metabolites from the flour (wheat, marula seed cake, mopane worm and mealworm), the samples went through a defatting step. This step was performed for 12 hours using n-hexane (Merck, Sigma-Aldrich, South Africa) as a solvent in a ratio of 4/25 (g/mL) and the extraction was repeated three times. The remaining n-hexane residue in the sample was removed using a vacuum oven at 50 °C overnight. The defatting process procedure was applied to three independent samples resulting in triplicate. The dried defeated flour was then stored at room temperature under vacuum until further use.

Extraction of the soluble phenolic compounds was performed on a microscale as described by Regnier *et al*. (1996). Different solvents [water; methanol (60%); ethanol

(70%); acetone and a mix consisting of methanol: acetone: water (7:7:1, v/v/v)] were tested to select the best solvent for each type of composite flours. The combined supernatants from the sample were reduced to 1 mL under vacuum and stored at 4 °C and used to measure the total phenolics index (TPI) as well as the antioxidant activity.

The concentration of phenolic compounds present in the flour samples was determined using the Folin-Ciocateau colourimetric method according to Singleton *et al.* (1999), with minor modifications. Briefly, a 5 µL flour extract was transferred into a 96 wells microtiter plate (Merck, Johannesburg, South Africa), 165 µL of distilled water, 50 µL of 20% sodium bicarbonate ($Na_2CO_3$) (freshly prepared) and 25 µL of FolinCiocalteau reagent (colourimetric indicator) were added to each well. Blank solutions were prepared in the same manner by substitution of the extract with distilled water. The contents of each well were carefully mixed using a clean Pasteur pipette (for each well) and the plate was incubated at 40 °C for 30 min.

The absorbance of the resulting blue colour was measured at 690 nm using a microplate reader (Spectramax 190, Molecular Devices, Sunnyvale, CA, USA) and the optical density was recorded in triplicate. In addition, standard solutions of gallic acid (Sigma-Aldrich, Johannesburg, South Africa) (ranging from 0 to 0.2 mg/L) were prepared and a calibration curve was constructed as a function of absorbance versus concentration. The resulting linear regression equation ($y=0.0029x$; $R^2 = 0.998$) was used to determine the Total Polyphenol Index (TPI) of soluble extracts. Values were expressed as mg GAE/100 g of defatted dried sample.

### 3.5.8 Antioxidant activity

The radical scavenging activity of the extracts obtained in Section 3.5.7 was measured by the ABTS [2,2-azino-nis (3-ethylbenzthiazoline-6-sulfuric acid)] radical cation decolouration assay, as described by Mattia *et al.* (2019).

The bleaching rate of ABTS+• in the presence of the sample was monitored at 734 nm using a Spectramax 190 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The ABTS+• bleaching was observed at 30 °C and the discolouration after 5 min was used as the measure of antioxidant activity. The radical scavenging activity was measured as Trolox Equivalents Antioxidant Capacity (mmol of Trolox eq. per 100 g of sample) and calculated by the ratio of the correlation coefficient of the dose-response curve of each sample and the correlation coefficient of the dose-response curve of the standard compound. TEAC and % Inhibition are measures of antioxidant activity, with % Inhibition providing a straightforward calculation of radical scavenging efficacy based on absorbance changes. Trolox as a standard enhances the comparability and relevance of these measurements in assessing antioxidant potential.

……….Equation 3.7

$$\text{Inhibition (\%)} = \frac{\text{A0}}{\text{A0} - \text{As}} \text{ x } 100$$

Where:

$A_0$ is the absorbance of the ABTS•+ solution without the antioxidant (control).

$A_s$ is the absorbance of the ABTS•+ solution with the antioxidant (sample).

## 3.6 STATISTICAL ANALYSIS

Data was analysed using Statgraphics Centurion software (Statpoint XVI version 16.1.17 Technologies Inc., The Plains, Virginia, USA) Analysis of variance (ANOVA) was performed and a p-value of 0.05 for the F-test was considered to indicate statistically significant differences between samples. Means were compared with the aid of Fisher`s LSD (Least Significant Difference) intervals. All analyses were performed in triplicate to ensure validity and measure of precision.

## 3.7 RESULTS AND DISCUSSION

### 3.7.1 Microbial safety

The Total Viable Count (TVC) and Total Fungal Count (TFC), act as a baseline for analysing the microbial composition of flours. The data reveal significant disparities in microbial counts among the flours. According to (Uzuegbu *et al*. 2012), the allowable ranges for both Total Viable Count (TVC) and Total Fungal Count (TFC) in flour are 1.7 x $10^2$ to 9.3 x $10^2$ cfu/mL, respectively, which are used to determine the safety criteria for flour. The findings displayed in Table 3.1 demonstrate the Total Viable Count (TVC) in colony-forming units per mil (cfu/mL) and Total Fungal Count (TFC) in colony-forming units per mil (cfu/mL). The findings provide insight into the microbial composition and safety of the various flours analysed. The standards provided can be used as reference values for comparison to assess the quality and safety of the samples.

Based on the results from the provided results, the total viable count (TVC) and total fungal count (TFC) in various flours. The highest TVC was observed in mopane worm flour at $5.10 \times 10^5$ cfu/mL, followed by marula seed cake flour with $1.75 \times 10^3$ cfu/mL and mealworm flour with $2.70 \times 10^2$ cfu/mL. These values exceed the minimal standards for safety with white wheat flour having the lowest TVC at <25 cfu/mL. The total fungal count was highest in mopane worm flour at $6.9 \times 10^3$ cfu/mL, followed by mealworm flour at $2.70 \times 10^2$ cfu/mL. Marula seed cake flour and white wheat flour both had TFC values <25 cfu/mL. TVC and TFC in mopane worm flour may be due to the conditions during the preparation and drying of the raw material before flour production. However, an effective level of toxin formation requires a large number of microorganisms between $10^6$-$10^7$ cfu/mL to negatively impact the safety of flour if consumed. of specific microorganisms like *Bacillus cereus*, Enterobacteriaceae, coliforms, *Staphylococcus aureus*, Salmonella spp. The absence of harmful organisms such as *Staphylococcus aureus,* Salmonella spp. and *E. coli* in commercially produced marula seed cake, mopane worm, and mealworm flours, is significant since South African regulations (SANS10049) require the absence of these pathogens in products to be considered safe.

Table 3.1: Microbiological analysis on White wheat, Marula Seed cake, Mealworm and Mopane worm flours

| Microbial count (CFU/mL) | White Wheat Flour | Mealworm Flour | Mopane Worm Flour | Marula Seed cake Flour | Standards |
|---|---|---|---|---|---|
| TVC | 25 | $2.70x10^2$ | $5.10x10^5$ | $1.75x10^3$ | $1.7x10^2$-$9.3x10^2$ |
| TFC | 25 | $5.6x10^2$ | $6.9x10^3$ | 25 | $1.7x10^2$ -$9.3x10^2$ |
| *Bacillus cereus* | NG | $3.8x10^2$ | NG | $2.2x10^2$ | ISO 7932:2004 less than 100 cfu/g |
| Coliforms | | $2.7x10^2$ | $1.5x10^4$ | $1.5x10^4$ | ISO 4831:2006 range of 1 to 100 cfu/g |
| *Escherichia coli* | NG | NG | NG | NG | ISO 7251:2005 absence |
| *Enterobacteriaceae* | | $3X10^2$ | $1.5x10^4$ | $1.5x10^4$ | ISO 21528-1:2004 a maximum of 100 cfu/g |
| *Salmonella* | - | - | - | - | ISO 6579:2017 Absence in 25 grams |
| *Staphylococcus aureus* | NG | NG | NG | NG | ISO 6888-1:1999 a limit of less than 100 cfu/g |

CFU: colony forming unit NG: No growth - = Absent TVC: Total viable count TFC: Total fungal count * Uzuegbu, C. O., & Eke, O. (2012)

### 3.7.2 Mycotoxins

Mycotoxins, fungal secondary metabolites with potent toxic properties, are a pervasive threat to food and feed safety worldwide. These compounds, produced by moulds such as *Aspergillus*, *Penicillium*, and *Fusarium* species, can contaminate a wide range of agricultural commodities during cultivation, harvest, storage, and processing stages. Studies have shown that mycotoxin exposure is bad for your health. It can cause cancer, damage to the liver and kidneys, weakened immune systems, and birth defects in both humans and animals (Wu, 2014; Marin *et al.,* 2013; Bennett & Klich, 2003). Given the potential risks posed by mycotoxins, regulatory agencies worldwide have established strict limits and guidelines for their presence in food and feed products (European Commission, 2006; Food and Agriculture Organisation, 2018).

A representative sample of each flour was screened for the presence of a variety of mycotoxins in marula seed cake, mopane worm, and mealworm flours. The results revealed no detection of mycotoxins within the analysed samples from marula seed cake, mopane worm and mealworm flours. This absence of mycotoxin contamination is of paramount importance, as it signifies compliance with regulatory standards and underscores the safety of the examined products for human consumption, no mycotoxins were detected in the analysed samples, the lack of mycotoxin contamination is critical because it indicates compliance with regulatory criteria and confirms the safety of the researched items for human consumption.

Table 3.2: Results of the mycotoxins of Marula Seed cake, Mealworm and Mopane worm flour compared to samples of wheat flour

| | Aflatoxin (ppb(µg/kg)) | | | | Fumonisin (ppb(µg/kg) | | | Deoxynivalenol (DON) (ppb(µg/kg)) | 15-Acetyl-DON (ppb/µg/kg)) | Ochratoxin A (ppb(µb/kg) | Zearalenone (ppb(µg/kg) | T2 ppb (µg/kg) | HT-2 (ppb(µg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LOQ | B1 | B2 | G1 | G2 | | | | | | | | | |
| Sample | 5 | 5 | 5 | 5 | 20 | 20 | 20 | 100 | 100 | 5 | 20 | 20 | 20 |
| White wheat Flour | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Mealworm flour | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Mopane four | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Marula seed cake | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND flour |

ND-Not detected LOQ-The Limit of Quantification.

### 3.7.3 Proximate composition of the flours

The nutritional and physicochemical qualities of raw materials play a crucial role in shaping the characteristics and quality of the final product (Singh *et al.,* 2020). The physicochemical qualities of the flours determine the quality of the final product (Pang, *et al*. (2021). Proximate composition was found to differ between the control (wheat flour), edible insect flours, and marula seed cake flour (Table 3.3).

The ash content was highest in mopane worm (20%) flour at 3.60%, followed by marula seed cake flour (20%) at 1.4% and the lowest value recorded in mealworm flour (20%) at 1.33% with white wheat flour at 0.53%, ash content. The moisture content was highest in mopane worm flour (10%) at 11.6%, followed by mealworm flour (10%) at 10.86%, white wheat flour at 10.9% and the lowest moisture content was found in marula seed cake (10%) at 10.1%. The higher content in mopane worm flour may impact its shelf life and processing characteristics. The marula seed cake (20%) flour exhibited the highest percentage fat content at 6.76%, followed by mopane worm (20%) flour at 5.03% and white wheat flour at 1.20%, the lowest fat content was observed in mealworm (20%) at 3.7%. The higher fat content suggests its potential for enhancing flavour and texture. Protein content was highest in mopane worm flour (20%) at 21.22%, followed by marula seed cake flour at 18.46%, with white wheat flour at 12.46% and the lowest protein was observed in mealworm flour (20%) at 17.57%. This indicates that mopane worm is a rich source of protein, making it suitable for protein fortification (Pihlanto *et al*., 2017).

Conversely, the dietary fibre content was highest in marula seed cake at 15.1%, followed by mopane worm flour at 6.6%, with white wheat flour at 2.7%, and the lowest dietary fibre content was found in mealworm flour at 3.9%. The high dietary fibre suggests its potential health benefits, particularly, digestive health. The physicochemical analysis conducted after product development showed that there was a difference between composite enriched flour such as (marula seed cake, mopane worm, mealworm and white wheat flour. The mopane worm flour exhibited higher values for protein, ash, and moisture content, it had lower fat and dietary fiber compared to the other flours. These findings underscore the importance of selecting appropriate flour types based on their physicochemical properties for specific food applications according to (Cacak-Pietrzak *et al.,* 2024).

Table 3.3: Proximate composition of Marula seed cake, mealworm and Mopane worm flours and their composites with white wheat flour

| | % [6] | Ash | Moisture | Fat | Protein | Dietary fibre |
|---|---|---|---|---|---|---|
| White Wheat Flour | | 0.53 | 10.90 | 1.20 | 12.46 | 2.7 |
| Mealworm Flour | | | 4.26 | 27.2 | 49.70 | 3.9 |
| Mopane worm Flour | 100 | | 7.59 | 10.1 | 60.63 | 6.6 |
| Marula Seed cake Flour | | | 3.79 | 32.7 | 38.00 | 15.1 |
| Mealworm Flour | | 0.60 | 10.13 | 1.93 | 13.87 | |
| Mopane worm Flour | 2.5 | 0.66 | 11.66 | 1.16 | 13.83 | |
| Marula Seed cake Flour | | 0.66 | 9.03 | 1.16 | 13.40 | |
| Mealworm Flour | | 0.66 | 10.60 | 1.76 | 14.59 | |
| Mopane worm Flour | 5 | 1.13 | 11.63 | 1.73 | 14.75 | |
| Marula Seed cake Flour | | 0.80 | 10.50 | 1.20 | 14.29 | |
| Mealworm Flour | | 0.93 | 10.86 | 3.76 | 16.55 | |
| Mopane worm Flour | 10 | 1.86 | 11.06 | 2.03 | 17.26 | |
| Marula Seed cake Flour | | 0.86 | 10.10 | 3.93 | 15.57 | |
| Mealworm Flour | | 1.26 | 10.63 | 3.93 | 17.57 | |
| Mopane worm Flour | 15 | 2.66 | 10.90 | 2.90 | 19.33 | |
| Marula Seed cake Flour | | 1.13 | 10.13 | 4.66 | 17.02 | |
| Mealworm Flour | | 1.33 | 10.16 | 5.03 | 17.57 | |
| Mopane worm Flour | 20 | 3.60 | 10.83 | 3.70 | 21.22 | |
| Marula Seed cake Flour | | 1.4 | 9.86 | 6.76 | 18.46 | |

[6] Percentages of the composite flours

### 3.7.4 Amino acids

Table 3.4 shows the amino acid profile of mealworm, mopane worm and marula seed cake flours, a critical factor influencing the nutritional quality and functionality of food products (Kaur & Singh, 2020; Savadogo *et al.,* 2017). Essential amino acids, cannot be synthesized by the human body and must be obtained from dietary sources. In the context of alternative protein sources, such as marula seed cake, mopane worm, and mealworm flours, understanding the essential amino acid content is essential for assessing their nutritional adequacy and suitability for human consumption (van Huis *et al.,* 2013; Yen, 2012). The relative proportions of essential amino acids in these flours can significantly impact the amino acid profile of products formulated with them. The essential amino acid profile of white wheat is significantly lower than that of mealworms, mopane worms, and marula seed cake, indicating its limitations as a protein source. The isoleucine content of white wheat flour was 0.42 g per 100 g protein, which was markedly less than mealworms (2.26%) and mopane worms (2.05%). Leucine content of white wheat flour was 0.86 g per 100 g protein, while mealworms provide a much higher amount at 3.95%, showing a deficiency in white wheat' amino acid profile. The lysine content of white wheat flour was 0.28 g per 100 g protein compared to mopane worms (3.42%) and mealworms (2.63%), making lysine the first limiting amino acid in wheat. Methionine content of white wheat flour was 0.24 g per 100 g protein, which was lower than both mealworms (0.73%) and mopane worms (0.75%). The phenylalanine content of white wheat flour was 0.6 g per 100 g protein compared to mealworms (2.11%) and mopane worms (2.13%). Threonine content of white wheat flour was 0.35 g per 100 g protein, while mealworms had 2.12%. The Tryptophan content of white wheat flour was low at 0.13 g, per 100 g protein, especially when compared to mealworms (0.57%) and mopane worms (0.55%). The valine content of white wheat was 0.52 g per 100 g protein, which was significantly less than that found in mealworms (3.42%) and mopane worms (2.79%). Histidine content of white wheat flour was 0.26 g, per 100 g protein while mealworms contain 1.71% and mopane worms have 1.37%. Amino acid composition of white wheat is inadequate when compared to these alternative protein sources, particularly in essential amino acids like lysine and leucine, highlighting its role as a less optimal choice for meeting dietary protein needs. The essential amino acid profile of white wheat was markedly inferior compared to more nutrient-dense options like human milk and various insects and marula seed cake.

Protein levels in mopane worms, ranging from 49.4% to 61%, compared to mealworms, which contain between 40.2% and 63.3% protein, present valuable nutritional benefits as sources of protein and essential amino acids (Hlongwane *et al*., 2021; Matomani, 2021). Glutamic acid emerged as the predominant amino acid in marula seed cake flour, followed by mopane worm flour, while the control white wheat flour exhibited comparatively lower levels. Similarly, arginine and tryptophan showed varying patterns across the flours, with noticeable differences in their percentages. These results provide crucial insights into the amino acid compositions of marula seed cake, mopane worm, and mealworm flours, essential for determining their healthfulness and safety as ingredients in composite flours. It is noteworthy that while certain amino acids may be abundant in specific flours, others may be scarce, understanding the importance of dietary diversity and balanced nutrition (FAO, 2013; WHO, 2018).

Table 3.4: Amino acid content of White wheat flour, Mealworm flour, Mopane worm flour Marula Seed cake compared to Human milk (Coultate, 1997)

| Amino Acids (% protein) | White wheat flour | Mealworm | Mopane worm | Marula seed cake | Human milk |
|---|---|---|---|---|---|
| **Cysteine** | 0.495 | 0.775±0.025 | 0.610 ±0.040 | 0.805±0.065 | 2 |
| **Methionine** | 0.240±0.010 | 0.725±0.045 | 0.750±0.040 | 0.390±0.040 | 1.5 |
| **Histidine** | 0.260±0.000 | 1.710±0.010 | 1.365±0.005 | 0.945±0.005 | 2.5 |
| **Serine** | 0.610±0.000 | 2.925±0.015 | 2.520±0.030 | 1.700±0.020 | 4.3 |
| **Arginine** | 0.460±0.000 | 2.925±0.015 | 2.660±0.050 | 5.175±0.015 | 3.8 |
| **Glycine** | 0.465±0.005 | 2.835±0.025 | 2.595±0.085 | 1.815±0.005 | 2.5 |
| **Aspartic acid** | 0.545±0.005 | 3.485±0.095 | 4.860±0.090 | 3.075±0.015 | 9.1 |
| **Glutamic acid** | 4.370±0.000 | 5.020±0.040 | 6.430±0.110 | 9.405±0.025 | 17.4 |
| **Threonine** | 0.345±0.005 | 2.115±0.005 | 2.500±0.040 | 0.875±0.005 | 4.5 |
| **Alanine** | 0.370±0.000 | 3.680±0.010 | 2.805±0.035 | 1.195±0.005 | 4.2 |
| **Proline** | 1.420±0.000 | 3.385±0.025 | 2.625±0.025 | 1.255±0.005 | 9.9 |
| **Lysine** | 0.280±0.000 | 2.630±0.010 | 3.415±0.055 | 0.800±0.000 | 7.1 |
| **Tyrosine** | 0.355±0.015 | 4.170±0.110 | 2.945±0.085 | 1.040±0.010 | 3 |
| **Valine** | 0.520±0.000 | 3.420±0.050 | 2.790±0.020 | 1.875±0.005 | 6.8 |
| **Isoleucine** | 0.415±0.005 | 2.255±0.025 | 2.050±0.000 | 1.500±0.010 | 5.3 |
| **Leucine** | 0.855±0.005 | 3.950±0.040 | 3.175±0.045 | 2.325±0.015 | 9.9 |
| **Phenylalanine** | 0.600±0.010 | 2.110±0.040 | 2.125±0.005 | 1.630±0.010 | 3.8 |
| **Tryptophan** | 0.125±0.005 | 0.570±0.010 | 0.545±0.005 | 0.450±0.010 | 2.3 |

White wheat flour (WWF), Mealworm flour (MWF), Mopane worm flour (MPWF), Marula seed cake flour (MSCF)

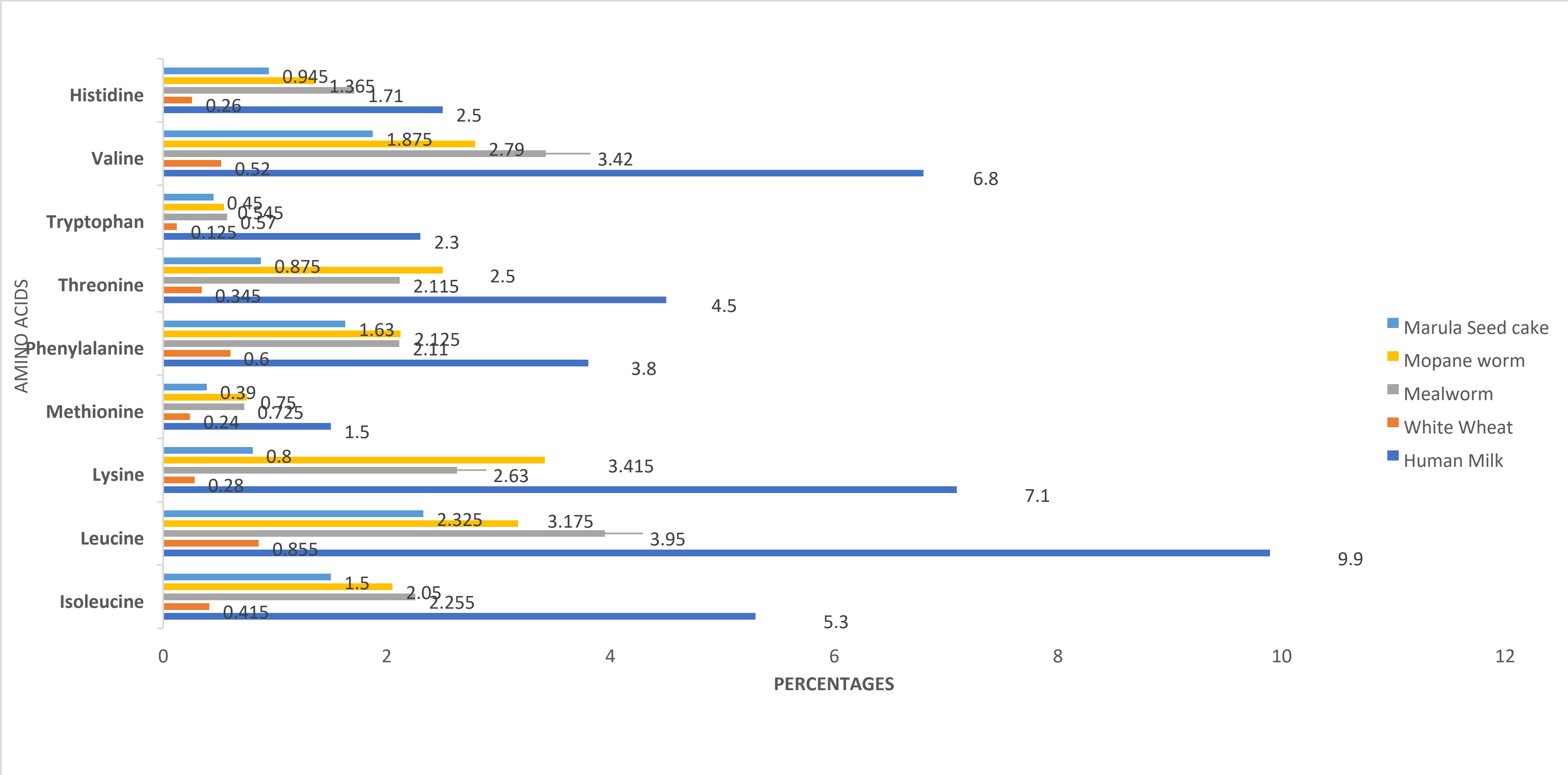


Figure 3.4: Essential amino acid content (g/100g protein) of Marula seed cake, Mealworm and Mopane worm flour samples compared to wheat flour and human milk (Coultate, 1997)

The amino acids may become essential under specific conditions, underscoring the necessity of consuming a balanced diet rich in essential amino acids and using human milk as a benchmark to evaluate the nutritional value of protein content in other foods, thereby reinforcing the need to align the amino acid composition of dietary proteins. It observes that, while there are variances, protein generated from insects differs significantly from human milk but is higher than plant protein sources such as white wheat and marula seed cake. The data imply that, despite their various sources, the protein of various diets produce generally similar amino acid compositions. This comparison highlights the possibility of alternative protein sources, such as insects, to help meet human nutrition needs, particularly in situations when traditional protein sources are scarce or unsustainable. Determining the necessary amino acids in alternative protein sources such as mealworm, mopane worm, and marula seed cake flours is crucial for assessing their health benefits and their suitability for human consumption. The relative proportions of essential amino acids have a major impact on the amino acid profile of products made from these flours. Marula seed cake flour revealed glutamic acid as the most abundant amino acid, followed by mopane worm flour, while wheat flour had exceptionally low quantities. Similarly, arginine and tryptophan distributions varied throughout the flours, indicating significant changes in proportion. The results give valuable information about the amino acid contents of marula seed cake, mopane worm, and mealworm flours. Amino acid composition provide that the food are safe and healthy as parts of composite flours. It was worth noting that while some amino acids are rich in some flours, others may be in short supply, emphasising the significance of dietary diversity and balance.

### 3.7.5 Total phenolic compound

Phenolics are a wide group of secondary metabolites found in most fruits and vegetables. They are classified as simple phenols or phenolic acids (with one phenol ring) and polyphenols (with at least two phenol rings). Typical examples include resorcinol, flavonoids, lignans, and tannins, among others, as detailed by many others (Stratil *et al.,* 2006).

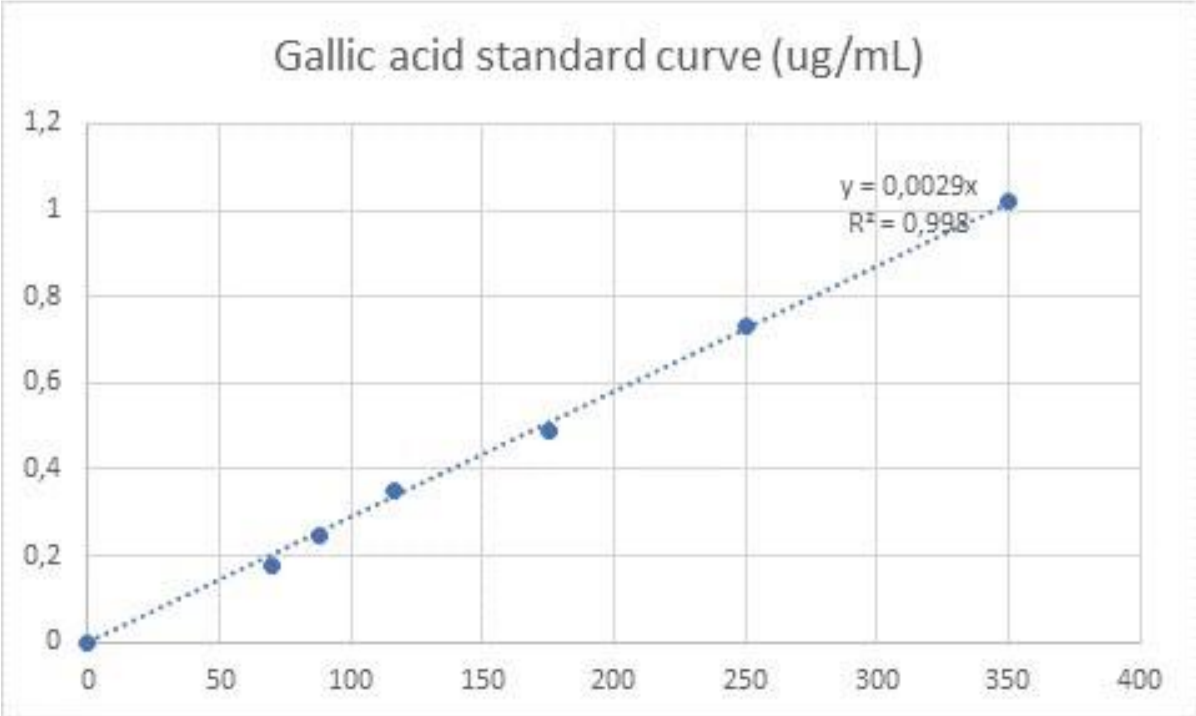

Figure 3.5: Gallic acid standard curve

The linear regression equation that resulted (y = 0.0029x $R^2$ = 0.998) was used to calculate the concentration of phenolic chemicals in the flour samples, and the results were reported as gallic acid equivalents mg GAE per 100 g of flour sample. In this section, the total polyphenol index (TPI) functions as a quantitative evaluation of the gross polyphenolic content of the soluble extracts of marula seed cake, Mopane worm, Mealworm, and white wheat flours. The total polyphenol index (TPI) in white wheat showed the highest value in water extract (441.209), followed by acetone (374.253) and mix solvents (209.816) and with the lowest value from methanol (195.632) and ethanol (170.115) as illustrated in Figure 3.6.

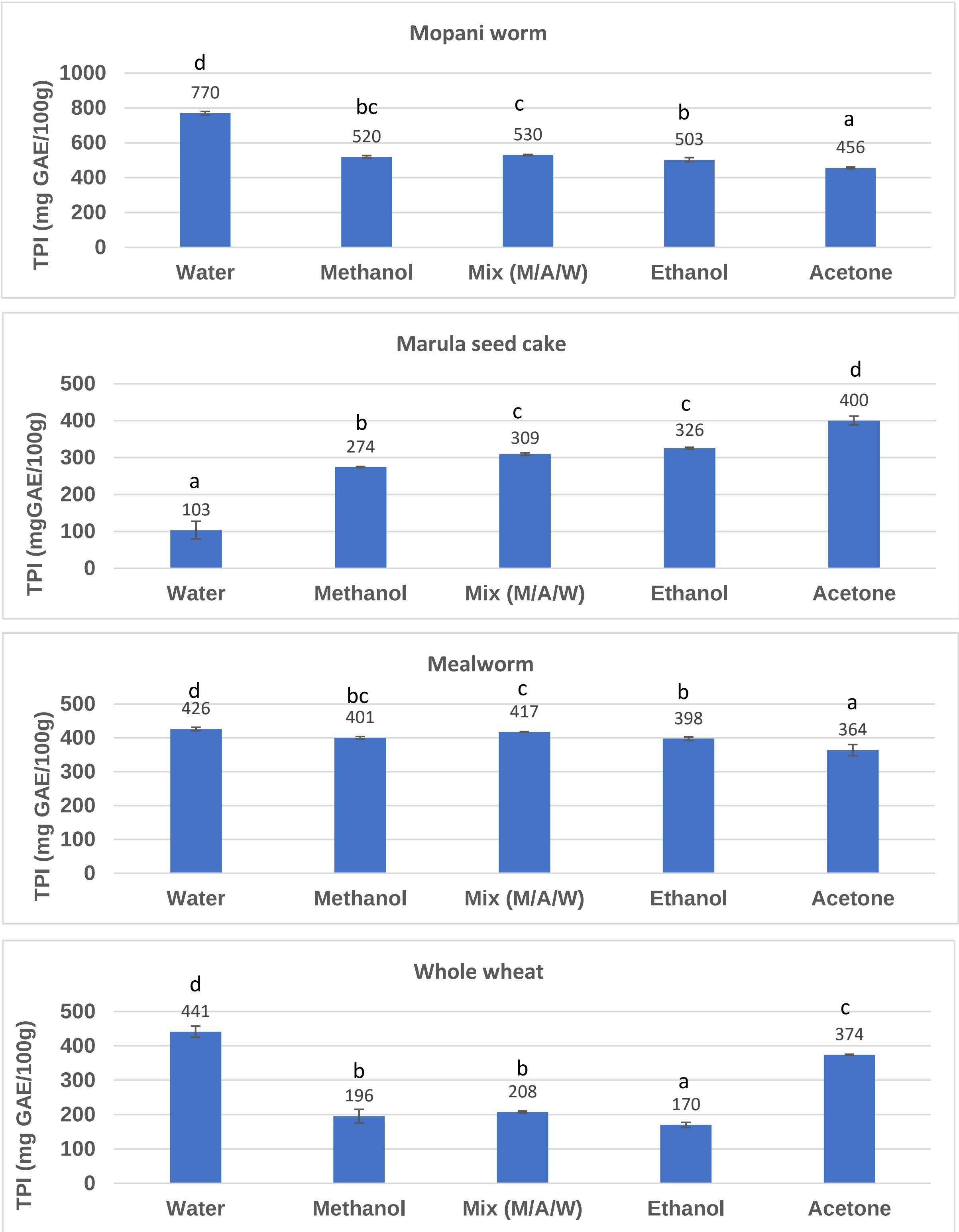


Figure 3.6: Total Polyphenol Index (TPI) of soluble extracts of marula seed cake, Mopane worm, Mealworm, and whole wheat flours according to the degree of polarity of the solvent (high to low).

In Figure 3.6, it can be observed that the water extract (426) of the mealworm and mixed solvent (417) of the mealworm were high as compared to the methanol and ethanol extract of the mealworm. The lowest TPI was acetone extract of mealworm at 364. Concerning the mopane worm, the total polyphenol index (TPI) of acetone and ethanol extracts can vary significantly based on the type of plant material and the extraction conditions used (456) and (503) respectively, than the water-soluble (770), mixed extract (530) and methanol (520). In contrast, the water extract from marula seed cake exhibited the lowest total polyphenol index at 103.45, while the acetone extract recorded the highest value at 400, as illustrated in Figure 3.6. The water-soluble extracts of whole wheat (441), mealworm (426), and mopane (770) display the highest values as compared to the water-soluble extract of marula seed cake (103). Figure 3.7 displayed the overall TPI of marula seed cake, Mopane worm, Mealworm, and whole wheat flour. The analysis shows that water-soluble extracts from whole wheat (441), mealworm, and mopane worm (770) yield higher TPI values compared to the significantly lower value observed in marula seed cake water extracts (400). This suggests that different extraction methods and sources significantly influence the total polyphenol content, with water and mixed solvents generally providing higher yields for certain materials, particularly in mopane worms.

In general, the trends indicate that the selection of a solvent for the extraction process has a notable effect on the Total Polyphenol Index (TPI) of extracts from various sources, the interactions between solvent type and flour type are influenced by solvent polarity, the chemical composition of the flours, extraction methodologies employed, solubility characteristics of polyphenols, and potential interactions among flour components. Understanding these factors crucial for optimizing extraction processes to enhance the antioxidant capacity and nutritional value of flour-based products. Discrepancies in the TPI across extracts indicate variations in the polyphenolic content in these food origins (Amidžić Klarić *et al.,* 2020). The antioxidant properties of polyphenols are recognized for their potential health advantages, such as diminishing the susceptibility to chronic ailments like cardiovascular disease and cancer (Tarasov *et al.,* 2023). Consequently, comprehending the polyphenol content in food origins aids in evaluating their nutritional worth and potential health-enhancing impacts. Selecting an appropriate solvent for extraction plays a pivotal role in determining the output and composition of polyphenolic compounds in food extractions. The results emphasize the significance of solvent selection in optimizing the efficacy of extractions and achieving precise evaluations of polyphenol content (Yeasmen & Orsat, 2023). Using various solvents, researchers can customize extraction methods to enhance the retrieval of specific polyphenols, thereby amplifying the nutritional and functional characteristics of food items.

The variation in the Total Polyphenol Index (TPI) among diverse food origins accentuates the diverse polyphenol profiles exhibited by these components. This diversity presents opportunities to include a variety of polyphenol-rich foods in diets, encouraging dietary variety and potentially improving overall health outcomes (Aravind *et al.,* 2021). Moreover, it underscores the possibility of non-traditional sources like insects (e.g., mealworm and Mopane worm) contributing to polyphenol intake and fulfilling nutritional requirements. The Utilisation of polyphenol-rich food extracts has attracted attention to the advancement of functional foods designed to enhance health and well-being. The discoveries offer insights into potential reservoirs of polyphenols for incorporation into functional food formulations. By integrating polyphenol-rich extracts into food items, producers can develop functional foods with heightened antioxidant qualities and potential health advantages (Olszewska *et al.,* 2020). Overall, the findings concerning the variation in Total Polyphenol Index (TPI) among diverse solvent extracts of different food origins enrich knowledge of the polyphenol content and potential health-promoting impacts of these constituents, with implications for both academic research and applied practices in the realms of food science and nutrition.

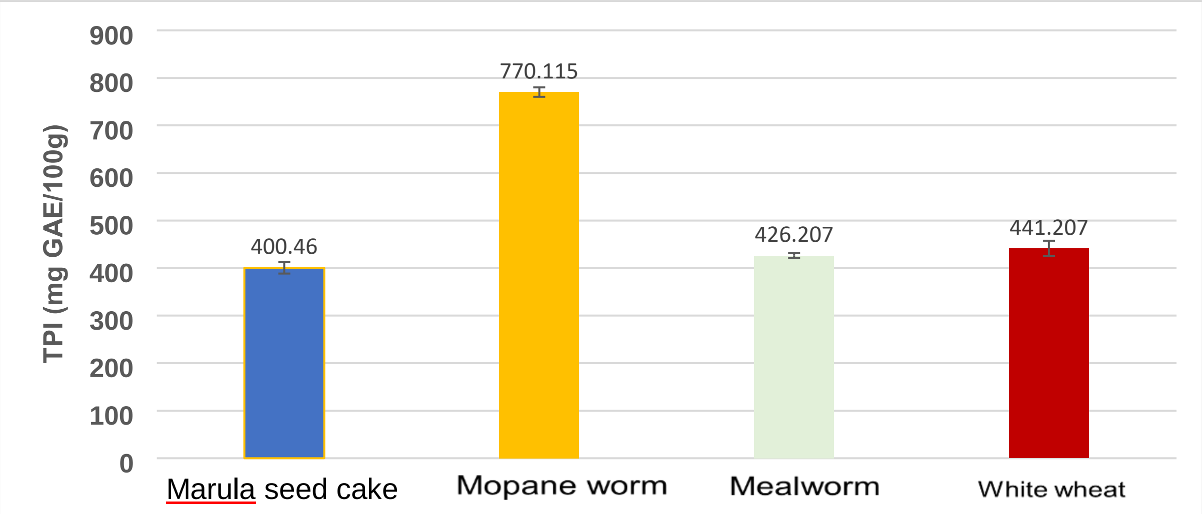


Figure 3.7: Total Polyphenol Index (TPI) of soluble extracts obtained by using the most suitable solvent for each flour type. Marula seed cake: acetone, Mopane worm: water, Mealworm: water, and whole wheat: water. Values are expressed as mg GAE/100 g of defatted dried sample and are the mean ± SD in triplicate. GAE, Gallic acid equivalent.

The Total Polyphenol Index (TPI) of different solvent extracts of whole wheat flour, mopane worm, marula seed cake, and mealworm gives us valuable information about the polyphenol content and possible health benefits of these foods. Phenolics, which include resorcinol, flavonoids, lignans, and tannins, are important secondary metabolites present in fruits and vegetables that affect both nutritional quality and food functionality. The extraction solvent significantly influences polyphenol TPI, often exhibiting extraction efficiency that was highest in solvents such as water and acetone, These differences in TPI among extracts reflect differences in polyphenolic concentrations between food origins. Such heterogeneity highlights the importance of solvent selection in optimising extraction performance and accurately estimating polyphenol concentration. Furthermore, the various polyphenol profiles of different food components offer chances for dietary variety and potential health benefits. Insects, such as mealworms and Mopane worms, may contribute to polyphenol intake while also meeting nutritional requirements.

### 3.7.6 Antioxidant activity

Antioxidants protect biological systems from oxidative stress-induced damage by scavenging free radicals and reactive oxygen species (ROS). This protective mechanism has received a lot of attention because of its potential use in avoiding cancer, cardiovascular disease, and neurodegenerative disorders. Researchers have developed a variety of methods to assess the antioxidant activity of compounds, including cellular assays, animal models, and *in vitro* assays such as the DPPH (2,2 diphenyl 1 picrylhydrazyl) scavenging assay, the ABTS (2,2' azino bis (3-ethylbenzothiazoline 6 sulfonic acid) assay, and the FRAP (Ferric Reducing Antioxidant Power) assay. Several studies have established the antioxidant capacity of natural substances such as polyphenols, flavonoids, carotenoids, and vitamins. For example, resveratrol, a polyphenol found in grapes and red wine, has been demonstrated to have powerful antioxidant capabilities that neutralise free radicals and reduce oxidative stress. The study of the antioxidant capacities of several food samples produced surprising results, revealing information on their nutritional properties.

Figure 3.8 depicts the variability in antioxidant capability among the examined extracts (composite mopane worm, marula seed cake, mealworm and white wheat flour extracts). Notably, the Mopane worm and whole wheat flour had the highest

antioxidant activity, with 2.751 mmol TE/100 g and 1.021 TE/100 g, respectively. The mealworm and Marula seed cake had the lowest values, with 1.007 mmol TE/100 g and 0.482 mmol TE/100 g, respectively. These findings highlight the varying antioxidant capabilities seen in various food products. A comparative examination of Trolox Equivalent Antioxidant Capacity (TEAC) levels after extract preparation, as described in Section
3.2, revealed no significant variations in mean TEAC values. This shows that the efficacy of antioxidants may be the same after extraction and baking. Such insights are critical to understanding the nutritional value of these foods. The observed changes in antioxidant characteristics, particularly the increased capabilities of the Mopane worm and whole wheat flour, are consistent with previous studies highlighting the nutritional value of edible insect flours and their antioxidant properties (Vanqa *et al.,* 2022). On the other hand, the lower values found in the mealworm and Marula seed cakes are in line with data showing that baked flour contain different amounts of antioxidants (Subiria-Cueto *et al.,* 2021). Hong *et al.* (2023) investigated the effect of dietary modifications on the bioactive properties of mealworm-derived protein derivatives. This demonstrates that customised dietary regimens can improve the nutritional and functional benefits of insect-derived proteins. Mealworms showed promising DPPH free radical scavenging activity (5.96 ± 0.95 mg of TEAC/g). Finally, extracts of Marula seed cake, Mopane worm, mealworm, and whole wheat flour are promising possibilities as antioxidant-rich supplements for a balanced nutritional diet. Even though there were early differences in how well the samples worked as antioxidants, the fact that there were no significant differences in the mean TEAC values after extraction suggests that the samples value outcomes may be closer to the white wheat flour.

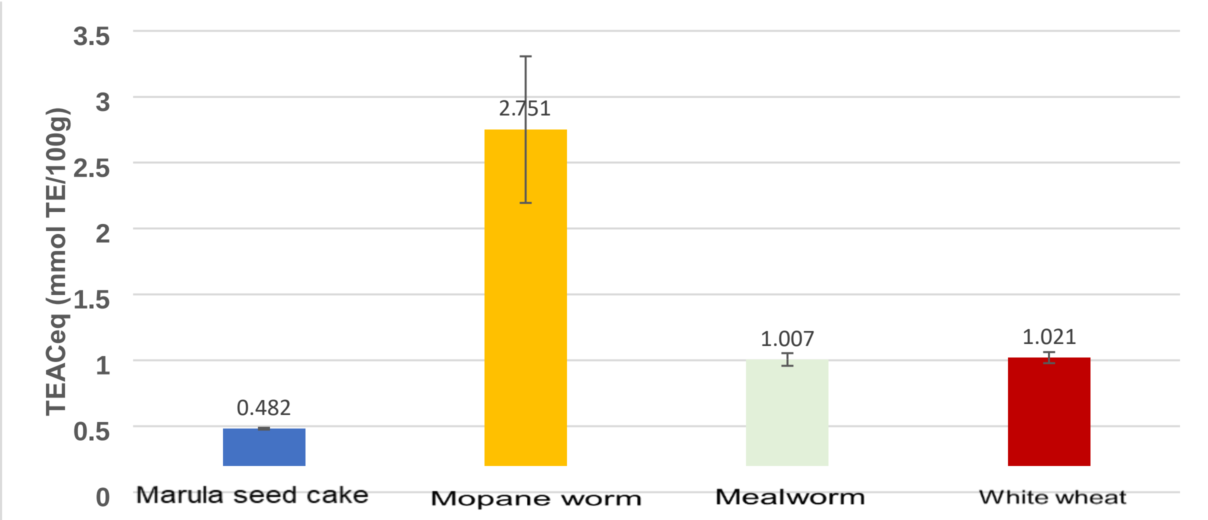


Figure 3.8: Trolox Equivalent Antioxidant Capacity (TEAC) of water-soluble extracts of Marula seed cake, Mopane worm, mealworm and white wheat flour. Values are expressed as TEACaq (mmol TE/100 g of defatted sample and are the mean ± SD in triplicate. TE, Trolox Equivalents.

## 3.8 CONCLUSION

Microbiology, nutritional value, amino acids, mycotoxins, phenolics, and antioxidants have all contributed to a comprehensive understanding of the factors that influence food quality and safety, while mopane worm flour had the highest microbial counts, the absence of harmful pathogens and compliance with regulations suggest its safety for consumption. Mopane worm and marula seed cake flours exhibited favorable nutritional profiles, including high protein and fiber. However, the high moisture content of mopane worm flour may impact its shelf life and processing characteristics. Overall, these alternative protein sources show promise as nutritious and functional ingredients in food formulations, but further research is needed to optimize their use and ensure consistent quality and safety. In the next Chapter, the impact of the incorporation of alternative flours into the dough, on the rheology properties and the final quality of bread, was assessed and it is uncovering new opportunities for improving bread quality and nutritional value.

# CHAPTER 4

# EFFECT OF MARULA SEED CAKE AND INSECT FLOURS ON DOUGH RHEOLOGY AND BREAD QUALITY

## 4.1 INTRODUCTION

Alternative protein and nutrient sources in food production have received significant interest due to concerns about sustainability, food security, and nutritional value (Burlingame *et al.,* 2019; Lynch *et al.,* 2020). Marula seed cake and insect flours are novel ingredients high in proteins, fibre, and bioactive substances with intriguing use in food formulations (Magoro *et al.,* 2017; van Huis *et al.,* 2013). Understanding their effect on dough rheology and bread quality is critical for optimising their use in bakery goods. Dough rheology has a significant impact on bread handling characteristics and final quality (Schofield *et al.,* 2019). A variety of factors, including component mix, hydration level, and processing procedures, influence the rheological qualities of dough. Marula seed cake and insect flours have distinct compositions that may interact differently with wheat flour, influencing dough behaviour and bread qualities (Balla *et al.,* 2018; Tang *et al.,* 2021). Furthermore, dough rheology inextricably links to bread quality, which includes texture, volume, crumb structure, and sensory features (Aguilera *et al.,* 2020). Changes in dough rheology caused by the addition of novel ingredients can have an impact on bread quality metrics, demanding further inquiry (Torbica *et al.,* 2018). Previous studies investigated the rheological characteristics and functioning of alternative protein sources in bakery goods (Oliviero *et al.,* 2019; Zaidul *et al.,* 2021). However, there is little literature regarding the effect of marula seed cake and insect flours on dough rheology and bread quality. Understanding how they affect these factors is critical for optimising formulations and improving bread's nutritional and taste properties. The study aims to investigate how marula seed cake and insect flour affect dough rheology and bread quality. By using analytical approaches like texture analysis, volume measurement and crumb structure analysis, insights into the interactions between alternate flours and wheat flour in dough systems can be obtained.

## 4.2 MATERIALS AND METHODS

The quality of baked goods, such as bread, is influenced by both the raw ingredients and the baking process. In Chapter 3, the physicochemical properties of the raw materials are presented. The impact of composite flour composition on the quality of the final baked product is examined in the current chapter, employing established procedures that generate reliable quantitative and/or qualitative findings. The experimental details are outlined in each section.

### 4.2.1 Preparing composite dough

The preparation of composite doughs entailed producing insect and marula seed cake flour in varying percentages (2.5%, 5%, 10%, 15%, and 20%). Table 4.1 summarises additional components and their sources. Individually blended composite flour in a polythene bag for 10 min. to ensure homogeneity, and then stored it in sealed plastic bags until it was ready for use. Composite flours were created by adjusting the quantities of insect and marula seed cake flours (2.5%, 5%, 10%, 15%, and 20%), with percentage of white wheat flour in Table 4.1 To ensure homogeneity, all composite flours were mixed in polythene bags for 10 min before storage.

### 4.2.2 Dough rheological characteristics

The mopane worm dough (Table 4.1), mealworm flour and marula seed cake flour show the rheological parameters of all formulations. To assess dough rheological behaviour and functional qualities, Mixolab (Chopin, Tripette et Renaud, Paris, France) was used by typical Chopin+ protocols. This procedure comprises heating and chilling the sample while mixing at a constant speed of 80 rpm. The amount of flour necessary for each assay was calculated using the Mixolab® programme, considering each sample's moisture content. Initial consistency (C1), stability time (min), minimum torque (Nm) during dough passage under mechanical and thermal constraints (C2), peak torque (Nm) during the heating stage (C3), minimum torque (Nm) during the heating process (C4), and torque obtained after cooling at 50 °C (C5) were all found on the Mixolab curve (Bonet *et al.,* 2006; Rosell *et al.,* 2010).

Table 4.1: Ingredients used in the development of composite bread and their source

| Ingredients | Sources |
|---|---|
| **Wheat flour** | RCL Foods (Ruto mills), South Africa |
| **Mopane worm** | Section 3.2.2 |
| **Mealworm** | Section 3.2.3 |
| **Marula seed cake** | Section 3.2.4 |
| **Anchor instant yeast** | Anchor yeast, Johannesburg, South Africa |
| **Sugar** | Pick n Pay, South Africa |
| **Salt** | Pick n Pay, South Africa |
| [7]**Format** | BICSA, South Africa |
| **Sunflower Oil** | Pick n Pay, South Africa |

Format is a commercial bread additive (Appendix 1 for specification sheet)

[7] Sodium Chloride, Sucrose, Vegetable Fats and Oils [Palm Oil (Palm Fruit, Palm Seed); Anti – Oxidant: BHA (E320)], Soya Flour, Modified Starch, Wheat Flour, Anti-Caking Agent (E170), Vegetable Oil [ Canola/Sunflower(BHT) Seed], Preservative(E282), Vegetable Fat and Oil [Canola/ Sunflower(BHT) Seed, Emulsifiers (E481,E471), Palm Oil (Palm Fruit, Palm Seed); Anti-Oxidant: BHA(320)], Oxidizing Agent(E300) and Enzymes

### 4.2.3 Bread baking

Table 4.2 presents the composite dough formulations, as well as the various wheat composite ratios. The flow chart represents the sequential processes in the bread-baking process, from ingredient preparation to final product packaging. This procedure includes several stages, including weighing, mixing, moulding, fermentation, proofing, baking, and cooling, all of which contribute to the bread's distinct flavour, texture, and appearance.

| **Processing Steps** | **Processing Steps** |
|---|---|
| **Weighing Ingredients** | |
| **Mixing Two step mixing:** **Water, sugar yeast (5 min to revive yeast) and other dried ingredients** | |
| **Moulding** | **770g of dough in bread baking pan** |
| **Proofing** | **In a proofer for 45 min at 40 °C** |
| **Baking** | **Bake the bread at 230 °C for 40 min** |
| **Cooling** | **For 1 hour at ambient temperature** |

Figure 4.1: Flow chart of the composite bread-baking process

Table 4.2 lists all dry ingredients to be used during the baking process. The dry baking ingredients were added to a mixing bowl while pre-dispersed water and oil were measured in a measuring cylinder. The combined dry components were mixed at a low speed for 2 min before the pre-dispersed oil and water were added. The mixer's (Inbest mixer, Taiwan) speed was increased to high speed for 8 min, for the batter to form. The final dough was measured (770 g), and placed in bread baking pans, left to rest for 8 min on the table. The proofer (Bakemark, United States) was set at 40 °C and the batter was proofed for 45 min at a humidity of 80%. The two-zone oven (ProBake oven, Canada) was heated to 230 °C and the bread was baked for 40 minutes. After baking, the bread was removed from the oven and allowed to cool at room temperature for 1 h and the height of the bread was measured using a ruler as described by Akubor and Obiegbuna (2014). The loaf was then sliced (12 mm) using a bread slicer (Taurus, cut master, Spain) and transferred to Ziploc bags to be stored for further evaluation using the approach described by Demirkesen *et al.* (2010).

Table 4.2: Formulations used for bread production

| **INGREDIENT (%)** | **Control** | **2.5%** | **5%** | **10%** | **15%** | **20%** |
|---|---|---|---|---|---|---|
| White Wheat bread flour | 100 | 97.5 | 95 | 90 | 85 | 80 |
| Alternative flours (Mopane, mealworm or marula seed cake) | 0 | 2.5 | 5 | 10 | 15 | 20 |
| Format/ | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 |
| Salt | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Sugar | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Yeast (wet) | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Oil | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Water | 60.00 | 60.00 | 60.00 | 60.00 | 60.00 | 60.00 |

The different concentrations of salt (1.50%), sugar (2.00%), yeast (2.00%) oil (3.00%) and format (0.85%) were held constant in all treatments (Amylase, wheat gluten, Lecithin, ascorbic acid)

### 4.2.4 C-Cell instrument

The C-Cell instrument (an entry-level digital imaging system) is an equipment manufactured by Calibre Control from the United Kingdom (AACC Method 10-18.01). It provides a comprehensive evaluation of baked goods by capturing high-resolution images of bread. It quantifies various parameters related to size, shape, and internal structure, allowing for precise control over baking processes.

The C-Cell requires a quick calibration process, taking about 2 min. to ensure accurate measurements. A clean slice of the baked product is placed in the sample drawer, ensuring that the surface is level with the guide rails for optimal imaging. Finally, the captured images are processed using bespoke software that analyses the key parameters (3 slices per loaf, random positions).

### 4.2.5 Colour of the crust and crumb

Quantitative colour descriptors of the crumb and crust of each loaf were determined using a Minolta Chroma Meter (Konica Minolta-CR-410, Osaka, Japan) based on the CIE L*a*b* system. Per the International Commission on Illumination's recommendations, measurements were taken at five positions on each exterior surface, including the top, bottom, sides, and ends of the bread samples. Colour variations were assessed using the CIE Lab* scale, with L* representing lightness, a* representing redness-greenness, and b* representing yellowness-blueness. The colour difference (ΔE) between whole wheat bread (control) and composite loaves of bread was calculated using the following equation:

..........Equation 4.1

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

The crust and crumb structure are crucial elements that define the quality and characteristics of a loaf of bread. The crust is the outer layer of the bread that forms during baking, while the crumb refers to the interior, spongy texture of the loaf.

Both the crust and crumb contribute to the overall appearance, texture, and eating experience of the bread. The crust is formed when the outer layer of the dough is exposed to high temperatures during baking. This causes the starch in the flour to gelatinize and the proteins to denature, creating a firm, crisp layer.

The crumb structure is the internal cellular network that gives bread its characteristic texture. It is formed by the expansion of gas bubbles within the dough during fermentation and baking. The size, distribution, and thickness of the cell walls in the crumb determine the texture and mouthfeel of the bread.

A well-structured crumb should be even, with small, uniform cells and thin cell walls for a soft, tender texture. Proper gluten development, gas retention during proofing, and controlled oven springs during baking are crucial for creating a desirable crumb structure. The crust and crumb structure are essential for assessing the quality and consistency of bread, through various techniques, such as image analysis and texture measurement, can be used to quantify the properties of the crust and crumb (Smith *et al.,* 2022).

### 4.2.6 Texture analysis

Texture analysis was conducted using a texture analyzer (TA.XT Plus Texture Analyzer, Stable Micro Systems, Godalming, Surrey, UK) by AACC procedures 74–09. The texture analyzer was equipped with a cylindrical probe measuring 36 mm in diameter to measure bread firmness, expressed as the compression peak force (N).

This method allowed for the evaluation of the baked goods' structural integrity and resistance to deformation, providing insights into changes in texture characteristics over time. Monitoring texture parameters throughout storage enabled the assessment of the product's shelf stability and potential changes in quality attributes associated with texture. References for the methodology include AACC procedures 74–09 for texture analysis, which measures texture over time as an indication of staling.

### 4.2.7 Microbiological analysis

The evaluation of the total number of microorganisms in the baked bread involved two methods: the total plate count (PCA) method and the yeast and mould count using the MEA method. For the total plate count (PCA) method, the bread samples were first diluted by preparing a 1:10 dilution of 10g of bread in 100 mL of sterile Ringer solution (Merck, Johannesburg, South Africa). The mixture was then homogenized for three min. at 250 rpm using a stomacher machine (Seward, England) in a sterile stomacher bag. A 10-fold dilution series by transferring 1 mL of the solution aliquot into 9 mL of sterile Ringer solution in sterile test tubes, followed by vortexing (Heidolph HEAX Top, Germany). Each dilution was plated in triplicate on Petri plates (90 mm, Merck, South Africa). After incubating the plates at 28 °C for 48 hours, visually determined the plate count. Colonies in the range of 30-300 were recorded, and the results were expressed in log cfu/mL. The yeast and mould count using the MEA method and bread samples were placed in the centre of MEA plates using a sterile needle. The plates were then incubated at 25 °C for 7 days, and the presence or absence of growth was visually observed. Any visible colonies were counted, and the results were expressed accordingly. These microbiological analyses provide valuable information about the microbial load and quality of the baked bread, aiding in determining its shelf life and safety for consumption.

### 4.2.8 Identification of Bacterial Growth

The 16S rRNA gene sequencing is a widely used technique for bacterial identification. This method targets the 16S ribosomal RNA gene, which is conserved across all bacteria but contains variable regions that can differentiate between species. Typically, the first 500 base pairs of the gene are sequenced, which is often sufficient for species-level identification.

This technique allows for the identification of both viable and non-viable organisms, including those with complex growth requirements and slow-growing bacteria. Sequence data can be matched against validated databases like MicroSEQ® or public databases such as the European Nucleotide Archive (ENA) for accurate identification.

#### *4.2.8.1 Polymerase Chain Reaction (PCR)*

PCR amplifies specific DNA sequences, enabling rapid identification of bacteria. Variants like Real-Time PCR and Nested PCR enhance sensitivity and specificity in detecting pathogens. PCR is particularly useful for identifying pathogens in clinical samples and can provide results much faster than traditional culture methods.

### 4.2.9 Identification of Yeast and Mould

For fungal identification, two primary sequencing approaches are employed: D2 region sequencing of the large subunit ribosomal RNA gene (D2 LSU) and sequencing of the Internal Transcribed Spacer (ITS) regions between the small and large subunit ribosomal RNA genes. Application: D2 LSU sequencing is commonly used for identifying molds and yeasts, while ITS sequencing provides high variability between closely related species, making it effective for distinguishing among different fungi. Both methods rely on comparisons with comprehensive sequence databases to confirm identities.

*4.2.9.1 MALDI-TOF Mass Spectrometry*

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry is another advanced technique used for microbial identification. This method analyzes the protein profiles of microorganisms to generate a unique fingerprint that can be matched against a database of known organisms. It is rapid and cost-effective but may require prior confirmation through sequencing for certain isolates.

*4.2.9.2 Shelf life*

The shelf life of baked goods was determined on fresh bread stored for 7 days under ambient conditions. In microbial quantification, plates with colonies within the permitted range of countable plates, have colony numbers ranging from 30 to 300. This range is utilised to provide both statistical reliability and convenience of counting. Plates outside of this range, dilutions, are discarded since they fail to meet the requirements. Plates with fewer than 30 colonies are prone to significant errors due to random variations in colony numbers. Conversely, plates with more than 300 colonies can be difficult to count accurately because colonies may be too dense, potentially leading to overlapping and counting errors. The ideal count range (30-300 colonies) ensures that the results are statistically valid and the counting process is manageable (Penn, 1991).

Plating was performed in triplicate and that the average number of colonies from the countable plates was calculated. The viable count (cfu/mL) was calculated using the following formula:

..........Equation 4.2

$$\text{Viable count (cfu/mL)} = \frac{NN}{VV \text{ x } DD}$$

Where:

*N* is the number of colonies   *V* is the volume plated in millilitres

*D* is the optimum counting dilution

## 4.3 STATISTICAL ANALYSIS

The statistical analysis was performed with SAS Software V8 (SAS Institute, Inc., Cary, NC, USA). A one-way ANOVA with Duncan's multiple-range correlation test was employed for the separation of means between samples. Statistica© Software, version 8 (StatSoft, Inc., Tulsa, Oklahoma, USA) was used for the linear regression analysis. The significance level was set at $p < 0.05$.

## 4.4 RESULTS AND DISCUSSION

### 4.4.1 Optimisation of composite flour blends for bread

Composite flour was utilised in varying proportions (2.5%, 5%, 10%, 15%, and 20%) to formulate flour blends while maintaining a constant water content of 60% (Table 4.2). Among the doughs prepared with composite flour, those with 15% and 20% ratios appeared to be less elastic and drier compared to the white wheat-based dough (control). Post-baking observations revealed that the white wheat dough yielded bread with a smooth crust and a consistent golden-brown colour, exhibiting satisfactory rise. White wheat flour's gluten proteins, which contribute to the dough's desirable viscoelastic properties, are known for their ability to produce high-quality bread. In contrast, the composite flour blend exhibited slower rising kinetics compared to the white wheat dough, resulting in bread with diminished volume, a dry crust, and uniform colouration (Perez-Fajardo *et al*., 2023).

### 4.4.2 Rheology measurements of composite flour blends

Table 4.3 provides a summary of the rheological parameters of various composite flour blends at different stages of the mixing and heating process, ranging from 2.5% to 20% ratios, mixed with water, gluten proteins from composite flours and wheat flour that form a three-dimensional matrix through the development of various molecular interactions, including di-sulfide, ionic, and hydrogen bonds (Wieser, 2007). The relative proportions of the composite ratios significantly impact the rheological properties of the products formulated with these flours. Several researchers (González *et al*., 2019; Haber *et al*., 2019; Gantner *et al.,* 2022) have already used Mixolab to look at how different combinations of insects and composite wheat affect the rheology of dough. Particularly, at C1, the composite flour blends containing mopane worm flour

(MPW) exhibited higher torque values compared to white wheat flour (control), primarily due to differences in protein content and composition between composite flour and white wheat flour.

The highest torque value was found in mopane worm ranging from 2,5% (1.513 Nm), 20% (1.51 Nm), 5% (1.373 Nm) and 10% (1.313 Nm), followed by marula seed cake 5% (1.195 Nm), 10% (1.165 Nm), 15% (1.085 Nm) and 20% (0.98 Nm), mealworm 5% (1.285 Nm) and 20% (0.955 Nm) were at the lower end compared to mopane worm. The highest stability of the formulated dough resulted from mealworm 15% (10.52 min), 10% (10.235 min), 5% (8.59 min), mopane worm 10% (10.035 min), 15% (8.365 min), and 2.5% (6.765 min) with marula seed cake 2.5% (7.64 min), 10% (6.82 min), 15% (5.685 min) and 20% (4.335 min) with the lowest stability values. These values contribute to understanding the rheological properties of the various composite flour formulations. (Table 4.3). The dough stability (measured in min.) did not change much between the composite formulations (ranging from 6.7 to 9.05 for 2.5% to 20% ratios compared to 8.67 for wheat), but the 10% formulation had a longer stability time of 10.2 min. This indicates that the 10% ratio mix possesses robust flour characteristics suitable for bread manufacturing applications (Kuktaite *et al.,* 2004).

At C2, while there was minimal difference between wheat flour and composite flours, the MPW ratio showed higher torque values than wheat, reflecting differences in protein content and composition. The C2 parameter, which shows how the gluten network grows with mechanical work and temperature, shows that higher values mean the dough keeps its shape better (Rosell *et al*., 2010). In terms of hot gel stability (C4), composite flours exhibited decreased torque due to their resistance to strong mechanical shear. This suggests that composite flour blends may have lower stability, potentially leading to a prolonged shelf life for bread (Collar, 2003). The C5 parameter, which measures retrogradation, indicated lower values in composite flour blends, suggesting reduced staling effects. Adding soluble dietary fibre, such as that present in insect flour, can slow retrogradation by maintaining dough moisture, while the addition of protein can further aid in moisture retention (Huang & Ma, 2019; Villasante *et al.,* 2022).

Table 4.3: Effect of composite flour ratios of Rheological characteristics

| | %[8] | C1Torque (Nm) | Stability (min) | C2Torque (Nm) | C3Torque (Nm) | C4Torque (Nm) | C5Torque (Nm) |
|---|---|---|---|---|---|---|---|
| **White Wheat** | | $1.145^{bc}\pm0.005$ | $8.675^{defg}\pm0.655$ | $0.485^{de}\pm0.005$ | $1.66^{f}\pm0.01$ | $1.6^{g}\pm0$ | $2.125^{cd}\pm0.395$ |
| **Mealworm** | | $1.26^{de}\pm0.04$ | $8.135^{cde}\pm0.185$ | $0.48^{de}\pm0.03$ | $1.615^{f}\pm0.205$ | $1.745^{h}\pm0.025$ | $2.58^{ef}\pm0.06$ |
| **Mopane worm** | **2.5** | $1.513^{f}\pm0.04$ | $6.765^{b}\pm0.735$ | $0.65^{gh}\pm0.015$ | $1.456^{def}\pm0.035$ | $2.026^{j}\pm0.0235$ | $2.975^{fgh}\pm0.032$ |
| **Marula seed cake** | | $1.17^{cd}\pm0.03$ | $7.64^{cd}\pm0.11$ | $0.435^{d}\pm0.015$ | $1.62^{f}\pm0.03$ | $1.54^{fg}\pm0.03$ | $2.295^{de}\pm0.015$ |
| **Mealworm** | | $1.285^{de}\pm0.025$ | $8.59^{fg}\pm0.11$ | $0.525^{f}\pm0.005$ | $1.152^{a}\pm0.258$ | $1.74^{h}\pm0.0015$ | $2.714^{efg}\pm0.042$ |
| **Mopane worm** | **5** | $1.373^{e}\pm0.045$ | $9.05^{fgh}\pm0.53$ | $0.623^{g}\pm0.02$ | $1.065^{a}\pm0.003$ | $2.012^{ij}\pm0.002$ | $3.052^{gh}\pm0.056$ |
| **Marula seed cake** | | $1.195^{cd}\pm0.005$ | $7.715^{cd}\pm0.085$ | $0.42^{cd}\pm0.01$ | $1.535^{ef}\pm0.015$ | $1.465^{e}\pm0.015.$ | $2.21^{cd}\pm0.01$ |
| **Mealworm** | | $1.07^{bc}\pm0.12$ | $10.235^{hi}\pm0.115$ | $0.52^{ef}\pm0.05$ | $1.308^{bcd}\pm0.0315$ | $1.513^{fg}\pm0.131$ | $2.28^{d}\pm0.19$ |
| **Mopane worm** | **10** | $1.313^{e}\pm0.03$ | $10.035^{ghi}\pm1.235$ | $0.62^{g}\pm0.01$ | $1.301^{bcd}\pm0.006$ | $1.97^{ij}\pm0.027$ | $2.983^{fgh}\pm0.04$ |
| **Marula seed cake** | | $1.165^{bc}\pm0.045$ | $6.82^{bc}\pm0.35$ | $0.4^{bc}\pm0.01$ | $1.37^{cde}\pm0.03$ | $1.305^{d}\pm0.035$ | $1.935^{b}\pm0.125$ |
| **Mealworm** | | $1.1^{cd}\pm0.01$ | $10.52^{i}\pm0.05$ | $0.56^{g}\pm0.06$ | $1.28^{abc}\pm0.03$ | $1.485^{fg}\pm0.025$ | $2.275^{d}\pm0.045$ |
| **Mopane worm** | **15** | $1.486^{f}\pm0$ | $8.365^{def}\pm0.015$ | $0.7h^{i}\pm0.005$ | $1.293^{bcd}\pm0.04$ | $1.984^{ij}\pm0.0135$ | $3.13^{h}\pm0.105$ |
| **Marula seed cake** | | $1.085^{ab}\pm0.005$ | $5.685^{b}\pm0.885$ | $0.315^{ab}\pm0.035$ | $1.18^{abc}\pm0.02$ | $1.075^{c}\pm0.035$ | $1.355^{a}\pm0.135$ |
| **Mealworm** | | $0.955^{a}\pm0.005$ | $8.635^{efg}\pm0.235$ | $0.44^{d}\pm0.01$ | $1.135^{ab}\pm0.015$ | $0.975^{b}\pm0.035$ | $1.955^{bc}\pm0.105$ |
| **Mopane worm** | **20** | $1.51^{f}\pm0.045$ | $8.925^{fg}\pm0.195$ | $0.733^{i}\pm0.01$ | $1.283^{bcd}\pm0.005$ | $1.926^{i}\pm0.02$ | $2.943^{fgh}\pm0.06$ |
| **Marula seed cake** | | $0.98^{a}\pm0$ | $4.335^{a}\pm0.265$ | $0.265^{a}\pm0.025$ | $1.075^{a}\pm0.005$ | $0.89^{a}\pm0.02$ | $1.3^{a}\pm0$ |

White wheat flour (WWF) Mealworm flour (MWF) Mopane worm flour (MPWF) Marula seed cake flour (MSCF)

Means in the same column with different superscripts are significantly different ($p_{<}$ <0,05)80

[8] Percentages of the composite flours

### 4.4.3 Baking performance of the bread

The bread baking test aims to evaluate the quality and performance of the flour and other ingredients to optimise the formulation of the bread. The visual appearance of the loaf plays an important role in the consumer's choice. In this study, the visual appearance was recorded to ensure consistency during the baking process, while the height of the loaves was recorded to evaluate the effect of the flour replacement on the quality of the final product. The C-Cell product analyser was used to objectively quantify key features that relate to raw material quality and process conditions.

As illustrated in Figures 4.2 and 4.3 all the fresh bread had an appealing brownish and crunchy crust, which could make the consumer more inclined to buy the product.

Figure 4.2: Marula seed cake loaves of bread with different substitutions

| | | | | |
|---|---|---|---|---|
| **2.5%** | **5%** | **10%** | **15%** | **20%** |

Figure 4.3: Mealworm loaves of bread with different substitutions

However, the loaves made with the mopane composite flour (Figure 4.4) were more compact and darker as the percentage of the insect's flour increased. A 15 and 20% replacement led to a very dense bread which was found not appealing.

| Control | 2.5% | 5% | 10% | 15% | 20% |
|---|---|---|---|---|---|

Figure 4.4: Mopane worm loaves of bread with different substitutions compared to the white wheat bread

The darker colour could be attributed to the mopane's flour which was grey to black like the dried worms used for the experiment. Such appearance should be considered as it will have a negative effect on the consumer's choice to purchase the loaf.

*4.4.3.1 Height*

Although the height of the loaf does not matter in the industry as long as the weight of the bread is clearly labelled, it indicates if the ingredients impact the consumer's perception while giving a good indication of the portioning of the composite ingredients on the overall quality.

In general, undersized bread can indicate poor quality control and consumers could assume that if the visual traits (shape /height) are not what they are used to, they will question the intrinsic attributes and will not consider the product.

Table 4.4 reports the height of the front part of the composite loaves of bread compared to the white wheat bread.

Table 4.4: Effect of composite breads in comparison to whole wheat of the bread height

| Flours | %[9] | Height (cm) |
|---|---|---|
| **White wheat** | 100 | 9.23[a] (0.32) |
| **Marula Seed cake** | 2.5 | 8.66[d] (0.09) |
| | 5 | 7.94[b] (0.75) |
| | 10 | 8.06[b] (0.10) |
| | 15 | 7.56[cd] (0.25) |
| | 20 | 7.25[d] (0.28) |
| **Mopane worm** | 2.5 | 9.03[a] (0.67) |
| | 5 | 9.10[a] (0.17) |
| | 10 | 8.63[b] (0.12) |
| | 15 | 8.33[c] (0.40) |
| | 20 | 7.50[cd] (0.20) |
| **Mealworm** | 2.5 | 9.17[a] (0.40) |
| | 5 | 8.96[a] (0.24) |
| | 10 | 8.23[b] (0.55) |
| | 15 | 7.41[c] (0.23) |
| | 20 | 7.26[cd] (0.45) |

[9] Percentages of the composite flours

Averages (n = 3) followed by the same upper-case letter did not differ significantly within a column at P≤0.05. The standard deviation for each set is given in brackets below the average.

The increased levels of flour substitution led to a significant ($p<0.05$) decrease in the height of all composite breads. This decrease in height may be due to the higher water absorption capacity of the alternative flours. However, as mentioned by Ma *et al*. (2007) flour for bread-making should possess a higher water absorption capacity at normal working consistencies so that the yield of dough, and hereafter bread, will be rather high. In addition, the absence of gluten in the alternative flour affects the height of the bread due to the inability of the blends to both expand (reduce air holding during mixing) and retain the carbon dioxide required for leavening (Cappelli, Oliva & Cini, 2020; Kowalczewski *et al*., 2019).

4.4.3.2 *Colour of the crust and crumbs*

Based on the data presented in Table 4.5, the colour characteristics of the baked bread's crust and crumb were evaluated. Colour plays a vital role in consumer acceptance of baked goods, alongside texture and volume. In general, formulations incorporating insect flours resulted in loaves with lower brightness (L*), higher redness (a*), and increased yellow tonality (b*). These colour changes were attributed to numerous factors, such as cooking duration, temperature, and the presence of composite bread. The colour of baked goods is often influenced by the colour of the raw materials used. The colour parameters (CIEL*ab**) of the composite bread at 10% mealworm (MW), mopane worm (MPW) and marula seed cake (MSC) were not directly correlated with the colour parameters of the crumbs. This suggests that breadmaking conditions during mixing, proofing, and baking could have altered the flour constituents compared to standard white wheat flour. The white wheat bread (control) had the greatest L* value, with 217.12 in the crust and 204.03 in the crumb. This suggests that the crust and crumb of whole wheat bread were lighter in colour or brighter than composite breads. Composite bread made with Mopane worm flour at 10% concentration, had the lowest L* value, with 128.07 in the crust and 142.54 in the crumb. This implies a darker colour or less brightness in the crust and crumb of bread baked with mopane worm flour. Composite bread made with Mopane worm flour at 2.5% concentration had the highest a* value 44.45 in the crust, this implies a greater redness in the crust of bread, while white wheat bread (control) had the lowest a* value in the crust (1.7). This suggests that white wheat bread's crust was less red than that of composite breads. Mopane worm bread at 20% concentration had the greatest b* value, measuring 77.67 in the crust and 77.67 in the crumb. Figures 4.5, 4.6, and 4.7 present critical visual representations that explain the findings of this study regarding colour differences. Each figure serves to illustrate key aspects of the research, enhancing the understanding of the data. At this concentration, the bread produced with mopane worm flour had a higher yellowness in both the crust and the crumb. White wheat bread had the lowest b* value in the crust at 58.15. This suggests that white wheat bread's crust was less yellow than composite breads from Table 4.5. The crumb colour of the composite loaves was darker, redder, and less yellow compared to white wheat bread. Marula seed cake bread results showed the most brownish crumbs, indicating the highest b* value. Similar crumb colours have been achieved using flours with colours approximating those of the composite insect and marula seed cake flours, such as lentils, beans, or chickpeas (Kohajdova *et al*., 2013). These colour parameter variations illustrate disparities in the crust and crumb colouration of composite breads, which can be related to their distinct compositions.

Table 4.5: Colour analysis of the crust of the composite breads compared to the white wheat bread

| | %[10] | L* | a* | b* | ΔEab |
|---|---|---|---|---|---|
| **White wheat bread** | 100 | $204.03^{e}$ | $14.71^{abc}$ | $81.41^{cdef}$ | - |
| **Marula Seed cake bread** | 2.5 | $179.60^{d}$ | $25.93^{efg}$ | $88.68^{fgh}$ | 28 |
| | 5 | $195.56^{e}$ | $13.92^{ab}$ | $80.52^{cdef}$ | 9 |
| | 10 | $178.07^{d}$ | $22.86^{def}$ | $83.04^{defg}$ | 27 |
| | 15 | $162.10^{c}$ | $31.70^{g}$ | $83.38^{efgh}$ | 46 |
| | 20 | $161.7^{c}$ | $26.59^{efg}$ | $77.47^{cde}$ | 44 |
| **Mopane worm bread** | 2.5 | $179.35^{d}$ | $22.80^{def}$ | $91.87^{gh}$ | 28 |
| | 5 | $176.36^{d}$ | $18.31^{bcd}$ | $83.87^{defg}$ | 28 |
| | 10 | $128.07^{d}$ | $23.39^{def}$ | $57.28^{a}$ | 80 |
| | 15 | $155.66^{c}$ | $18.08^{bcd}$ | $74.03^{bcd}$ | 49 |
| | 20 | $155.25^{c}$ | $10.08^{a}$ | $64.19^{ab}$ | 52 |
| **Mealworm bread** | 2.5 | $141.87^{b}$ | $44.45^{h}$ | $77.67^{cde}$ | 69 |
| | 5 | $182.13^{d}$ | $26.89^{fg}$ | $94.66^{h}$ | 28 |
| | 10 | $178.78^{d}$ | $19.85^{bcde}$ | $80.55^{cdef}$ | 26 |
| | 15 | $158.57^{c}$ | $23.34^{def}$ | $75.97^{cd}$ | 47 |
| | 20 | $157.02^{c}$ | $21.66^{cdef}$ | $72.44^{bc}$ | 46 |

Means in the same column with different superscripts is significantly different ($p<0,05$)

[10] Percentages of the composite flours

Table 4.6: Colour analysis of the crumb of the composite breads compared to the white wheat bread (Continued)

| Treatment | %[11] | L* | a* | b* | ΔEab |
|---|---|---|---|---|---|
| **White wheat bread** | 100 | $217.12^{l}$ | $1.7^{a}$ | $58.15^{cd}$ | - |
| **Marula Seed cake bread** | 2.5 | $200.48^{jk}$ | $7.74^{bcd}$ | $50.54^{ab}$ | 19 |
| | 5 | $192.33^{k}$ | $5.48^{ab}$ | $52.18^{b}$ | 25 |
| | 10 | $192.38^{hi}$ | $10.09^{cdef}$ | $51.18_{ab}$ | 27 |
| | 15 | $184.06^{ef}$ | $10.95^{def}$ | $47.79^{a}$ | 36 |
| | 20 | $176.40^{d}$ | $12.89^{ef}$ | $47.64^{a}$ | 44 |
| **Mopane worm bread** | 2.5 | $194.94^{de}$ | $1.28^{h}$ | $55.58^{h}$ | 22 |
| | 5 | $189.42^{d}$ | $1.26^{g}$ | $54.1^{g}$ | 28 |
| | 10 | $142.54^{a}$ | $3.32^{h}$ | $46.35^{c}$ | 76 |
| | 15 | $157.69^{b}$ | $2.77^{g}$ | $51.85^{f}$ | 60 |
| | 20 | $166.05^{b}$ | $1.91^{cdef}$ | $50.55^{e}$ | 52 |
| **Mealworm bread** | 2.5 | $204.90^{k}$ | $5.79^{ab}$ | $61.22^{de}$ | 13 |
| | 5 | $194.92^{ij}$ | $6.35^{bc}$ | $58.86^{cd}$ | 23 |
| | 10 | $187.72^{fg}$ | $8.98^{def}$ | $59.74^{cd}$ | 30 |
| | 15 | $175.22^{d}$ | $11.29^{def}$ | $60.09^{cd}$ | 42 |
| | 20 | $163.41^{c}$ | $13.57^{f}$ | $59.75^{cd}$ | 55 |

Means in the same column with different superscripts are significantly different (P<0,005).

White wheat flour (WWF) Mealworm flour (MWF) Mopane worm flour (MPWF) Marula seed cake flour (MSCF)

[11] Percentages of the composite flours

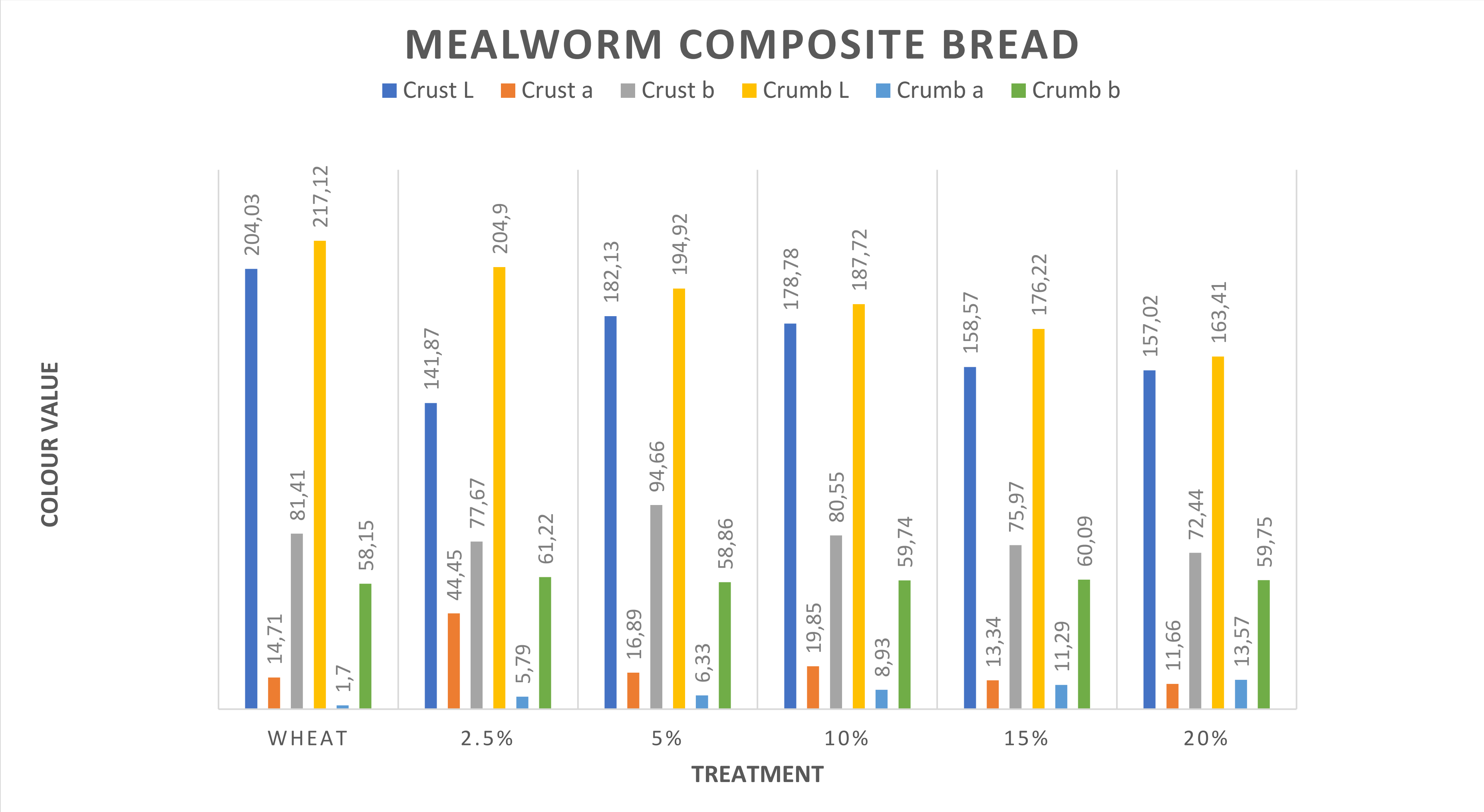


Figure 4.5: Crust & crumb colour of mealworm bread samples containing composite flours compared to standard white wheat

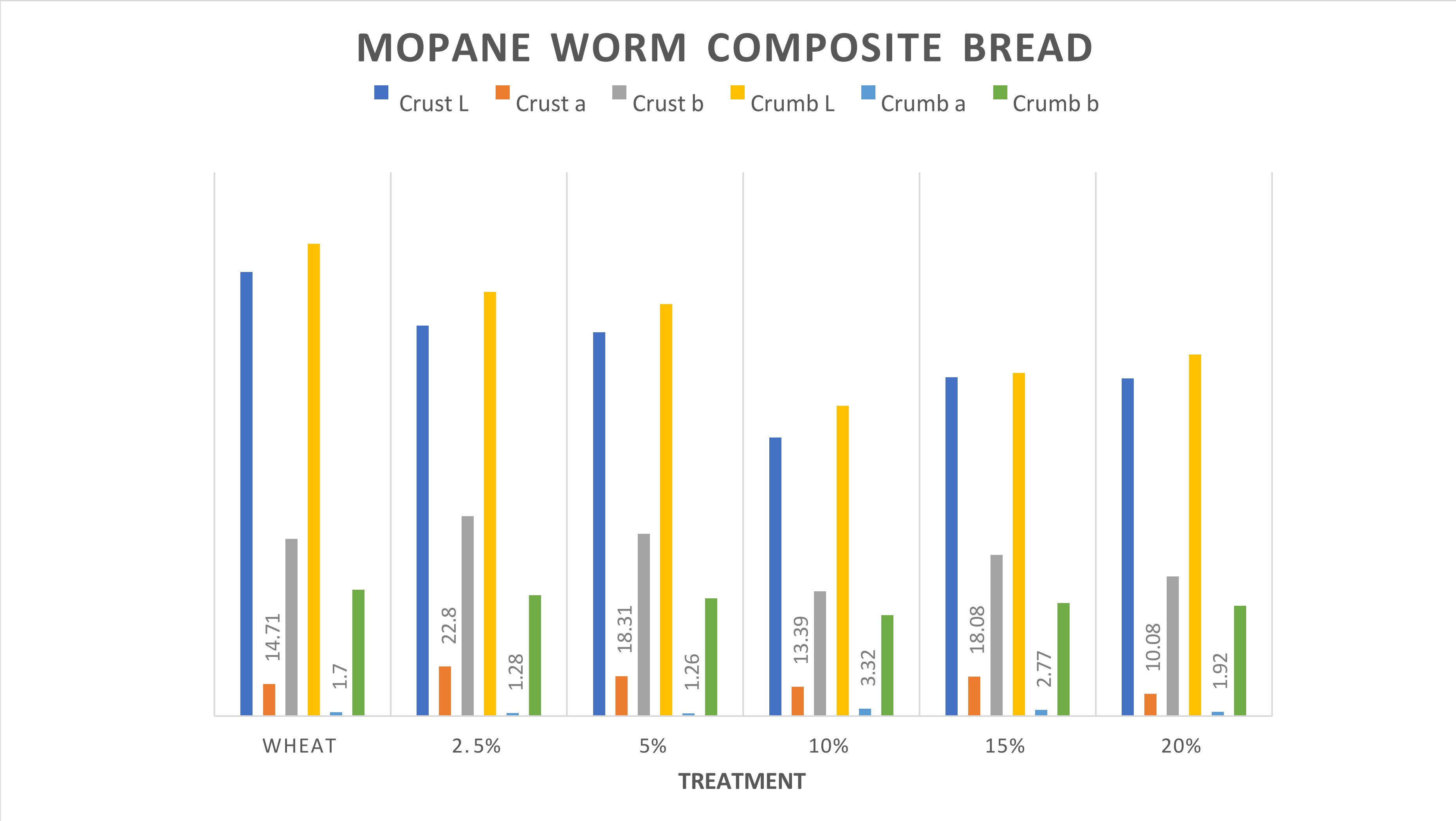


Figure 4.6: Crust & crumb Colour of mopane worm bread samples containing composite flours compared to standard white wheat

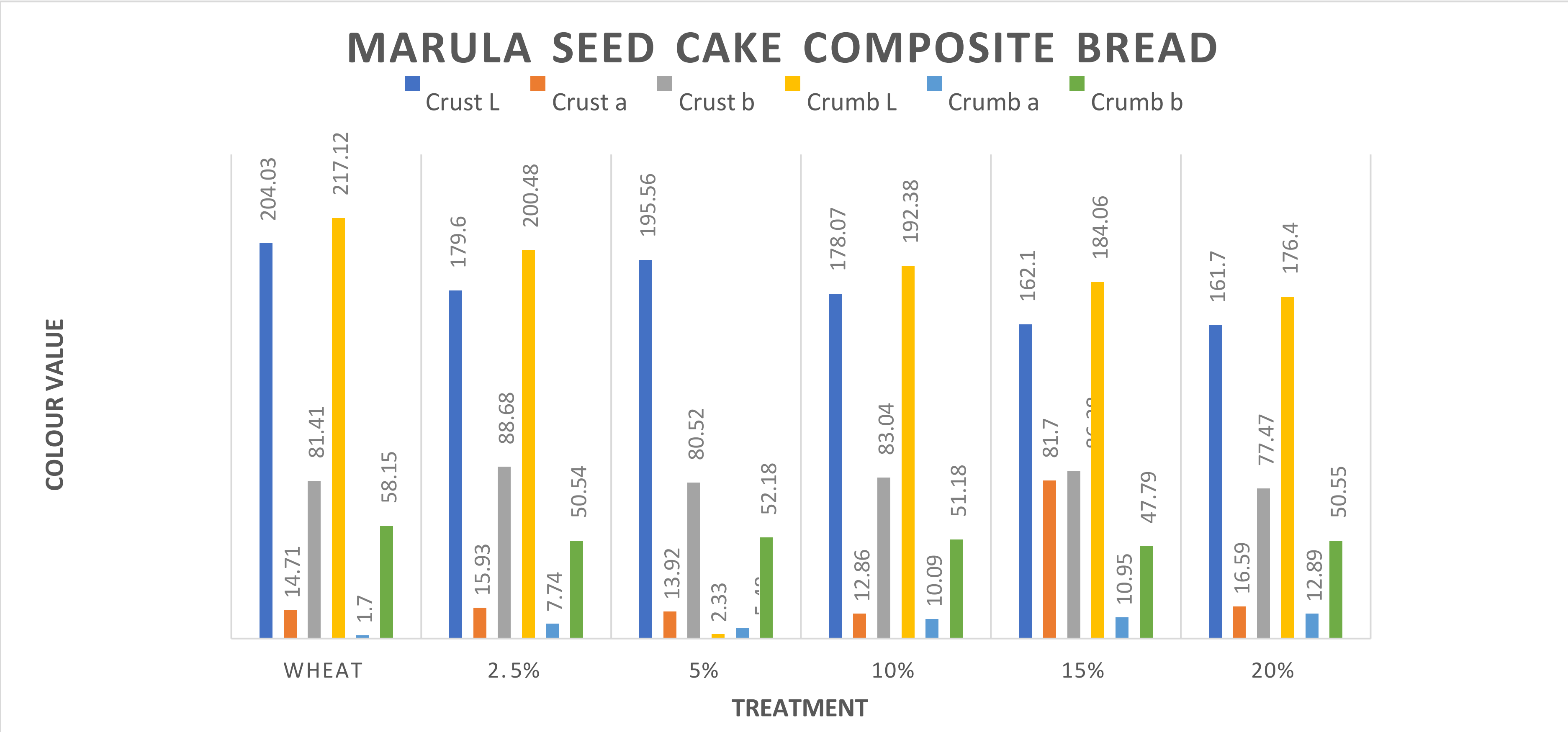


Figure 4.7: Crust & crumb Colour of marula seed cake bread samples containing composite flours compared to standard white wheat

*4.4.3.3 C-Cell analysis*

A comprehensive evaluation of the internal structure of bread slices was done using the C-cell imaging system focusing on key metrics such as cell diameter, area of cells, slice area, and wall thickness. The mealworm composite bread had the largest average cell diameter at 3.10 mm, indicating a more open and airier crumb structure compared to the white wheat bread with a diameter of 2.68 mm, indicating a moderately open crumb structure. The mopane worm composite bread had a slightly smaller diameter at 2.51 mm, while the marula seed cake composite bread had the smallest average cell diameter at 2.45 mm, indicating a denser crumb structure. The mealworm sample had the highest area of cells at 52.04%, representing the proportion of the total slice area occupied by the cells' air pockets. The white wheat bread showed a similar area of cells at 50.7%, indicating good aeration. Both mopane worm (50.68%) and marula seed cake (50.08%) composite breads had slightly lower areas of cells, suggesting less aeration in their respective crumb structures.

Figure 4.8 illustrates the density characteristics of mopane worm, marula seed acke and mealworm composite breads as analysed using the C-Cell imaging system. This figure provides a detailed visual representation of the internal crumb structure of the bread, which is crucial for understanding its quality and texture. The slice areas varied among samples, with the mealworm sample having the largest slice area at 8890 $mm^2$ (2,5%), which may contribute to its overall texture, with the white wheat slice area at 8206 $mm^2$ indicating the total surface area of the sliced bread, while the marula seed cake and mopane worm samples had smaller slice areas (7939 $mm^2$ and 7503 $mm^2$, respectively), that may affect how each type of bread retains moisture and air during baking. The results from the wall thickness were relatively consistent across most samples but were slightly thicker in the mealworm sample (0.60 mm) compared to others like the white wheat (0.58 mm) and marula seed cake (0.56 mm) and the mopane worm flour had a wall thickness of 0.57 mm, indicating that all samples maintained similar structural integrity but varied slightly in terms of wall thickness (Li *et al*., 2023).

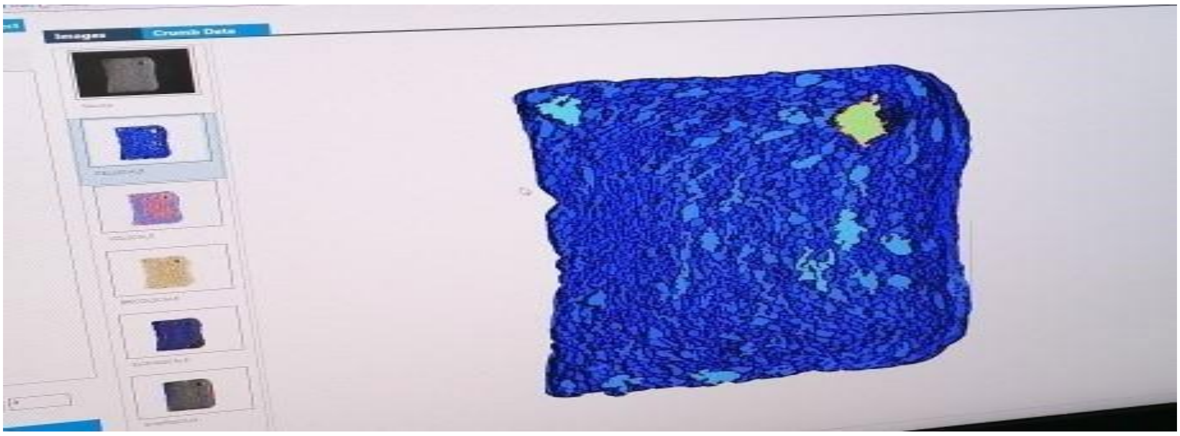

Figure 4.8: Density characteristics of mopane worm composite bread under C-Cell

The mealworm composite bread stood out with larger cell diameters and higher areas of cells, contributing to a lighter texture, but both the mopane worm and marula seed cake composite breads exhibited denser structures with smaller cell sizes and areas, which may influence their baking. The consistent wall thickness across samples indicates that all types maintained structural integrity during baking. However, the proximate composition of mopane worm composite bread, which had more protein and less fat than mealworm composite bread, may interfere with appropriate dough development during fermentation. This interference may contribute to the weaker gluten network's low gas retention ability, especially in bread produced with mopane worm composite bread at ratios of 2.5%, 5%, 10%, 15%, and 20% (Table 4.6). It is important to note that, while some composite breads may yield smaller bread sizes than the control, but they may have additional nutritional benefits that need further investigation and optimisation (Whitworth *et al.,* 2008).

Table 4.7: The effect of baking performance on composite bread

| Sample | %[12] | Cell diameter (mm) | Area of cells (%) | Slice area ($mm^2$) | Wall thickness (mm) | |
|---|---|---|---|---|---|---|
| **White Wheat** | | $2.68^{c}$ ±0.16 | $50.7^{e}$ ±1.05 | $8206.31^{f}$±344.98 | $0.58^{bcde}$±0.02 | c |
| **Mealworm** | | $3.1^{c}$ ±0.34 | $52.04^{e}$ ±1.05 | $8890.1^{g}$±502.78 | $0.6^{efg}$±0.02 | c |
| **Mopane worm** | **2.5** | $2.51^{c}$ ±0.11 | $50.68^{e}$ ±0.44 | $7503^{de}$±395.46 | $0.57^{bc}$±0.01 | c |
| **Marula seed cake** | | $2.45^{c}$ ±0.21 | $50.08^{e}$ ±0.85 | $7939.41^{ef}$±284.22 | $0.56^{bcde}$±0.02 | c |
| **Mealworm** | | $2.86^{c}$ ±0.16 | $50.29^{e}$ ±0.42 | $7992.96^{f}$±234.02 | $0.6^{fg}$±0.01 | c |
| **Mopane worm** | **5** | $2.37^{c}$ ±0.16 | $50.54^{e}$ ±0.55 | $7495.09^{bcd}$±341.48 | $0.56^{bc}$±0.01 | c |
| **Marula seed cake** | | $2.7^{c}$ ±0.35 | $50.39^{e}$ ±0.93 | $7451.81^{bcd}$±279.65 | $0.58^{cdef}$±0.01 | c |
| **Mealworm** | | $2.86^{c}$ ±0.16 | $50.29^{c}$±0.42 | $7992.96^{cde}$±234.02 | $0.6^{fg}$±0.01 | c |
| **Mopane worm** | **10** | $2.23^{c}$ ±0.13 | $48.88^{f}$±0.63 | $6998.14^{a}$±417.03 | $0.54^{a}$±0.01 | c |
| **Marula seed cake** | | $2.88^{c}$ ±0.1 | $51.21^{d}$±0.47 | $7675.42^{def}$±199.9 | $0.6^{g}$±0.01 | c |
| **Mealworm** | | $2.55^{b}$±0.21 | $49.69^{b}$±0.62 | $6665.22^{abc}$±323.27 | $0.57^{bcd}$±0.01 | b |
| **Mopane worm** | **15** | $1.94^{c}$ ±0.82 | $42.06^{d}$±1.71 | $5793.41^{abc}$±2379.87 | $0.46^{ab}$±0.19 | c |
| **Marula seed cake** | | $2.45^{c}$ ±0.13 | $49.5^{c}$±0.43 | $7326^{cde}$±185.53 | $0.56^{bc}$±0.01 | c |
| **Mealworm** | | $2.49^{a}$±0.15 | $48.45^{a}$±0.64 | $6874.84^{ab}$±158.41 | $0.57^{defg}$±0.57 | a |
| **Mopane worm** | **20** | $0.78^{c}$ ±1.11 | $16.24^{d}$±2.29 | $2151.38^{a}$±3043.11 | $0.18^{ab}$±0.26 | c |
| **Marula seed cake** | | $2.68^{d}$±0.1 | $49.96^{g}$±0.58 | $6847.23^{ab}$±203.27 | $0.58^{cdefg}$±0 | d |

Means in the same column with different superscripts is significantly different ($p < 0,05$)

[12] Percentages of the composite flours

### 4.4.4 Texture analysis

Texture is critical for understanding bread's properties and customer acceptance (Gómez *et al.,* 2018). Texture includes a variety of properties such as crumb softness, chewiness, resilience, and overall mouthfeel, all of which have a substantial impact on the eating experience. Firmness, springiness, cohesiveness, and chewiness are popular texture analysis characteristics used to characterise bread's physical attributes (Ahmed *et al.,* 2019). These properties are frequently measured with devices such as texture analysers, which apply controlled stresses to bread samples and record the mechanical responses.

Understanding how different ingredients and processing procedures affect the texture of composite flours in breadmaking was essential for optimizing product quality. The mealworm, mopane worm, and marula seed cake composite flours affected bread firmness when compared to white wheat bread (Figures 4.9, 4.10, 4.11). Mealworm composite bread had a lower hardness than white wheat bread. As the percentage of mealworm flour grew, the bread became less firm. Throughout the testing period (Days 1-7), all samples increased in hardness, with the lowest reading at 15% mealworm bread and the highest at 5%. This contrasts with the increased hardness of white wheat bread over the same period.

The inclusion of mealworm flour hindered water absorption due to its high-fat content, resulting in increased hardness over time. This result is similar to recent studies on seaweed composite bread Mamat *et al.* (2014), which found that the addition of composite flours boosted the bread's hardness, resulting in significant increases in quality measures. Loaves of bread prepared with mopane worm and marula seed cake composite flours also hardened with time, but in different ways than mealworm and white wheat bread loaves. The mopane worm flour, mealworm flour, had a significant fat content that decreased water absorption. The composite breads were firmer than white wheat bread, with mealworm having the most similar softness profile to white wheat bread, followed by mopane worm and marula seed cake. All composite bread formulations were consistently firmer than white wheat bread, indicating long-term firmness. Mart-Alfonso *et al*. (2018) suggest that the lower viscosity and increased fat content of composite bread may influence its texture

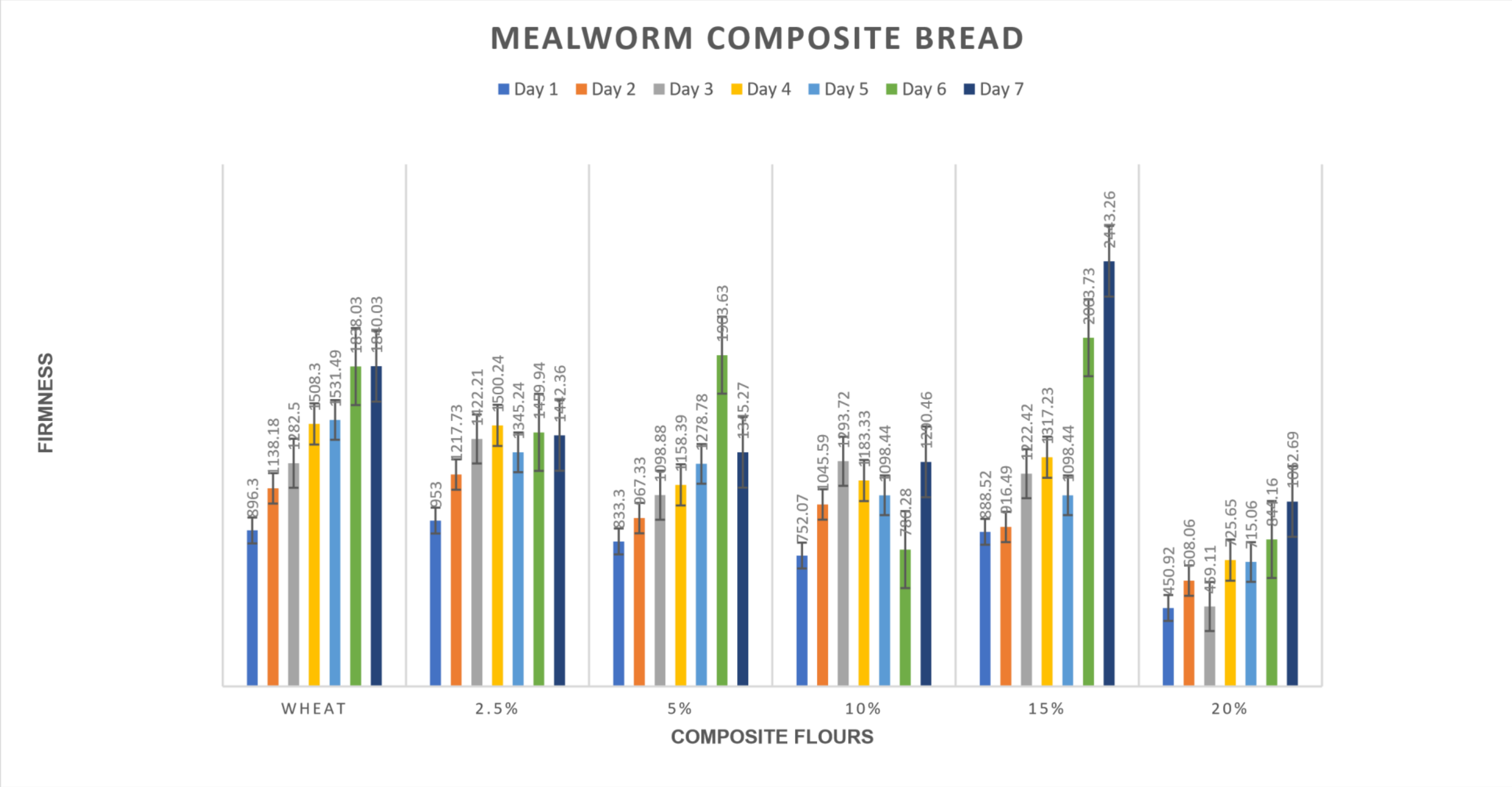


Figure 4.9: Firmness of mealworm bread samples containing composite flours compared to standard wheat (WWB)

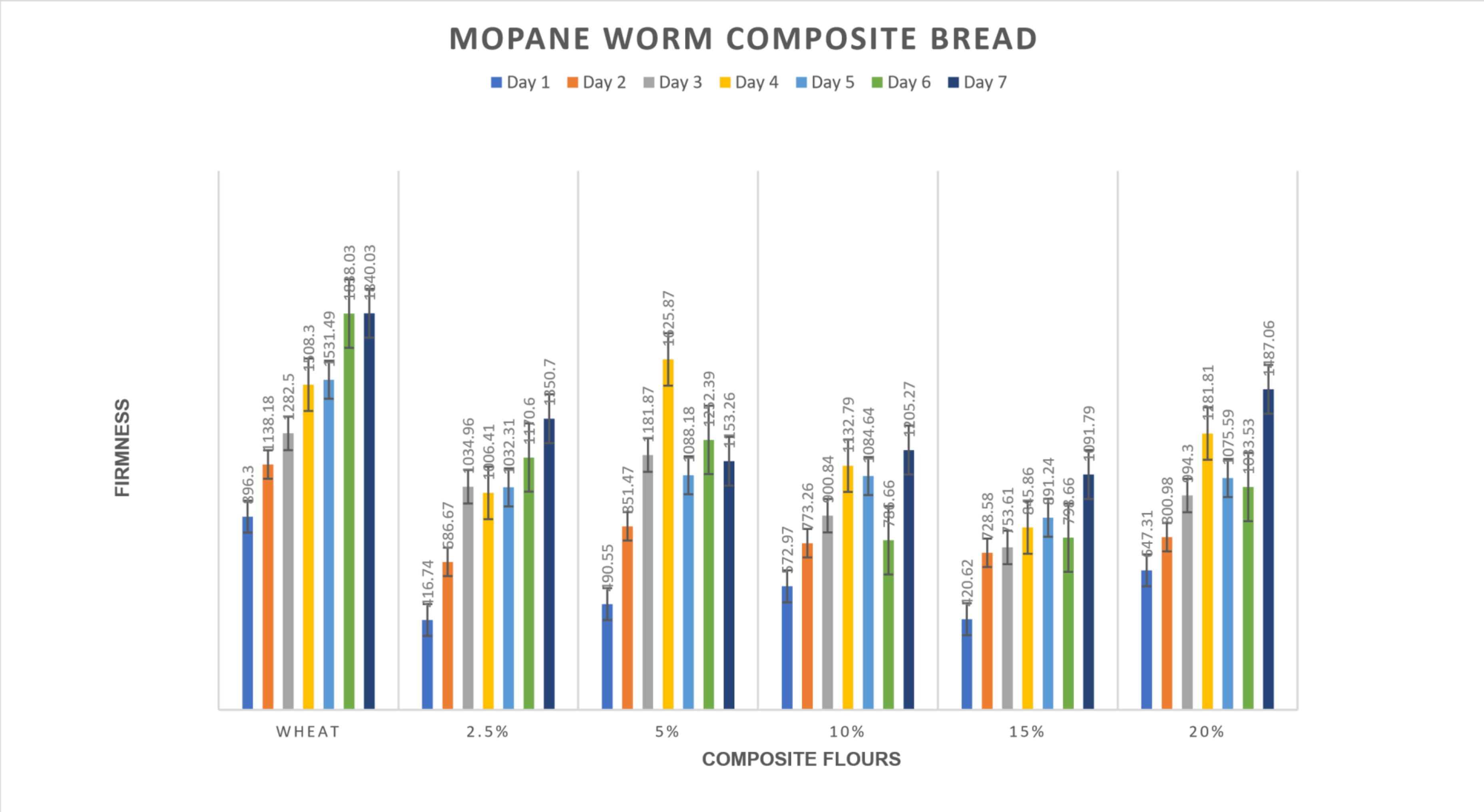


Figure 4.10: Firmness of mopane bread samples containing composite flours compared to standard wheat (WWB)

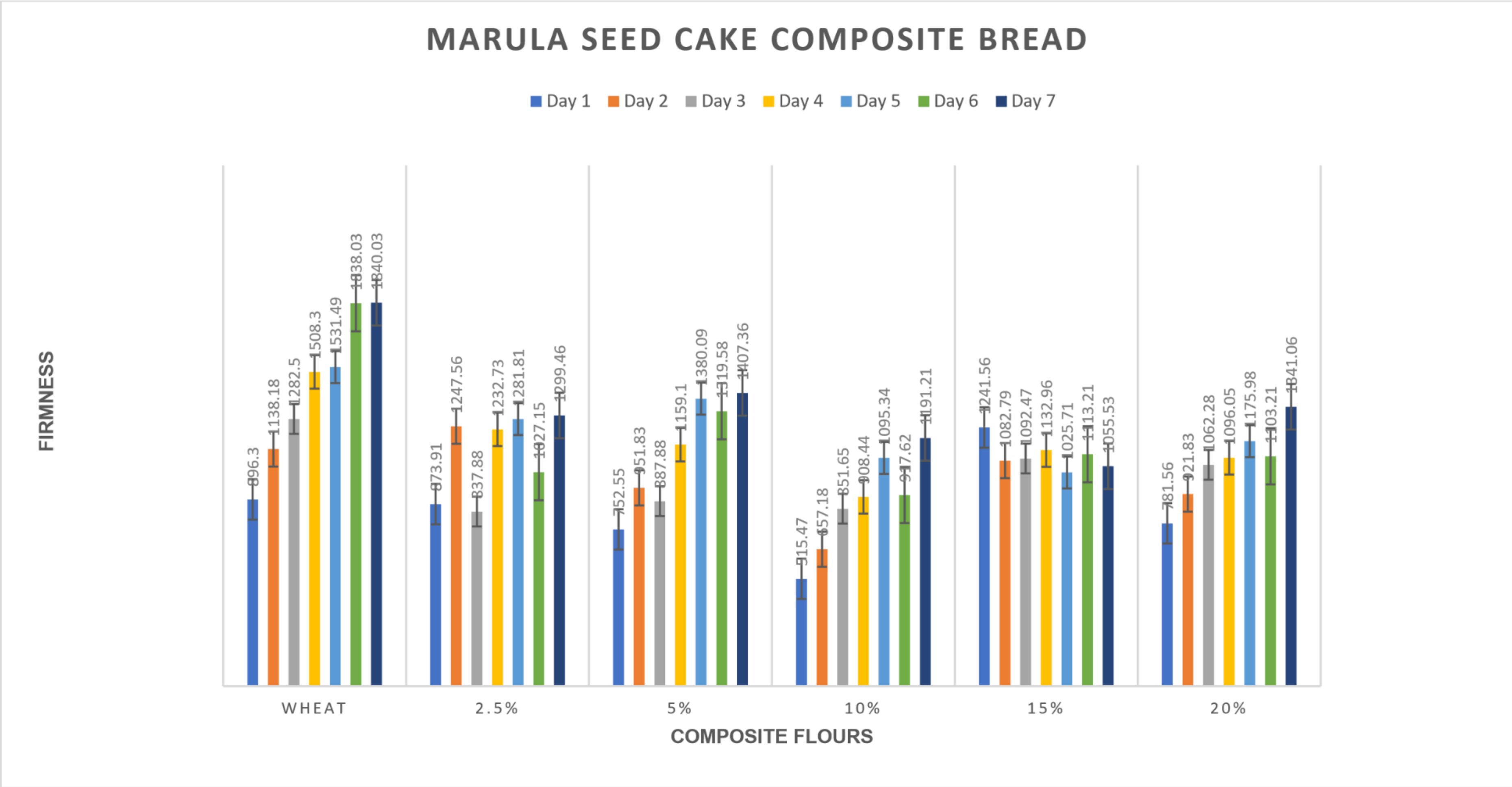


Figure 4.11: Firmness of marula seed cake bread samples containing composite flours compared to standard wheat

### 4.4.5 Shelf life of bread

The results presented in Table 4.7 provide insights into the microbial counts of various bread types mealworm bread mopane worm bread, and marula seed cake bread at different inclusion levels (2.5%, 5%, 10%, 15%, and 20%) over five days. The control samples for both PCA and MEA showed negligible microbial counts (< 25 cfu/mL), indicating that the base bread was free from significant microbial contamination. The introduction of alternative loaves of bread, on day 1, the TVC for mealworm bread at 5% inclusion was recorded at 5.3 x $10^2$ cfu/mL, while mopane worm bread and marula seed cake bread showed negligible counts. This indicates that mealworm bread may have a higher initial microbial load compared to the other loaves of bread (Mmthiyane *et al*., 2017). The MEA results showed similar trends, with mopane worm bread having a notably high count of 8.3 x $10^7$ cfu/mL. suggesting a significant level of fungal contamination.

The TVC for mealworms increased to 5.3 x $10^2$ cfu/g, while MPWF showed a notable rise to 4.4 x $10^4$ cfu/mL. This increase indicates that microbial activity was growing, particularly in the mopane worm. The results on the MEA plates for day 2 revealed that mopane worm bread had counts of 8.3 x $10^6$ cfu/mL g, indicating a sustained presence of fungi. TVC for mopane worm bread remained stable at 8.5 x $10^3$ cfu/mL, while mopane worm bread showed a slight decrease to 6.3 x $10^3$ cfu/mL, this suggests that the microbial growth may be stabilizing. The MEA results showed that MPWF maintained a count of 5.7 x $10^3$ cfu/mL, indicating consistent fungal presence.

Mealworm bread exhibited a significant increase in the TVC to 3.2 x $10^6$ cfu/mL, indicating a potential spoilage risk, while mopane worm bread also showed an increase to 6.8 x $10^4$ cfu/mL. Mealworm bread peaked at 7.8 x $10^7$ cfu/mL, while mopane worm bread also showed high counts of 5.6 x $10^6$ cfu/mL. This suggests that both breads are prone to high microbial growth over time, particularly the mealworm bread. The inclusion of alternative flours, particularly mealworm flour and mopane worm flour, significantly affects the microbial quality of the bread over time. The high microbial counts observed, especially in mealworm bread, raises concerns regarding the safety and shelf life of products made with these flours. The absence of harmful microorganisms such as *Staphylococcus aureus* and Salmonella spp. in the samples is reassuring, but the elevated total viable and fungal counts suggest that careful monitoring and management of microbial loads are essential in the processing of these alternative flours (Uzuegbu *et al*., 2012).

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Table 4.8: Microbial counts for composite bread over 5 days

| | | **5%** | | | **10%** | | | **15%** | | | **20%** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **MWB** | **MPWB** | **MSCB** | **MWB** | **MPWB** | **MSCB** | **MWB** | **MPWB** | **MSCB** | **MWB** | **MPWB** | **MSCB** |
| **Control** | TVC | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 |
| | TFC | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 |
| **Day 1** | TVC | $5.3x10^{2}$ | <25 | <25 | $1.2x10^{5}$ | $8.7x10^{3}$ | $1.4x10^{5}$ | < 25 | $9.3x10^{4}$ | < 25 | $8.5x10^{3}$ | $7.7x10^{3}$ | < 25 |
| | TFC | < 25 | $8.3x10^{7}$ | $5.1x10^{2}$ | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 |
| **Day 2** | TVC | $5.3x10^{2}$ | >25 | $5.4X10^{3}$ | $1.2x10^{5}$ | $4.4x10^{4}$ | $7.6x10^{4}$ | < 25 | < 25 | < 25 | $9.6x10^{3}$ | < 25 | <25 |
| | TFC | < 25 | < 25 | < 25 | < 25 | < 25 | < 25 | $8.5x10^{3}$ | $8.3x10^{6}$ | < 25 | $5.9x10^{3}$ | < 25 | $4.9x10^{5}$ |
| **Day 3** | TVC | $8.5x10^{3}$ | $6.3x10^{3}$ | $7.1x10^{3}$ | $9.9x10^{3}$ | $4.5x10^{3}$ | $4.9x10^{3}$ | < 25 | $5.5x10^{3}$ | < 25 | < 25 | $5.3x10^{5}$ | < 25 |
| | TFC | < 25 | $5.7x10^{3}$ | < 25 | < 25 | $6.4x10^{3}$ | < 25 | < 25 | < 25 | < 25 | < 25 | $5.5x10^{3}$ | < 25 |
| **Day 4** | TVC | $3.2x10^{6}$ | $6.8x10^{4}$ | $5.1x10^{4}$ | $7.5x10^{4}$ | $4.4x10^{5}$ | $8.2x10^{3}$ | < 25 | $4.4x10^{5}$ | < 25 | $3.7x10^{4}$ | $8.4x10^{4}$ | $5.6x10^{2}$ |
| | TFC | $8.6x10^{3}$ | $9.3x10^{3}$ | $6.5x10^{4}$ | $8.9x10^{3}$ | $1.4x10^{4}$ | $9.4x10^{5}$ | < 25 | $6.1x10^{5}$ | $4.2x10^{2}$ | $1.0x10^{4}$ | $8.8x10^{4}$ | $5.2x10^{2}$ |
| **Day 5** | TVC | $7.8x10^{7}$ | $5.6x10^{6}$ | < 25 | $3.8x10^{5}$ | $5.1x10^{7}$ | $5.9x10^{6}$ | $5.4x10^{4}$ | $7.7x10^{6}$ | $6.8x10^{6}$ | $3.4x10^{6}$ | < 25 | < 25 |
| | TFC | $4.8x10^{4}$ | < 25 | $6.3x10^{4}$ | < 25 | $7.0x10^{5}$ | $4.0x10^{4}$ | < 25 | $7.6x10^{6}$ | $4.7x10^{4}$ | $1.0x10^{5}$ | < 25 | $5.2x10^{5}$ |

The microbial analysis of various flour types, including white wheat flour, mealworm flour, mopane worm flour, and marula seed cake flour, revealed the presence of specific bacterial and fungal species. Table 4.9 provides insights into the microbial composition of these alternative flours and their potential implications for food safety and quality. Several factors may account for the absence of bacteria in baked bread products. The pre-treatment processes and working conditions employed during the production of composite bread likely contributed to a reduction in spoilage bacteria. Additionally, the heat applied during baking is effective in eliminating most microbes present in the dough (Coda *et al*., 2011). The results indicate that while WWF showed no growth of bacteria or fungi, the alternative flours exhibited the presence of various microorganisms. *Aspergilus niger*, is a common fungus with industrial applications and widespread presence in food. The *Bacillus safensis*, known for its plant growthpromoting properties and biotechnological uses, was also detected. The presence of *Cronobacter sakazakii*, associated with neonatal illnesses, was specifically noted in the mopane worm bread with *Bacillus subtilis* recognized as safe for consumption, and *Rhizopus oeyzae* which produces fumaric acid, was found in the marula seed cake bread (Caulier *et al.* 2019). These findings underscore the necessity for stringent food safety measures throughout the manufacturing and storage processes. They also highlight the acceptable microbiological quality of the composite bread during storage, suggesting that while some microbial presence is noted, it does not compromise the overall safety of the products.

Table 4.9: Detection and identification of microbial growth from composite bread

| Sample ID | White wheat bread | Mealworm bread | Mopane worm bread | Marula seed cake bread | Literature range |
|---|---|---|---|---|---|
| **Bacterial growth** | NG | *Bacillus safensis-* | *Bacillis velezensis* | *Bacillis subtilis* | - |
| **Yeast and Mould** | NG | *Aspergillus niger* | *Cronobacter sakazakii* | *Rhizopus delemar* | - |

NG: No growth; - = Absent;

## 4.5 PROXIMATE VALUE OF COMPOSITE BREAD

The proximate analysis of various breads revealed significant differences in their protein, fat, and dietary fiber content. White wheat bread contains 8.08 % protein, 1.46 % fat, and 1.78 % dietary fiber, serving as a common staple in many diets. In contrast, mealworm composite bread had a higher protein content of 11.81 % and a notable increase in fat at 3.37 %, along with 1.9 % dietary fiber, representing a notable increase of 46 %. highlighting its potential as a nutritious alternative. Mopane worm composite bread stood out with the highest protein level at 15 %, substantial fat content of 12.9 %, and 2.34 % dietary fiber, making it an excellent source of essential nutrients with a notable high overall increase of 59 %. Marula seed cake composite bread presents a balanced profile with 10.66% protein, 3.91 % fat, and the highest dietary fiber content at 3 %, reflecting a increase of 32 % overall. These alternative flours not only provide higher protein and fat levels compared to traditional white wheat flour but also contribute to increased dietary fiber intake, promoting a more nutritious diet.

Table 4.10: Proximate composition value of standard white wheat bread compared to composite breads of wheat with mealworm, mopane worm and marula seed cake flours at 15% substitution.

| | **%[13]** | **Protein** | **Fat** | **Dietary fibre** | **Increase(%)** |
|---|---|---|---|---|---|
| White Wheat Bread | | 8.08 | 1.46 | 1.8 | |
| Mealworm composite bread | | 11.81 | 3.37 | 1.9 | 46 |
| Mopane worm composite bread | 15 | 12.9 | 2.34 | 1.9 | 59 |
| Marula Seed cake composite bread | | 10.66 | 3.91 | 3 | 32 |

[13] Percentage with the best performing composite bread

## 4.6 COST CALCULATION

The analysis of the costs associated with various composite bread formulations indicates that the marula seed cake 15% bread is the most cost-effective option among those evaluated. Table 4.11 detailed breakdown of the costs for each type of bread, including standard bread, mopane bread 15%, mealworm bread 15%, and marula seed cake bread 15%. The cost calculation for composite bread using mealworm, mopane worm, and marula seed cake flour demonstrates substantial economic advantages over market protein bread (Table 4.11).

The marula seed cake formulation stands out due to its lower overall cost compared to other composite breads, particularly the mealworm bread, which is significantly more expensive due to the high cost of mealworm flour at R340.18 per kg. The marula seed cake flour, priced at R36 per kg, offers a more affordable alternative while still providing nutritional benefits.

The total cost for the marula seed cake bread is R9.15 per loaf, which is higher than the standard bread at R7.68 but lower than the mopane bread at R11.44 per loaf and it is notably less than the mealworm bread at R30.18 per loaf.

Table 4.11: Costing comparisons for the composite bread

| **Bread Type** | % | **Total cost per loaf (R)** |
|---|---|---|
| White wheat bread | | R7.68 |
| Mopane Bread | 15 | R11.44 |
| Mealworm Bread | | R30.18 |
| Marula seed cake Bread | | R9.15 |

The cost is based on the ingredients only excluding other overalls like electricity, labour, packaging and retailing

The results indicate that incorporating marula seed cake not only reduces costs but also enhances the nutritional profile of the bread, making it an attractive option for consumers seeking healthier and more sustainable food choices. This analysis supports the growing interest in composite flours as a means to diversify ingredients and improve food security in regions where traditional wheat flour prices can be volatile. These calculations provide the estimated nutritional value and cost of the bread recipe based on the provided ingredients and their respective prices. The 15 % composite flour resulted in bread with a desirable balance of thickness and hardness, as well as increased nutritional value due to composite ingredients.

## 4.7 CONCLUSION

Based on the research findings regarding the microbial analysis of various flours, including white wheat flour, mealworm flour, mopane worm flour, and marula seed cake flour, several recommendations for ideal outcomes from the different bread treatments at varying inclusion levels (2.5%, 5%, 10%, 15%, and 20%) can be made. The control sample exhibited the lowest Total Viable Count (TVC) and Total Fungal Count (TFC), both below the acceptable limits, indicating a high level of microbial safety. As such, loaves of bread made primarily with white wheat flour should be prioritized for products requiring strict safety standards.

At lower inclusion levels (2.5% and 5%), mopane worm bread showed acceptable microbial counts, making it suitable for incorporation without significantly impacting safety. However, as the percentage increased, particularly at 15% and 20%, the TVC and TFC rose sharply, indicating a potential spoilage risk, therefore, limiting mopane worm bread to 5% or less, where the mopane worm composite bread exhibited the highest microbial counts, particularly at 10% and above. To ensure safety, it is advisable to use mopane worm flour at a maximum of 5% in bread formulations. Marula seed cake composite bread with relatively low microbial counts can be included at higher levels (up to 20%) without compromising safety. Breads formulated with mealworm flour and mopane worm flour at a 10% inclusion level, enhanced the protein content, with mealworm (16.55) and mopane worm flour contributing (17.26) protein. Therefore, using these flours at 10% can effectively fortify bread with higher protein. marula seed cake composite bread had the highest dietary fiber content (15.1%). Incorporating marula seed cake composite bread at levels of 10% to 20% can enhance the fiber content of bread. The mealworm composite bread stood out with larger cell diameters and higher areas of cells, contributing to a lighter texture, but both the mopane worm and marula seed cake composite breads exhibited denser structures with smaller cell sizes and areas, which may influence their baking. The consistent wall thickness across samples indicates that all types maintain structural integrity during baking. The inclusion of insect flours and marula seed cake resulted in darker and more colourful bread, which may appeal to consumers looking for unique products, the brightness (L* value) decreased with higher inclusion levels of mopane worm flour.

Bread production utilizing alternative flours with a 15% inclusion level enhanced protein and fiber content. Mopane worm flour should be treated again before the drying and milling processes, to bring the microbiological load down during further studies.

The total cost of the bread ingredients (excluding overheads) for the marula seed cake bread was R9.15 per loaf, which was lower than the mopane bread at R11.44 per loaf but higher than the standard bread at R7.68. It was notably less than the mealworm bread at R30.18 per loaf.

Thus, at the end of the study, it can be stated that incorporating insects or marula seed cake into a staple item like bread could be a viable option to improve the nutritional status of the South African population.

# CHAPTER 5

# GENERAL CONCLUSIONS AND RECOMMENDATIONS

## 5.1 ACHIEVEMENT OF OBJECTIVES

The present study aimed to characterize the nutritional properties of composite flours, obtained from mealworm, mopane worm and marula seed cake originating in Southern Africa. Important functional properties studied included the safety, stability and baking qualities of different flours. In addition, a major aim of the study was to develop a composite bread with acceptable quality characteristics compared to wheat flour on the rheological properties, baking performance and overall quality of the composite flours were investigated. The objectives set out in Section 1.5 were achieved using standard methods. Where necessary methods were modified to accommodate the specific characteristics of the composite flours.

The general properties of the composite flours indicate that it is composed primarily of proteins, higher than that of wheat. The high protein makes the composite flour suitable as ingredients in food products where increased levels of proteins are required. Compared to wheat, composite flours were darker, redder and more yellow, due to the presence of polyphenols in the seed, as well as the natural pigmentation of the insects. The colour of the flour negatively affected the colour of the final baked product.

The principle ratio of 15%, analysed from the different composite flours showed that the 15% was distinguishable from the other ratios, but closer compared to wheat flour. The composite was distinguishable from one another based on nutritional value, with protein and fibre standing out compared to white wheat flour.

During the development of composite bread containing mealworm, mopane worm and marula seed cake flour, respectively, the formulation required the addition of different ratios (2.5%, 5%, 10% 15% and 20%) compared to the wheat formulation. The addition of the different ratios in the formulations changed the rheological properties of the breads. This in turn, after baking, resulted in bread with improved volume and better crumbs with a more uniform gas distribution at the 2.5% to 15% ratios, higher than the15% ratio showing a decreased volume and crumbs with weak gas distribution compared to wheat bread.

## 5.2 CONTRIBUTION OF THE STUDY

This is the first study that investigated the characteristics of composite flour from mealworm, mopane worm and marula seed cake on the flour/bread in South Africa. These composite flours were subjected to identical environmental conditions, thus reducing variabilities based on environmental factors.

This study provides new knowledge of how composite flours from different ratios can be different in terms of the flours' general characteristics, such as functional and rheological properties. Thus, it would be important to select the composite flour based on the application of the flour. The results of this study have been presented to the scientific community at the following proceedings: SAAFoST Conference, Cape Town, 2023. Tshwane University of Technology, Science Week, 2021.

1-Van Rooi, C.F., Meiring, B., Regnier, T., Cappelli, A., The effect of marula seed cake, mopane worm and mealworm on dough rheology and bread quality. Oral presentation at the SAAFoST 25$^{th}$ Biennial International Congress and Exhibition, 28-30 August 2023, Cape Town, South Africa.

2-Van Rooi, C.F., Meiring, B., Regnier, T., Cappelli A. Potential of marula seed cake for bread production, Oral presentation, Tshwane University of Technology, Science week 2021, Tshwane, South Africa.

## 5.3 SHORTCOMINGS OF THE STUDY

Given the fact that all objectives were achieved, minor shortcomings were identified:

- Since the TUT ethics committee had been dissolved at the time the sensory had to be canceled before the study concluded.
- The 15% and 20% ratios need to be modified to improve the volume and gas distribution of the bread. As a result, this should be investigated further.
- The 15% ratio was ideal for further development of the composite bread.

Due to the differences in the protein, functional, and thermal properties of the flours, the effect of ratio differences on the quality of composite bread should be investigated further.

## 5.4 RECOMMENDATIONS AND FUTURE WORK

Based on the findings of this study, the following recommendations for further research can be made: Determining the nutritional value, microbial identification, mycotoxins, and structural characteristics of ratios from different composite flours.

- Investigating the effect of improved ratios on the quality characteristics of composite bread.
- Investigating additional applications for composite flour in the food industry.

# CHAPTER 6

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# APPENDICES

APPENDIX 1 FORMAT INGREDIENT LIST

**Quality**

**MANAGEMENT SYSTEM**

**Doc No.:** PRP-PISB%BP-02

**Page** 1 of 3

**EFFECTIVE DATE:**

**REV: DEPARTMENT: NPD ISO Clause:** 7.3.3

25.01.22 1.4 **RESPONSIBILITY: NPD Technologist Approved By:** National Product Development Manager

**Product Information – SB 5% BREAD PREMIX**

*All information and recommendations are based on tests and research believed to be reliable. No guarantee of their accuracy is made however. Since the manufacturers have no control over the conditions under which the products are transported to, stored, handled or used by purchasers, all recommendations and sales are made on the condition that the manufacturers and sellers will not be held liable for any damages resulting from their use. No representative of the manufacturers has any authority to waive or change the above provisions, but our technical staffs is available to assist purchasers in adapting the fore mentioned products to their needs and circumstances. Nothing contained herein shall imply a recommendation to infringe any patents now or hereafter in existence.*

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SOUTH BAKELS (PTY) LTD

235 Main Rd, Martindale, Johannesburg, Gauteng

Telephone 0027 11 673 2100 Fax 0027 11 477 9073

sbakels@sbakels.co.za A member of the International BAKELS Group

**Product Name: SB 5% Bread Premix**

**Product Code:** 17200

**Description:** A richly tan-coloured, soft paste that has a homogeneous dispersion of bread improver particles.

**Intended Use/Abuse: SB 5% BREAD PREMIX** is an all-in-one bread premix with a superior blend of quality bread improvers making it suitable for the production of excellent white and brown bread.

**Not suitable for consumers suffering with coeliac disease, Soya and other gluten and Soya related allergies.**

Damaged packaging can result in product contamination. Prevent exposure to air or strong vapour to maintain organoleptic and sensory properties of the product.

**Ingredients:** Sodium Chloride, Sucrose, Vegetable Fats and Oils [Palm Oil (Palm Fruit, Palm Seed); Anti – Oxidant: BHA (E320)], Soya Flour, Modified Starch, Wheat Flour, Anti-Caking Agent (E170), Vegetable Oil [ Canola/Sunflower(BHT) Seed], Preservative(E282), Vegetable Fat and Oil [Canola/ Sunflower(BHT) Seed, Emulsifiers (E481,E471), Palm Oil (Palm Fruit, Palm Seed); Anti-Oxidant: BHA(320)], Oxidizing Agent(E300) and Enzymes.

APPENDIX 2 CALCULATIONS OF COST (BASED ON INGREDIENTS OVERHEADS NOT INCLUDED)

**Bread formulation: Standard bread**

| Ingredient | Batch (g) | Per loaf (g) | Cost per unit | Cost (R) per kg or liter | Cost per loaf | Notes |
|---|---|---|---|---|---|---|
| White wheat flour | 2000 | 461,08 | R184.95/12.5 kg | R14,80 | R6,82 | Makro Snowflake white bread flour |
| Format | 20 | 4,61 | R496.1/10kg | R49,61 | R0,23 | Aries Format |
| Salt | 40 | 9,22 | R18.95/kg | R18,95 | R0,17 | Makro Cerebos salt |
| Sugar | 20 | 4,61 | R204.95/10 kg | R20,50 | R0,09 | Makro Illovo white sugar |
| Yeast | 40 | 9,22 | R215.00/10 kg | R21,50 | R0,20 | Anchor anchor yeast |
| Oil | 20 | 4,61 | R179.95/5 liter | R35,99 | R0,17 | Makro Sunfoil sunflower oil |
| Water | 1200 | 276,65 | | | R0,00 | |
| **Total** | **3340** | **770,00** | | | **R7,68** | |

**Bread formulation: Mopane 15% bread**

| Ingredient | Batch (g) | Per loaf (g) | Cost per unit | Cost (R) per kg or liter | Cost per loaf | Notes |
|---|---|---|---|---|---|---|
| White wheat flour | 1700 | 391,92 | R184.95/12.5 kg | R14,80 | R5,80 | Makro Snowflake white bread flour |
| Mopane flour | 300 | 69,16 | R691.20/10 kg | R69,12 | R4,78 | Marabastad street market |
| Format | 20 | 4,61 | R496.1/10kg | R49,61 | R0,23 | Aries Format |
| Salt | 40 | 9,22 | R18.95/kg | R18,95 | R0,17 | Makro Cerebos salt |

| | | | | | | |
|---|---|---|---|---|---|---|
| Sugar | 20 | 4,61 | R204.95/10 kg | R20,50 | R0,09 | Makro Illovo white sugar |
| Yeast | 40 | 9,22 | R215.00/10 kg | R21,50 | R0,20 | Anchor anchor yeast |
| Oil | 20 | 4,61 | R179.95/5 liter | R35,99 | R0,17 | Makro Sunfoil sunflower oil |
| Water | 1200 | 276,65 | | | R0,00 | |
| **Total** | **3340** | **770,00** | | | **R11,44** | |

**Bread formulation: Mealworm 15% bread**

| **Ingredient** | **Batch (g)** | **Per loaf (g)** | **Cost per unit** | **Cost (R) per kg or liter** | **Cost per loaf** | **Notes** |
|---|---|---|---|---|---|---|
| White wheat flour | 1700 | 391,92 | R184.95/12.5 kg | R14,80 | R5,80 | Makro Snowflake white bread flour |
| Mealworm flour | 300 | 69,16 | R3400/10 kg | R340,00 | R23,51 | Marabastad street market |
| Format | 20 | 4,61 | R496.1/10kg | R49,61 | R0,23 | Aries Format |
| Salt | 40 | 9,22 | R18.95/kg | R18,95 | R0,17 | Makro Cerebos salt |
| Sugar | 20 | 4,61 | R204.95/10 kg | R20,50 | R0,09 | Makro Illovo white sugar |
| Yeast | 40 | 9,22 | R215.00/10 kg | R21,50 | R0,20 | Anchor anchor yeast |
| Oil | 20 | 4,61 | R179.95/5 liter | R35,99 | R0,17 | Makro Sunfoil sunflower oil |
| Water | 1200 | 276,65 | | | R0,00 | |
| **Total** | **3340** | **770,00** | | | **R30,18** | |

**Bread formulation: Marula seed cake 15% bread**

| Ingredient | Batch (g) | Per loaf (g) | Cost per unit | Cost (R) per kg or liter | Cost per loaf | Notes |
|---|---|---|---|---|---|---|
| White wheat flour | 1700 | 391,92 | R184.95/12.5 kg | R14,80 | R5,80 | Makro Snowflake white bread flour |
| Marula seedacke flour | 300 | 69,16 | R360/10 kg | R36,00 | R2,49 | Marabastad street market |
| Format | 20 | 4,61 | R496.1/10kg | R49,61 | R0,23 | Aries Format |
| Salt | 40 | 9,22 | R18.95/kg | R18,95 | R0,17 | Makro Cerebos salt |
| Sugar | 20 | 4,61 | R204.95/10 kg | R20,50 | R0,09 | Makro Illovo white sugar |
| Yeast | 40 | 9,22 | R215.00/10 kg | R21,50 | R0,20 | Anchor anchor yeast |
| Oil | 20 | 4,61 | R179.95/5 liter | R35,99 | R0,17 | Makro Sunfoil sunflower oil |
| Water | 1200 | 276,65 | | | R0,00 | |
| **Total** | **3340** | **770,00** | | | **R9,15** | |