

**DEVELOPMENT OF A READY-TO-EAT SORGHUM SNACK SUPPLEMENTED WITH  
SESAME AND BAOBAB FRUIT FOR NUTRITIONAL AND SENSORY QUALITY**

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TECHNOLOGY**

**DEPARTMENT OF FOOD SCIENCE, NUTRITION AND TECHNOLOGY  
FACULTY OF AGRICULTURE  
UNIVERSITY OF NAIROBI**

**2022**

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I, **Kimani David Edgar**, declare that this thesis is my original work and to the best of my knowledge, has not been previously submitted for an award in any other university or institution.

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Date: 22<sup>nd</sup> February 2022

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## **DEDICATION**

This work is dedicated to my late grandfather Joe Ephraim Kimani and my dear mother Catherine Wangari Kimani to whom her love and sacrifice remains my eternal strength.

*Ex nihil nihilo fit* (Nothing comes from nothing)

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Forever love (*amor para siempre*)

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## **LIST OF ACRONYMS**

<b>ANOVA</b>	Analysis of Variance
<b>ASAL</b>	Arid and semi-arid lands
<b>AOA</b>	Antioxidant Activity
<b>AOAC</b>	Association of Official Analytical Collaboration International
<b>CFU</b>	Colony forming units
<b>DM</b>	Dry matter
<b>FFA</b>	Free Fatty Acid
<b>FSF1</b>	Fermented Sorghum Flour Formulation 1
<b>FSF2</b>	Fermented Sorghum Flour Formulation 2
<b>FSF3</b>	Fermented Sorghum Flour Formulation 3
<b>FSF4</b>	Fermented Sorghum Flour Formulation 4
<b>GDP</b>	Gross Domestic Product
<b>LAB</b>	Lactic acid bacteria
<b>MSF1</b>	Malted Sorghum Flour Formulation 1
<b>MSF2</b>	Malted Sorghum Flour Formulation 2
<b>MSF3</b>	Malted Sorghum Flour Formulation 3
<b>MSF4</b>	Malted Sorghum Flour Formulation 4
<b>MT</b>	Metric tons
<b>NCDs</b>	Non-communicable diseases
<b>ND</b>	Not Detected
<b>RDA</b>	Recommended Dietary Allowance
<b>RDI</b>	Recommended Dietary Intake

<b>RSF1</b>	Roasted Sorghum Flour Formulation 1
<b>RSF2</b>	Roasted Sorghum Flour Formulation 2
<b>RSF3</b>	Roasted Sorghum Flour Formulation 3
<b>RSF4</b>	Roasted Sorghum Flour Formulation 4
<b>RTE</b>	Ready-to-eat
<b>PUFA</b>	Polyunsaturated fatty acids
<b>PV</b>	Peroxide Value
<b>SSA</b>	Sub-Saharan Africa
<b>TFC</b>	Total Flavonoid Content
<b>TPC</b>	Total Phenolic Content
<b>TVC</b>	Total Viable Count
<b>WHO</b>	World Health Organizations

# TURNITIN ORIGINALITY REPORT



APPROVED BY DR CATHERINE KUNYANGA

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## GENERAL ABSTRACT

Orphan crops of sorghum, sesame and baobab fruit remain underutilized despite their nutritional and commercial advantages to modern high value food products that can address food and nutrition security in majority of sub-Saharan Africa. This study aimed at evaluating the nutritional, antinutrients and shelf-life stability of a ready to eat snack bar as influenced by the processing method and storage conditions. The study employed an experimental study design of a 3 × 4 factorial arrangement with three factors of malting, fermenting and roasting with four supplementation blends of 60:25:15; 70:20:10; 80:15:5; and 100:0:0 of sorghum, sesame and baobab fruit pulp respectively. Raw unprocessed samples of sorghum, sesame and baobab fruit pulp were acted as controls.

The results showed that the nutritional composition of the snack bars made by supplementing with sesame and baobab fruit improved significantly ( $p < 0.05$ ). The protein and fat content improved significantly at 25% sesame and 15% baobab fruit pulp supplementation with roasted formulation RSF1 recording the highest at 16.74% for protein, and fermented sorghum formulation FSF1 at 19.73% for fat content respectively. Fiber content ranged between 5.59g/100g and 9.46 g/100g with formulations FSF4, RSF1, and MSF1 exhibiting high contents at 10.46%, 8.18% and 7.9% respectively. The mineral contents were significantly different ( $p < 0.05$ ) to the control samples with Iron levels ranging between 5.46 mg/100g and 14.611 mg/100g, with roasted formulation RSF1 having high content at 14.61 mg/100g as compared to MSF1 at 11.44 mg/100g, and FSF1 at 11.45 mg/100g respectively at 25% sesame and 15% baobab fruit pulp supplementation. Calcium levels in the snack formulations ranged between 82 mg/100g and 246 mg/100g, with malted formulation MSF1 at 25% sesame and 15% baobab fruit pulp supplementation having high content at 246.7 mg/100g, followed by RSF1 and FSF1 at 227.2 mg/100g and 171.5 mg/100g respectively. Zinc concentrations were significant for roasted and malted formulations at 25% sesame and 15% baobab fruit pulp supplementation at 4.82 mg/100g and 4.98 mg/100g respectively.

The carbohydrate content in roasted sorghum snacks ranging between 48.20-59.85%, malted sorghum snacks between 48.54-59.71%, and fermented sorghum snacks between 46.37-60.31%. The calculated energy content ranged between 397-426.9kcal/100g in roasted sorghum formulations, 387.1-428.8 kcal/100g for malted sorghum formulations and between 377.3-425.1

kcal/100g for fermented sorghum formulations respectively. The sensory evaluation of the snacks was done by use of a 5-point hedonic scale and revealed significance differences ( $p < 0.05$ ) in color, taste and overall acceptability with mean scores above 3.5. The aroma and crunchiness of the snacks were found to not be significant ( $p > 0.05$ ) with mean scores of 3 indicating neither like or dislike. Snack bars with no added baobab were found to be generally acceptable with RSF4 ( $3.853 \pm 0.99$ ), MSF4 ( $3.529 \pm 0.99$ ) and FSF4 ( $3.676 \pm 1.34$ ) being the most preferred.

The effect of processing method on antinutrients in the snack bars differed significantly ( $p < 0.05$ ). Roasting averagely reduced tannic content by 82.71%, phytates by 53.26%. Malting process decreased on average the tannic content by 78.66%, phytates by 48.89%, while fermentation was 78.71% on tannins and 51.54% phytates respectively. The phenolic content retention in the snack bars was significantly different ( $p < 0.05$ ) with average retentions at 59.58% for roasted formulations, 59.6% for malted formulations and 58.31% for fermented sorghum formulations respectively.

The microbial and physicochemical properties of the snack bars were within acceptable limit up to day 3 of accelerated shelf-life storage at  $55 \pm 2^\circ\text{C}$ . Snack bars stored in Flexible package exhibited better keeping quality than Kraft and Poly/PE-coated packages. The mean count of TVC and yeast and molds for samples stored in kraft and poly/PE-coated packages were highest at the second day of storage as compared to the flexible package which recorded high mean counts at day 3 of storage ( $p < 0.05$ ). The *S. aureus* mean counts were found to be of acceptable limits of  $10^2 \log \text{cfu g}^{-1}$ . The pathogenic microorganisms were not detected in the formulations during the duration of storage.

The oxidative stability of the snack bar formulations was significant ( $p < 0.05$ ) among the packaging materials with Kraft package exhibiting faster detection after day 2 of accelerated storage period at 5.084 meq  $\text{O}_2/\text{kg}$ . Autooxidation was detected in poly/PE-coated and Flexible packages at day 3 of accelerated storage at 4.942 meq  $\text{O}_2/\text{kg}$  and 2.031 meq  $\text{O}_2/\text{kg}$  respectively. The FFA content among the snack formulations was not significantly different ( $p > 0.05$ ) during the accelerated shelf-life period in the three packaging materials. Oxidative stability of the snack formulations was best after three months of storage in Flexible packaging material as compared to Kraft and poly/PE-coated materials.

The study concludes that sorghum, sesame and baobab are viable crop alternatives for food and nutrition security and innovative opportunities in food product development. This can support the

economic wellbeing to sorghum farmers, micro-processors along the sorghum value chain. However, to achieve full utilization of orphan crops, more research should be extended on accessible processing technologies to achieve these objectives.

## **CHAPTER ONE: GENERAL INTRODUCTION**

### **1.1 BACKGROUND INFORMATION**

Cereal grains are a principal component of human diets, with archeological evidence pointing their long historical role in shaping human civilizations. This is particularly so as various studies have estimated that cereals provide half of the world's daily caloric intake daily as a result of their direct consumption (Sarwar *et al.*, 2013). Among the Polish diets, Laskowski *et al.* (2019) found that cereals and their products contribute 30.4% of total dietary energy needs. Therefore, cereals in food and nutrition security cannot be underestimated.

Sorghum (*Sorghum bicolor* L. Moench) is a major traditional course cereal in the developing world where it is an important subsistence crop. Sorghum is noted for its drought and pest resistance properties; hence the cereal can adapt in harsh ecological conditions. In sub-Saharan Africa, Iqbal and Iqbal. (2015) notes that sorghum now accounts for 43% of the cultivated cereals. In Kenya, sorghum is primarily grown in Western and the Lower Eastern ASAL parts of the country. Recent data puts amount of sorghum harvested in SSA at 29 million MT vis-à-vis Kenya at 206 thousand MT (FAO, 2018). It thus translate that sorghum is an important source of macro and micronutrients for the marginalized populations (Adetayo *et al.*, 2013).

Sorghum, in comparison to other cereals fares well in its range of macro- and micro-nutrients. Current information and consumer trends have bent the curve towards bioactive components in plant-based foods which have been epidemiologically linked with reducing risk to lifestyle-based diseases. Sorghum is a major source of beneficial phytochemicals with added therapeutic effects against NCDs such as cardiovascular diseases, cancers, diabetes, and obesity. The burden of NCDs on mortality rates in 2016 was 41 million of the 57 million deaths globally, a 71% representation (WHO, 2018). Studies have profiled beneficial bioactive compounds in sorghum inclusive of but not limited to phenolic acids, tannins, flavonoids, anthocyanins, 3-deoxyanthocyanidins (Awika and Rooney, 2004; Dykes and Rooney, 2006; Sidhu *et al.*, 2007; de Morais Cardoso *et al.*, 2017). In addition, the cereal is heralded for its resistant starch and fiber component which is key in regulating glycaemic responses (Taylor *et al.*, 2015).

Sorghum uses are diverse as food, beverage, feed products, and such uses vary regionally. In addition, sorghum is gluten free and hence suitable to coeliacs. Range of sorghum based products

include cakes, bread, cookies, pasta, snack foods in addition to bioethanol production (Taylor *et al.*, 2006; Aruna and Visarada, 2018). In Kenya, sorghum is mostly milled into flour for preparation of *uji*, a thin gruel, *ugali*, a thick gruel, composite flour (Kilambya., 2013; Njagi *et al.*, 2019). However, despite sorghum's nutritional quality and drought resistant traits, the cereal remains largely underutilized in the face of mounting food and nutrition security challenges globally (Iqbal and Iqbal., 2015).

Sesame (*Sesamum indicum*), so-called "Queen of oil seeds" is an important oil crop ranked fifth after soybean, groundnut, sunflower and mustard. Sesame seeds are important sources of protein, dietary fiber, micronutrients, lignans, tocopherols and phytosterols (Elleuch *et al.*, 2011). Major sesame growing regions are China, India, Nigeria, Sudan and Tanzania. In Kenya, Coastal counties of Kwale, Kilifi, Lamu, and Western counties of Busia, Bungoma and Kakamega comprise sesame growing zones (Koitilio *et al.*, 2018).

Sesame has been used in culinary preparations of *tahini*, decoration of breads and cookies, edible oil that is used in frying purposes (Amoo *et al.*, 2017; Hegde, 2012). Sesame oil has been heralded for its oxidative stability (Anilakumar *et al.*, 2010), enhanced flavors when roasted and as an ingredient in processing of margarine and soaps (Amoo *et al.*, 2017). In Kenya, sesame seeds products are limited to snack balls and as toppings in baked goods. Hence, its use is narrow in Kenya.

Baobab (*Adansonia digitata* L., Malvaceae) is a tree associated with semi-arid regions of Africa due to its economic and nutritional importance it provides (Aluko *et al.*, 2016). The tree withstands long periods of drought and high temperatures in SSA ASAL zones and they are grown mostly for their fruit and leaves. In West Africa, baobab leaves are dried into powder for preparation of sauces, whilst in some segments of Zimbabwe, the baobab leaves serves as vegetable substitutes (Muthai *et al.*, 2017; Zahrau *et al.*, 2014). The fruit pulp once ground into powder, is used in processing of juices, sweets, snacks and in alcoholic beverage fermentation (Kaboré *et al.*, 2011). In Kenya, baobab seeds, are harnessed into *mabuyu*, a sweetened essence that is coated on the baobab seeds and sold widely in Kenyan streets. The lower Eastern and Coastal areas of Kenya are baobab growing zones but the economic potential of this tree is yet to be harnessed fully. Thus, baobab tree remains largely undomesticated and underutilized.

Thus, this study aimed at developing a sorghum-based snack bar with acceptable nutritional and sensory acceptable and illustrate the value addition and processing techniques of locally available materials into an economic viable product.

## **1.2 STATEMENT OF THE RESEARCH PROBLEM**

Course cereals have been christened as poor man's crop and thus have remain neglected in the sphere of commercialized food systems, research and development. Sorghum is an indigenous course cereal, drought resistant and capable of adapting to harsh climatic and soil conditions whose cultivation and utilization has been on the decline as compared to exotic cereals of maize, wheat and rice. Consequently, its value chain remains weak, unstructured and mired in governance and policy weak links. However, efforts have been directed to improve the uptake of sorghum such as in commercial beer processing. In SSA, sorghum is used in processing traditional foods such as semi-leavened breads, fermented and non-fermented foods, cakes and thin and thick gruels. Unfortunately, the barrier remains in bringing forth sorghum into mainstream food baskets of the populace. In particular, has been a narrow range of products that are necessary to bridge the cultural divide that places sorghum as a "poor man's crop". Prevalent low knowledge on farming, utilization and processing of sorghum have been blamed for slowing the expansion of sorghum into the mainstream cuisines and products.

Nutritional balance and fulfillment remain an essential plank. More so, the WHO and research papers have put forth the theory of nutrition shift marked by proliferation of highly processed foods with little nutritional value. Conversely, sorghum is heralded as a nutritionally rich cereal with its range of phytochemicals that have been epidemiologically linked with reducing the risk to some illnesses. Unfortunately, knowledge on the nutritional superiority of sorghum is low and as a consequence, a segment of the populace is unaware of such information. This is afflicted by available sorghum-based foods which are gruels that are unpopular with vast majority of the youth. The present study aims at developing a RTE sorghum snack incorporated with a blend of sesame and baobab fruit powder with enhanced nutritional and sensory quality as well as other bioactive compounds with health benefits.

### 1.3 JUSTIFICATION OF THE STUDY

Course cereals, such as sorghum remain neglected in mainstream food baskets, policy making and investments in research and development. This is against a backdrop of adverse effects of climate change, increased population and thus the negative consequences to food and nutrition security. Taylor *et al.*, (2006) asserts sorghum to be drought resistant, requiring little inputs for growth. Sorghum is vital for people in SSA and Asia, but the cereal remains underutilized as most of harvested grain is used as animal feed (Taylor *et al.*, 2006; Shimelis *et al.*, 2016). Njagi *et al.*, (2019) observes the sorghum value chain is disjointed and characterized with inconsistent quality products, poor market linkages, and lack of competitive edge among consumers. In the era of sustained campaigns on healthy diets, sorghum is yet to bridge the ‘poor man’s crop’ tag thereby depressing production and productivity. Kilambya (2013) breaks down the fate of sorghum harvested in Kenya where 53% is milled into flour, 24% is processed as beer, 10% is processed as animal feed and 11% of the cereal goes to waste. However, sorghum potentiality as human food and beverage source needs to be fully exploited. An initiative by East Africa Brewery Limited (EABL) to contract sorghum for use in malt processing of beer in Western Kenya has seen an improved productivity with farmers enjoying better bottom lines. Novel and traditional foods such as gluten free breads, cakes and cookies, tortillas, snack foods, and malt drinks are proof of sorghum potentiality (Taylor *et al.*, 2006; Ratnavathi, 2014; Alavi *et al.*, 2018; Alavi *et al.*, 2019).

A sorghum-based snack bar is a convenient, shelf stable and nutritionally rich in comparison to range of products that are highly processed. Fortification with sesame and baobab fruit powder in processing of a sorghum-based snack aims at nutritionally balance with improved macro- and micronutrient according to WHO guidelines. Sesame is an excellent source of protein, essential oil, lignans, and tocopherols (Hegde, 2012) while baobab is laden with vitamin C, zinc, calcium and potassium (Aluko *et al.*, 2016; Nouruddeen *et al.*, 2016). Various studies have outlined the composition and range of sorghum phytochemicals and their related health benefits (Awika and Rooney, 2004; Dykes and Rooney, 2006; Girard and Awika, 2018; Serna-saldivar *et al.*, 2019). This is in addition to sorghum being a gluten-free cereal thus coeliacs can enjoy their resultant products. A sorghum-based lunch bar snack will aim to bring forth sorghum as a mainstream snack thus improving its standing among popular cereals of wheat, maize and rice thus diversifying and adding to range of products.

## **1.4 STUDY OBJECTIVES**

### **1.4.1 OVERALL OBJECTIVE**

To develop a sorghum-based snack supplemented with sesame and baobab fruit powder for nutritional and sensory quality.

### **1.4.2 SPECIFIC OBJECTIVES**

1. To formulate a sorghum-based snack supplemented with sesame and baobab fruit powder
2. To determine the nutritional and biochemical composition of the raw food ingredients and the sorghum-based snack.
3. To evaluate the anti-nutrient content in the food ingredients and the developed sorghum-based snack
4. To determine the functional properties of the sorghum-based snack.
5. To evaluate the acceptability of the developed ready-to-eat sorghum-based snack

## **1.5 HYPOTHESES**

1. An acceptable, and shelf stable snack bars can be developed from sorghum, sesame and baobab fruit pulp powder.
2. The biochemical and sensory acceptability of the developed product are of acceptable standards.
3. The processing modes have a significant effect on the anti-nutrient and phytochemicals presence in the developed product.
4. The developed product is shelf stable under aerobic and vacuum packaging conditions.



## CHAPTER TWO: LITERATURE REVIEW

### 2.1 OVERVIEW OF CEREAL VALUE CHAIN

Food and nutrition security is the biggest challenge of our times, owing to the demand for food is increasing with the growing population. Majority of human source of food has been contributed cereals, legumes, roots and tubers for food and nutrition security (Daryanto *et al.*, 2017). Major cereals of maize, wheat and rice, and minor cereals of barley, sorghum oats, rye and millet provide 56% of energy and 50% of protein consumed by majority of people globally (Daryanto *et al.*, 2017). According to FAOSTAT, (2019) data, world cereal production peaked at 2.979 billion tons in 2019, up from 2.91 billion tons in 2018 underlying their dominant place of cereals in the global food supply chain. In Europe, cereals production contribute to 20% of the global output with 61% used as animal feed and only 24% for human consumption (Schils *et al.*, 2018). In the United States, cereals in particular corn is used as corn starch and corn-syrup sweeteners and food, and lately in production of industrial ethanol (Awika, 2011). In Africa, FAOSTAT, (2019) data shows cereal production to be 2.04 billion tons of cereal production in 2019, compared to 2.077 billion tons in 2018.

Maize and rice are predominant in warm climates of sub-Saharan Africa and India as they are susceptible to drought conditions (Awika, 2011). Wheat and Barley does well in the temperate regions of Europe and North America, while sorghum and millet are best suited for drought-stress areas (Yu *et al.*, 2019).

Cereals have remained ubiquitous in the global food security as they are not only staple crops with a rich source of proteins, carbohydrates, vitamins, minerals, fats and oils, but they constitute the crops that are cultivated in greater quantities per acreage (Yu *et al.*, 2019). In developing countries, the contribution of calories from cereals account up to 60%, and can be as high as 80% in the poorest countries (Awika, 2011). In majority of subs-Saharan Africa, agriculture contributes up to 55% of the continent's GDP, with 85% of the population depending on the sector for food security and a source of livelihood (Kogo *et al.*, 2021). The measure of a country's food security in African countries is by cereal yield output in a given season. In Kenya, an average of 35-40 million bags of maize are usually harvested, with maize demand as high as 90% of the households in rural and

urban areas depending on it as a staple food and thus a major source of carbohydrates (Nyoro, 2002; Otieno, 2020).

The productivity of cereals in the recent years has come under pressure from effects of climate change such as drought, aflatoxin contamination and over reliance of once cereal over indigenous drought resistant cereals. Global warming has brought adverse weather changes with incidences of prolonged droughts, water stress, and erratic rainfall affecting yields (O'Leary *et al.*, 2018). In 2017, Kenya experienced an extended period of drought which resulted in maize yield failures and precipitating the government to import maize and halt the impending food shortage (Otieno, 2020).

The challenge of postharvest losses of cereals through mycotoxins continues to affect the food and nutrition security in majority of sub-Saharan countries. Aflatoxins are a potent toxins when consumed in cereals, as exemplified by aflatoxicosis in Kenya in 1981 and 2004 where acute toxicity lead to deaths (Nabwire *et al.*, 2020). Issues of poor handling and sorting, improper drying of harvested cereals contributes to most losses (Nabwire *et al.*, 2020). In Kenya, losses of maize at the National Cereals and Produce Board (NCPB) due to aflatoxin poisoning has led to losses of needed cereals which ultimately forces the country to import the deficit to avoid a food security crisis.

Cereal's utilization still permeates our diets due to their nutritional content, despite the growing challenges to their production and utilization. Wheat has continued its wide usage in the baking industry in production of bread, cakes, biscuits among others (Alavi *et al.*, 2018). Diversification of cereal uses has seen sorghum being used in beer processing (Taylor and Duodu, 2019), flakes, cakes, bread (Ratnavathi and Chavan, 2016), maize in production of industrial ethanol (Awika, 2011), rice flours (Wu *et al.*, 2019) which have been used in production of noodles, tortillas and corn chips. Therefore, cereals have continued to play a role in utilization through new product innovations and modifications to fulfill current dietary and nutrition shifts.

## **2.2 NUTRITIONAL QUALITY OF SORGHUM**

### **2.2.1 Proximate composition of sorghum**

The proximate composition of sorghum has some distinct nutritional profile in comparison to other cereals. The pericarp of the sorghum is fiber rich, while protein, fat and minerals are localized in the germ, whilst the endosperm is predominantly starch and protein (Serna-saldivar *et al.*, 2019).

The protein are localized in the endosperm, germ and pericarp at a range of 6-18% and are largely composed of fractions of water soluble albumins, salt-soluble globulins, alcohol soluble prolamins, and acid-alkali soluble glutelins (Awika, 2014). Albumins and globulins are localized in the germ, whilst the endosperm are endowed with glutelins and prolamins (Bean *et al.*, 2019).

The crude fat content in sorghum ranges at 3% which compares favorably to rice and wheat (Kulamarva *et al.*, 2009). The sorghum's germ and the aleurone layers are major sources of lipid content (Kulamarva *et al.*, 2009). The predominant crude fat content include oleic acid (31.1-48.9%), linoleic acid (1.7-3.9%) which constitutes 80% of the lipid content (Awika, 2014), stearic acid (1.1-2.6%), palmitic acid (11.7-20.2%) and palmitoleic acid (0.4-0.6%) (Stefoska-Needham *et al.*, 2015). In addition, the dietary fiber of sorghum is localized at the pericarp and endosperm cell walls and decortication reduces the content of the fiber significantly (Serna-saldivar *et al.*, 2019).

Some of the vitamins in sorghum include the B-complex vitamins of thiamine, riboflavin, Vitamin B6, biotin, and niacin. The mineral composition in sorghum compares to millet, higher in maize but lower to wheat (Stefoska-Needham *et al.*, 2015). The pericarp and the germ contain significant composition of micronutrients with phosphorous and potassium present (Awika, 2014). However, the phytic content has an effect on the bioavailability of phosphorous, though processing operations such as malting and fermentation have been effective in improving the mineral content (Serna-saldivar *et al.*, 2019). Sorghum is noted for its low calcium content in comparison to finger millet (Gull *et al.*, 2014).

### **2.2.1 Phytochemicals and antinutrients in sorghum**

In plants, phenolic compounds possess antioxidant activity with potential health benefits such as acting as a natural defense against diseases and oxidative stress (Serna-saldivar *et al.*, 2019). The phenolic acids are the dominant substances which form a major component in the cell wall structure of cereal grains (Girard and Awika, 2018). The cinnamic and benzoic acids are the derivatives of phenolic acid with the former being the abundant substance (Awika and Rooney, 2004). The phenolic compounds show elevated antioxidant activity which is evidenced by their knack to scavenge free radicals (Stefoska-Needham *et al.*, 2015).

In fruits and vegetables, phenolic compounds have a strong influence on color and flavor, and are found in free form and easily extractable, but in sorghum, they are in bound form esterified to the cell wall and not easily extractable (Awika and Rooney, 2004; Girard and Awika, 2018). The bound phenolic acid account for 70% in sorghum and are mainly liable for the extensive crosslinking traits in the cereals cell wall (Duodu and Awika, 2019). The unbound form of phenolic acids are limiting in sorghum which is not a rarity as most monomeric phenolic compounds in nature (Dicko *et al.*, 2006; Duodu and Awika, 2019).

Flavonoids are found in pigmented varieties of sorghum with the exception of white sorghum, and they are potent antioxidant properties (Duodu and Awika, 2019). The flavonoids in cereal exists in relatively low quantities as opposed to fruits and flowers where they contribute to pigmentation that is essential for pollination (Girard and Awika, 2018). A wide array of flavonoids found in sorghum are at high levels than other cereal grains (Awika, 2014).

Non-communicable disease of obesity, cardiovascular diseases, colon diseases are largely associated with oxidative stress (Awika and Rooney, 2004) and sorghum is gaining interest due to its significant antioxidant activity in vitro relative to other cereal grains (Awika, 2014). The extent of beneficial effect of phytochemicals in the human diet in vivo remain unclear due to limited clinical research studies undertaken. However, the role of sorghum has seen an uptake in in vitro investigations relating to their antioxidant and anti-inflammatory properties. The discourse is between in vivo and in vitro studies where definitive correlations are yet to be established (Serna-saldivar *et al.*, 2019). It has been noted that the capability of ingested dietary antioxidants to scavenge radicals in vivo are restricted by poor absorption and the elaborate endogenous antioxidant system (Awika, 2014).

### 2.3 NUTRITIONAL VALUE OF SESAME SEEDS

The protein and oil content in sesame seed are influenced by genetic and environmental factors. Some of the content in sesame are 17-32% protein, 48-55% oil, 14-16% sugar, 6-8% fiber and 5-7% ash content (Hegde, 2012). Sesame protein is localized on the seed's outer layers and is in the range of 17-32% and averages at 25% (Hegde, 2012). The dominant protein fraction in sesame is globulin and is composed of fractions of  $\alpha$ -globulin and  $\beta$ -globulin (Prakash and Naik, 2014). In addition, the proteins are high in the amino acids methionine and tryptophan (Hegde, 2012). Tryptophan is limiting in other oilseed crops but it is abundant in sesame. However, the availability of amino acids are affected by processing method, whilst heat treatment is known to improve their digestibility (Onsaard, 2012).

The seed oil content is significant content in comparison to other oil seed crops with various studies reporting oil content in sesame to be in the range of 40-50% which is dependent on variety and environmental conditions (Asghar and Majeed, 2013; Prakash and Naik, 2014) with oleic and linoleic acid the predominant fractions (Hegde, 2012). In addition, the oil content is resistant to the effects of oxidative rancidity. This auto-oxidation characteristic is partly due to the significant of tocopherol (Hegde, 2012; Hwang, 2005) and lignan contents Sesamin, sesamol, sesamol and sesaminol which are the main lignans in sesame seed (Bodoira *et al.*, 2017). The unsaturated fatty acid fraction are essential in lowering of blood cholesterol levels and hence reduces the risk of heart related ailments, sesame seed oil forms an essential part in human nutrition (Anilakumar *et al.*, 2010).

Sesame seeds contain 20-25% of carbohydrate content (Onsaard, 2012). The crude fiber content is present in the seed's husk with reported content ranging at 3-6%. Predominant mineral content includes calcium, phosphorous and iron. The mineral content ranges at 4-7%. Calcium predominates the husk and its content lowers when the seed is dehulled (Hegde, 2012).

The micronutrient content in sesame is significant with potassium, phosphorous, magnesium, calcium and sodium (Prakash and Naik, 2014). The potassium content is high has a role in the synthesis of protein and amino acid, whilst calcium role is essential in the development of bone (Prakash and Naik, 2014). The bulk of mineral content in sesame is present in its oil thereby making it a highly nutritious and acceptable food material.

## 2.4 CEREAL-BASED SNACKS AND CONFECTIONERIES

Cereals are an important component in our dietary systems and have a dominant presence in our food systems and they are the first source of calories and proteins to diets of humans (Behera and Srivastav, 2018). The growth of cereal based snacks has been fueled by rising consumer focus on dietary requirements such as low salt content, low cholesterol levels, low or no sugar content, low calories and high proteins and vitamins (Behera and Srivastav, 2018). Thus, there has been a proliferation of cereal related bakery and non-bakery products which are intended to be shelf stable, appealing and convenient (Mir *et al.*, 2019) and include but not limited to breads, breakfast cereals, noodles, flaked and popped products, and lunch bars. Consequently, cereal snacks products are widely accepted and consumed by varied consumer groups which is coupled by their convenience and long shelf-lives (Behera and Srivastav, 2018).

Wheat products constitute important staple foods globally. Wheat is unique among cereal flours due to its characteristics that enable it to be extensively used in a range of baked products that are sensory acceptable to consumers (Attenburrow *et al.*, 1990). Wheat contains the gluten proteins which serves as a storage protein fraction (Shewry, 2019) are heat stable and possess viscoelastic properties which have positive effect on rheological properties of expansion, shape and texture of resultant baked goods (Biesiekierski, 2017; Ortolan and Steel, 2017; Ma *et al.*, 2019).

Whilst wheat products are mainstream, a segment of the population have an autoimmune disorder due to gluten proteins (Scherf *et al.*, 2016). Advances in product development have thus fueled use of pseudo-cereals in product formulation and consumer awareness on healthy eating. Schober *et al.* (2005) produced a gluten free bread from varying sorghum hybrids and corn starch. Wanjuu *et al.* (2018) produced a composite bread with 30% orange flesh sweet potato and 70% wheat flour and investigated its physiochemical properties. The studied showed the composite bread to have improved  $\beta$ -carotene content and overall bread qualities and had a longer shelf life as compared to bread with 100% wheat. Flores-Silva *et al.* (2015) developed gluten free snacks using a blend of plantain-chickpea and maize. The study showed the developed snacks to have high fiber content, low glycemic index and had overall acceptability when compared to commercially produced chili snacks. Rai *et al.*, (2014) developed cookies from blends of rice and maize flours and sorghum and pearl millet flours. Kaur and Aggarwal. (2017) developed a maize-potato tortilla chips which had low protein and total phenolic content as compared to the control which had 100% maize. Mir *et*

*al.* (2019) developed a gluten free extruded snack based on brown rice and chestnut flour. Espinoza-Moreno *et al.* (2016) extruded a snack from transgenic maize and black common bean that had higher protein content, total dietary fiber and antioxidant values.

Thus, cereals play a huge role in human diets as shown by various products. In addition, the use of pseudo-cereals has improved the product ranges largely due to consumer awareness on healthy eating. The advantages accrued by cereal snacks of convenience, stable shelf life and consumer acceptability will further enhance innovations and processing methods.

## **2.5 EFFECT OF FERMENTATION, MALTING AND ROASTING ON SORGHUM**

### **2.5.1 Fermentation**

The processing technique of fermenting cereals is among the oldest practice that dates to ancient Egyptians who produced bread and beer by use of yeasts and lactic acid bacteria (Hammes *et al.*, 2005). Fermentation involves activation of the endogenous enzymes which are responsible in hydrolyzing stored starch content (Nkhata *et al.*, 2018). Traditionally, sorghum has been spontaneously fermented by activation of naturally occurring microbes found on the cereal kernels (Taylor and Kruger, 2019) or via the back-slopping method (Dlamini *et al.*, 2007). In fermenting cereals, the milled flour is mixed with water at ratios of 1:2-3 and left to ferment at 25°C- 37°C for a period of 24-72 h and up to 8 days (Taylor and Kruger, 2019). The overall effect of fermenting sorghum is to induce desirable biochemical changes, improve palatability and shelf life and functionality of sorghum (Debabandya *et al.*, 2017; Mohapatra *et al.*, 2019).

Thus, fermentation has a profound effect on the biochemical composition in sorghum. In studies, it has been shown that LAB fermentation leads to a decrease in the carbohydrate and fat content largely due to respiration (Taylor and Kruger, 2019) due to fermentation of extracellular enzymes that break down the stored starch that is enmeshed in a protein matrix (Debabandya *et al.*, 2017). A study by Mohapatra *et al.* (2019) on effect of processing sorghum observed a decrease in fat from 4.7% to 3.6%, and an increase in protein content. On the contrary, fermenting sorghum increases the digestibility of lysine and protein attributed to hydrolysis of stored proteins (Taylor and Kruger, 2019). The degradation of tannins and phytates by fermentation that complexes stored

proteins thereby improves digestibility whilst also reducing baking problems in sorghum dough (Debabandya *et al.*, 2017).

Dietary fiber is an important plank in glycemic index among diabetics and general belief is fermentation improves its content. Studies have shown either no effect on total dietary fiber or a reduction by 12% with observed changes due to degradation of xylanases (Taylor and Kruger, 2019). In the mineral content, fermentation cannot destroy or synthesize them thus no net change. Most minerals are complexed with anti-nutrients such as phytates which lowers their bioavailability (Taylor and Kruger, 2019). Loss of mineral content is attributed to leaching of water-soluble minerals into the fermentation media (Mohapatra *et al.*, 2019).

Sorghum is heralded for its range of bioactive properties (Awika and Rooney, 2004) which have been epidemiologically linked in reducing some risks to some diseases (Duodu and Awika, 2019). Lactic acid fermentation has been shown to reduce total phenolic acids, tannin and phytates contents in sorghum (Mohapatra *et al.*, 2019; Taylor and Kruger, 2019). These are similar observations by Mohapatra *et al.* (2019) who reported a reduction in total phenolics when sorghum flour is fermented. Dlamini *et al.*, (2007) observed a 49-68% reduction in tannins in fermented sorghum porridge. Sorghum tannins form complexes with iron and zinc thus reducing their bioaccessibility but lactic acid fermentation reduces their levels (Taylor and Kruger, 2019). Fermentation is an effective barrier to enteropathogenic bacteria due to the lactic acid bacteria (LAB) producing bacteriocins, ethanol and organic acids that lower the pH which inhibits their growth (Debabandya *et al.*, 2017).

### **2.5.2 Sprouting and Malting**

The process of limited germination of grains is a traditional technique that has been applied to improve nutritional, sensory and technological properties of various food types (Ojha *et al.*, 2018a). Broadly, malting involves cleaning the grains, steeping in water at ratios of 1:3 (w/v) for 12-24 h, then spreading the grains and keeping them damp and allowing them to sprout for 48-72 h or longer (Taylor and Kruger, 2019). After sprouting, the grains are dried, deculmed and milled into flour for use in various food products. In SSA, malting sorghum is used in production of traditional beers with most countries around the world increasingly adopting its application in processing lager beer and malt beverages (Taylor and Duodu, 2015).



Malting sorghum grain synthesizes its amylases, proteases and inherent enzymes, and their hydrolysis often modifies the structure and component of the grain. Changes attributed to malting include release and/or metabolism of macro- and micronutrients, phenolics, anti-nutrients (Taylor and Kruger, 2019). Various authors seem to vary on the effect of sprouting on protein content. Taylor and Kruger (2019) suggests that sprouting slightly increases protein content owing to respiration of carbohydrates but once deculming is done, the removal of protein rich germ ultimately lowers protein content. Omary and others (2012) proposes that decreased protein content can be down to steeping time, temperatures, frequency of rinsing and sprouting vigor. Onyango *et al.* (2013) studied effect of malting on sorghum and pearl millet and observed increased protein digestibility from 48% to 68% and 21.5% and 34.5% respectively.

The effect of malting on sorghum phenolics have yielded mixed results though most studies have found a decrease (Taylor and Kruger, 2019). Most studies have proposed leaching of phenolic compounds during steeping and germination that provide the aqueous environment that facilitates their solubilization (Taylor and Duodu, 2015). (Khoddami *et al.*, (2017) reported a 10% decrease in TPC upon malting three sorghum cultivars for 96 h. Taylor and Kruger. (2019) observes some increases in phenolic compounds due to their breakdown and their aggregation. Dicko *et al.* (2006) evaluated sprouting effect of red and white sorghum varieties on their phenolic contents after 72 h. The study showed red varieties had some increased phenolic content while the white sorghum variety decreased at the same time. Phenolic acids are soluble in water and thus, by steeping, the phenolics are solubilized as the grains take in water, which partly explains reduced concentration (Duodu, 2014a). Sprouting is marked by a decrease in tannins and phytates which are known to complex minerals, starch and proteins thus reducing their bioaccessibility (Dlamini *et al.*, 2007; Ojha *et al.*, 2018). Decrease in tannins has been attributed to leaching losses during steeping (Ogbonna *et al.*, 2012). Sprouting sorghum synthesizes the tannins that complexes starch and proteins thus improving their bioaccessibility (Khoddami *et al.*, 2017). Furthermore, sprouting is noted for its substantial 10-fold decrease in cyanogenic glycoside levels in the grains approximately to 400 ppm (Taylor and Kruger, 2019).

### **2.5.3 Thermal Processing of Sorghum**

Sorghum is subjected to various thermal processes such as wet cooking, steam cooking, extrusion, baking and roasting (Taylor and Duodu, 2015). Some thermal processing involves water as a medium for supplying heat and can also be limiting in some processes such as in baking biscuits and cookies, popping and puffing of whole grain kernels and flours (Taylor and Kruger, 2019). Heat treatment to sorghum has effects on nutritional quality of foods and functionality of sorghum.

#### **2.5.3.1 Heat Moist treatment**

Starch digestibility in cereals has gained interest; however, effect of thermal treatment varies among various studies. Taylor and Emmambux (2010) points to cross-linking of endosperm proteins as a factor affecting starch digestibility and gelatinization especially in moist heat treatment of sorghum. Sun *et al.* (2014) studied the effect of heat moisture treatment on sorghum starch and sorghum flour at moisture contents of 20% and 25%. The study found heat moisture treatment had a more pronounced effect on solubility, swelling power and crystallinity in sorghum flour as compared to sorghum starches. However, both set of samples had improved texture after heat moist treatment which is desirable in processing sorghum based products (Sun *et al.*, 2014). Saravanabavan *et al.* (2013) investigated the effect of popping three varieties of red, pop and *maldandi* sorghum varieties on their starch digestibility. The study found that popping sorghum decreases resistant starch thus improving on starch digestibility. In addition, popping sorghum has been found to cause the starch granules to shatter whose effect disrupts the starch-protein matrix which allows for gelatinization and improve digestibility of proteins (Saravanabavan *et al.*, 2013).

The effect of thermal processing on phenolic content is of great research concern due to the epidemiological benefits of sorghum phenolics. Thus, proposed theories on varying degrees of phenolic and antioxidant properties put forth include release of bound phenolics, heat degradation, oxidation and polymerization of phenolics into simple molecules, formation of Maillard products, and complexation of phenolics with other food components (Taylor and Duodu, 2015; Taylor and Kruger, 2019).

It has been generally found that wet cooking of sorghum has an overall reduction in TPC, but with minimal effect on phytate levels (Taylor and Kruger, 2019). This is due to leaching of phenolics in water and migration of phenolics in sorghum's endosperm which forms complex network with protein and starch (Taylor and Duodu, 2015).

### **2.5.3.2 Extrusion**

Sorghum is noted for complexing its stored protein which has an effect of its digestibility thus bioaccessibility and thermal processing methods have had an effect on their availability. Taylor and Kruger. (2019) notes that wet cooking reduces protein digestibility by polymerization of the disulfide bonds in the endosperm proteins. Notably, extrusion cooking improves protein digestibility by physical disruption of the endosperm structure that encapsulates sorghum proteins (Taylor and Kruger, 2019). Ezeogu *et al.* (2008) investigated the influence of cooking conditions on the protein matrix of sorghum and maize. The study findings showed that cooking sorghum tends to form more knotty structures as compared to maize protein matrix. The consequence is formation of greater disulphide bonds in sorghum than maize which leads to low digestibility in sorghum flours (Ezeogu *et al.*, 2008).

Extrusion cooking has an overall effect on reduction on phenolic and phytate levels of tannin and non-tannin sorghum types (Taylor and Kruger, 2019). Cardoso *et al.* (2015) evaluated effects of extrusion and dry heating sorghum's phenolic profile. Extrusion has a bigger impact reduction of flavanones, flavones and 3-deoxyanthocyanidins as compared to dry heat cooking method. Cardoso *et al.* (2015) points to heat sensitivity of flavanones and flavones and effects of thermal degradation for their reduced levels.

### **2.5.3.3 Roasting**

Irondi *et al.* (2019) investigated effect of roasting on phenolic and antioxidant profile of raw and roasted sorghum and observed an increase in antioxidant activity as phenolic compounds decreased with increase in roasting temperature. It is postulated that formation of Maillard products have some antioxidant activity and lowers the total phenolic content (TPC) levels (Irondi *et al.*, 2019; Taylor and Duodu, 2015). In microwave roasting of sorghum, Sharanagat *et al.* (2019) observed an increase in TPC and AOA, a reduced total flavonoid content (TFC) than in unroasted sorghum flour. It is indicated that extractable phenolic acids do not possess antioxidant activity (AOA), whilst reduction of flavonoid content also alters antioxidant levels in sorghum (Sharanagat *et al.*, 2019).

Dry heat treatment such as baking has found to reduce tannin levels, but has been shown to have improved phenolic levels in total phenolics (Taylor and Kruger, 2019). Cardoso *et al.* (2014) evaluated the effects of dry and wet cooking sorghum on phenolic contents, AOA, 3-

deoxyanthocyanidins, tocopherols and carotenoids in sorghum. The study concluded that in general, dry heating of sorghum does not often affect the phenolic compounds profile while in wet heat processing, it reduces them. Wu *et al.* (2013) developed sorghum tea from a red sorghum variety by successive processes of soaking, steaming and roasting. Wu *et al.* (2013) observed significant decreases in TPC, TFC and procyanidins content in soaking and steaming processes but significant increases when roasted. Later studies by Xiong *et al.* (2019) used white sorghum grain variety to develop sorghum tea by consecutive processes of soaking, steaming and roasting. However, Xiong *et al.* (2019) noted increase in TFC upon soaking and steaming, while TPC remained unaffected. The levels of TPC increased upon roasting which correlates to (Wu *et al.*, 2013).

## **2.6 SESAME SEED UTILIZATION AND PROCESSING**

### **2.6.1 Sesame seed utilization and production**

Sesame (*Sesamum indicum* L.) is a chief oil crop of the Pedaliaceae family, cultivated for its rich protein seed and edible oil (Elleuch *et al.*, 2011). Sesame is widely grown in some parts of Africa and Asia and has been christened as the ‘Queen of oilseed crop’ with the crop ranking fifth after soybean, groundnut, sunflower and mustard (Asghar *et al.*, 2014; Koitilio *et al.*, 2018; Pathak *et al.*, 2014). The primary products of sesame seed are its whole seed, seed oil and meal which have diverse uses across the food industry (Anilakumar *et al.*, 2010).

Sesame seeds are a rich source of proteins (Anilakumar *et al.*, 2010), essential oils (Hegde, 2012) and phytochemicals comprising tocopherols (Hwang, 2005), sesame lignans of sesamin, sesamol, sesamol and sesaminol (Bodoira *et al.*, 2017). Sesame seeds have carved a niche in various local and industrial applications owing to their superior nutritional and functional properties. Sesame seeds have been used to decorate baked goods while its paste has been used to flavor salads and sauces, and in processing of *tehneh* a common Middle East sweetened snack and *halva* which is common in Greece and parts of Asia (Gorrepati *et al.*, 2015; Elleuch *et al.*, 2011). Sesame seed contributes up to 90% of the edible oil production worldwide (Das and Bhattacharjee, 2015), and is noted with a pleasant flavor which can be consumed without the need for further purification processes such as winterization (Hassan, 2013; Abdulmalik *et al.*, 2015). The sesame seed oil has found uses in the food industry such as in roasting, frying and stewing of

various foods such as fish, meat and vegetables and as a base in salad dressings (Gorrepati *et al.*, 2015; Chakraborty *et al.*, 2017).

### **2.6.2 Sesame seed processing**

To fully exploit the potential of sesame seed's nutritional, sensory and functional qualities, the seeds are usually dehulled and roasted prior to use. The sesame seed coat has relatively high on the anti-nutrient oxalic acid and fiber content which if not removed, imparts a bitter taste to resultant seeds (Elleuch *et al.*, 2011). Previous studies have put forth either physical, mechanical or chemical methods for dehulling sesame seeds and improving their functional properties.

Inyang *et al.*, (1996) dehulled sesame seeds by soaking the seeds in 10% NaCl solution for 14 h, followed by concurrent actions of washing with tap water and rubbing the seeds so as to decorticate off the hull and reduce salinity. The study noted a slight decrease in crude protein and ash content, however, due to its simplicity, the authors recommend the procedure. Similar observations were made by Bamigboye *et al.*, (2010), who soaked the seeds in distilled water for 30 minutes, and thereafter rubbed with hand to remove the hull, which was separated by drying in oven and thereafter winnowed to remove the hulls.

Chemical means of dehulling sesame seeds have been investigated and their effect on their color and structural integrity. Carbonell-Barrachina *et al.* (2009) investigated chemical dehulling on the effect of sesame seeds color and microstructure. Seeds were soaked in sodium hypochlorite (NaClO), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), concentrated HCl, solid NaOH, solid sodium metabisulfite (Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>), solid sodium bisulfite (NaHSO<sub>3</sub>), and concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) and the seeds thereafter washed with tap water whilst rubbing them, air dried and hulls separated by winnowing. The study concludes chemical dehulling induces minimal damage to its microstructure, thus retains their light color and not bitter which is suitable for food purposes.

Roasting process is a basic precursor to sesame products due to resultant desirable changes in their physical, chemical and nutritional properties of the seeds (Rizki *et al.*, 2015). Roasting temperatures preferred are generally 150-200°C for 10-20 minutes to induce pleasant aroma and taste (Berk *et al.*, 2019). This is achieved by a balance of time and temperature on  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness) of sesame seeds during roasting. However, undesirable contaminants are usually formed when sesame seeds are subjected to increased heat levels by forming contaminants during maillard reaction. For instance, Berk *et al.* (2019) investigated

maillard reaction reactions at varying temperatures of 150, 180, 200, and 220°C noted increase in hydroxymethylfurfural (HMF), acrylamide levels and  $\alpha$ -dicarbonyl compounds with increase heat load. Kahyaoglu and Kaya. (2006) reported increase in  $L^*$  values at 120 °C, while higher temperatures led to decline in  $L^*$  values while Berk *et al.* (2019) reported increase in  $a^*$  values up to 200°C, while  $b^*$  values decreased with increase in roasting temperatures which shows loss of yellow color and increased brownness.

Sesame seed oil, is highly stable against oxidative rancidity partly due to its high tocopherol content (Wan *et al.*, 2015). Thus, studies have observed that while roasting of sesame seeds imparts desirable flavor to its oil, the treatment can have desirable and undesirable effects. In addition, roasting sesame seeds has been reported to improve FRAP, ABTS radical scavenging activity and DPPH too (Lawal *et al.*, 2019) which has been correlated to improved stability of sesame oil against peroxidation.

## **2.7 BAOBAB**

### **2.7.1 Production and nutritional quality**

In sub-Saharan, wild tree fruits form an important part of their diets and source of income too (Parkouda *et al.*, 2012). Most of these indigenous fruit trees are largely consumed without minimal processing operations, and they form an important nutritional source to most communities in sub-Saharan Africa (Gebauer *et al.*, 2016). These trees include baobab (*Adansonia digitata*), coconut (*Cocos nucifera*), horned melon (*Cucumis metuliferus*), pumpkin (*Cucurbita maxima*), kei apple (*Dovyalis caffra*) among others (AOCC, 2018).

The edible portions of baobab have adequate biochemical composition among the indigenous tree family. The fruit pulp is noted for its vitamin c content, while its seeds are rich in mineral content, in particular calcium, zinc and iron (Amarteifio and Mosase, 2009). Stadlmayr *et al.* (2020) studied the nutritional composition of baobab fruit pulp in several locations of Kenya. The study reported high vitamin C ( $175\pm 62$  mg/100g), calcium ( $375\pm 93$  mg/100g), and potassium ( $1006\pm 280$  mg/100g). Similar studies by Muthai *et al.* (2017) collaborate the fact that the edible portions of baobab (fruit pulp, seeds, and leaves) are important source of vitamin C and mineral content. Whilst the tree remains underutilized, the baobab tree has potential to improve food and nutrition security, there are economic advantages yet to be realized. Therein the imperative to re-look the importance of baobab in our diets.

### **2.7.2 Products from Baobab**

The baobab tree is a revered species in various African communities due to the multi-duplicity of uses the tree parts provides (Gebauer *et al.*, 2002; Kaboré *et al.*, 2011). Edible parts of the tree include the fruit pulp, which is eaten raw, or processed in forms of juices, jams and beers (Stadlmayr *et al.*, 2020), the leaves (as vegetables) (Zahrau *et al.*, 2014), the seeds which can be roasted and eaten as snacks (Kaboré *et al.*, 2011), but in Kenya, they are usually coated with color and sugar mix and sold as ‘*mabuyu*’ (Gebauer *et al.*, 2016). The seeds provide oil when pressed (Aluko *et al.*, 2016) and serve as thickeners for soups (Kamatou *et al.*, 2011). The tree’s fibrous bark is utilized in making of ropes, bags (Stadlmayr *et al.*, 2020) with the fruit’s woody shell used in manufacture of musical instruments, lamp shades, and curio items (Gebauer *et al.*, 2016).

### **2.8 KNOWLEDGE GAPS**

The use of orphan crops has been shown to provide an avenue for new product development and diversification. This is important so, especially the challenge of food and nutrition security in the 21<sup>st</sup> century. The challenge therein lies in use of processing technologies that provides a balance in nutritional and functional properties of such food products. A combination of modern processing and traditional techniques have the potential to have a net positive effect on nutritional and functional balance. However, the effect of such processing methods on the biochemical, functional and sensory properties need systematic evaluation so as to establish their effect. This will provide the case for increased use of orphan crops in product diversification.

# **<sup>1</sup>CHAPTER THREE: NUTRITIONAL AND SENSORY QUALITY OF A SORGHUM SNACK SUPPLEMENTED WITH SESAME AND BAOBAB FRUIT POWDER**

## **ABSTRACT**

Sorghum, sesame seeds and baobab fruit are commercially viable crops which remain underutilized in sub-Saharan Africa with potential for use in development of high-quality value-added products for food and nutritional security. This study aimed at evaluating the nutritional and sensory attributes of a ready to eat snack bar developed from sorghum supplemented with sesame and baobab fruit pulp powder. The study was set in a 3 × 4 factorial design with three levels of sorghum processing modes of roasting, malting and fermentation and four blends <sup>2</sup>(60:25:15; 70:20:10; 80:15:5; 100:0:0).

The moisture content ranged between 6.38% and 10.28%, total fiber content ranged between 5.59g/100g and 10.455g/100g while protein and fat content ranged between 11.28g/100g and 16.74g/100g and 9.65g/100g and 18.58g/100g respectively. The carbohydrates content in the snack bars ranged between 46.37g/100g and 60.31g/100g, while energy content averaged 426.33 kcal/100g for raw materials and 414.38 kcal/100g for formulated snack bars. Concentrations of Iron, calcium and zinc ranged between 5.46 mg/100g and 14.611 mg/100g, 82 mg/100g and 246 mg/100g, and 1.377 mg/100g and 4.98 mg/100g respectively.

Sensory evaluation of the bars formulations was based on a 5-point hedonic scale and revealed significance differences ( $p < 0.05$ ) in color, taste and overall acceptability (Appendix 1). The aroma and crunchiness of the snacks were found not significant. Snack bars with no added baobab were found to be generally acceptable with RSF4 ( $3.853 \pm 0.99$ ), MSF4 ( $3.529 \pm 0.99$ ) and FSF4 ( $3.676 \pm 1.34$ ) being the most preferred.

The study found underutilized crops have the versatility to improve the range of products and spur innovation in new product development.

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<sup>2</sup> Formulation blends labeled F1- Formulation 1, F2 – Formulation 2, F3 – Formulation 3, F4 – Formulation 4 respectively



### 3.1 INTRODUCTION

Sorghum is an important underutilized cereal in Africa due to its drought resistance (Chikuta *et al.*, 2014). Ranked the fifth most important cereal, sorghum provides protein and energy through gruels to many people in sub-Saharan Africa (Peleme *et al.*, 2002). The biochemical composition in sorghum structure generally compares with other cereals with some minor compositions (Taylor and Kruger, 2019). Sorghum is high in fiber content, protein though deficient in lysine content, starch and good distribution of micronutrients albeit in low levels (Serna-saldivar *et al.*, 2019). In addition, sorghum has limiting levels of Sulphur containing amino acids of cysteine and methionine (Peleme *et al.*, 2002). Hence, there is need to complement the biochemical composition of sorghum with an oil seed such as sesame for protein and PUFA (Hegde, 2012) and baobab fruit which is noted for its ascorbic acid and mineral content (Aluko *et al.*, 2016).

On the other hand, sesame (*Sesamum indicum* L.) is an important oil seed that is widely grown in some parts of Africa and Asia (Asghar *et al.*, 2014). Sesame seed is noted for its high protein content at ranges of 17-32% and abundant quantities of oil at 40-50% and laden with tocopherols (Gharby *et al.*, 2015). In addition, sesame is rich in calcium, phosphorous and iron (Onsaard, 2012). Thus, sesame seeds have found a multitude of uses across the food industry such as processing of margarines, oil, sauces (Hiremath *et al.*, 2010) including but not limited to production of soaps, lubricants in the non-food niche (Nyongesa *et al.*, 2013). However, despite the nutritional and industrial importance of sesame, its cultivation and yield remains low in Kenya averaging 400 kg ha<sup>-1</sup> (Nyongesa *et al.*, 2013), with its range of applications limited to roasted seeds and sesame oil (Koitolio *et al.*, 2018).

Further, it has been shown that Baobab (*Adansonia digitata* L., Malvaceae) is localized in Lower Eastern and coastal parts of Kenya where it remains as a wild undomesticated tree (Kinuthia *et al.*, 2017). The importance of baobab is underpinned as the tree is composed of edible leaves, seeds and fruit pulp (Kinuthia *et al.*, 2017). The baobab fruit pulp is particularly noted for its high ascorbic acid content reported at 337 mg/100g pulp (Momanyi *et al.*, 2019). The pulp has significant levels of micronutrients particularly calcium, zinc and potassium (Aluko *et al.*, 2016), however, it has low levels of protein and fat content (Momanyi *et al.*, 2019).

These orphan crops have been underutilized in production of value-added products for commercialization. Therefore, there is need for product diversification that are nutritious,

convenient aligned to increased consumer awareness. Popkin (1999) noted increased nutrition shift towards consumption of superior grains of rice, wheat and maize while indigenous cereals such as sorghum have been neglected and christened a poor man's crop (Hadebe *et al.*, 2017; Orr, 2017). Snacks are ready-to-eat products which have been characterized by high calorie, low nutritional density and has contributed to increased incidences of lifestyle diseases such as diabetes and cardiovascular diseases (Popkin, 2015; Bhurosy and Jeewon, 2016). Development of sorghum-based snacks has yet to be fully exploited, and which has the potential to improve its utilization.

In particular is sorghum, which is laden with anti-nutrients that can chelate available micronutrients (Singh *et al.*, 2016). As a result, sorghum is reported to have a poor starch and protein digestibility (Taylor, 2017). Traditional processing methods of malting and fermentation have a profound effect on the digestibility of sorghum. Previous studies have shown that fermentation and malting processes leads to a surge in the endogenous activity of sorghum via de novo activation of inherent phytases (Onyango *et al.*, 2013). Roasting has the effect of imparting desirable sensory qualities whilst also denaturing anti-nutrient factors such as trypsin inhibitors (Adedeji *et al.*, 2015; Msheliza *et al.*, 2018).

The potential of incorporating these ingredients in developing a nutritious ready-to-eat snack bar will improve their utilization, whilst alleviating consumer health concerns regarding snacks. This study was aimed at formulating and analyzing the effect of sorghum treatment methods and incorporation of sesame and baobab on the nutritional and biochemical composition of the developed snack bar.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Sample Preparation

Sorghum (*Sorghum bicolor* L. Moench) and sesame (*Sesamum indicum* L.) seeds were sourced from <sup>3</sup>Kangemi market, Nairobi while dried baobab fruits were sourced from Makueni County, Kenya. Preliminary steps of cleaning, grading, removal of broken kernels and foreign matter were done at the Department of Food Science, Nutrition and Technology, College of Veterinary Sciences, University of Nairobi.

Sorghum grains were prepared in three batches through malting, fermentation and roasting processes. The sorghum grains were steeped in water (2:1, w/v) for 18 hours, the malted batch was placed in damp muslin cloths and allowed to germinate for 72 hours. For the roasted batch, the steeped grains were air-oven dried at 105°C for 3 hours, and thereafter roasted at 180°C for 15 minutes in an open pan. For the fermented batch, the steeped cereals were oven dried at 105°C for three hours and milled into 1 mm particle size. The flour was added portable water and spontaneous fermentation by lactic acid bacteria under anaerobic conditions for 48 hours. The fermented flour was oven dried at 105°C for three hours and milled back into 1 mm flour.

Sesame seeds were cleaned, and steeped in water for 18 hours. Sesame seeds were dehulled by method described by Inyang & Ekanem. (1996) with some modifications. The steeped seeds were soaked in 10% NaCl solution for 12 hours. The seeds were thereafter consecutively washed thoroughly with water and rubbed by hands so as to decorticate them. The water was drained off and the seeds air oven-dried at 65 °C for three hours. The dried seeds were separated from the hulls by winnowing them and then pan roasted at 110 °C for 15 minutes in a pan to impart desirable sensory qualities.

The baobab fruits were cleaned and the dried pulp scrapped out with a knife on clean containers. The seeds were separated from the scraped-out pulp. The pulp was crushed in a blender (Krupps, Model Type KB703, Mayenne - France) which reduced the pulp to fine particles of 1 mm.

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<sup>3</sup> Kangemi market – Local market located in the outskirt of Nairobi County, Kenya

### 3.2.2 Formulation(s) of ready-to-eat snack bars

The formulations consisting of fermented, malted and roasted sorghum, roasted sesame and baobab fruit were modeled in a  $3 \times 4$  full factorial experiment by Nutrisurvey 2007 version (Erhardt 2007) and Momanyi *et al.* (2020). Baobab fruit pulp powder substitution levels were determined by Momanyi *et al.* (2020) owing to their astringency nature in levels above 20%. Table 3.1 shows the factors and formulations developed.

**Table 3. 1: Experimental design of the various formulations**

Formulation	Factors		
	Roasted	Malted	Fermented
Formulation 1	RSF1	MSF1	FSF1
Formulation 2	RSF2	MSF2	FSF2
Formulation 3	RSF3	MSF3	FSF3
Formulation 4	RSF4	MSF4	FSF4

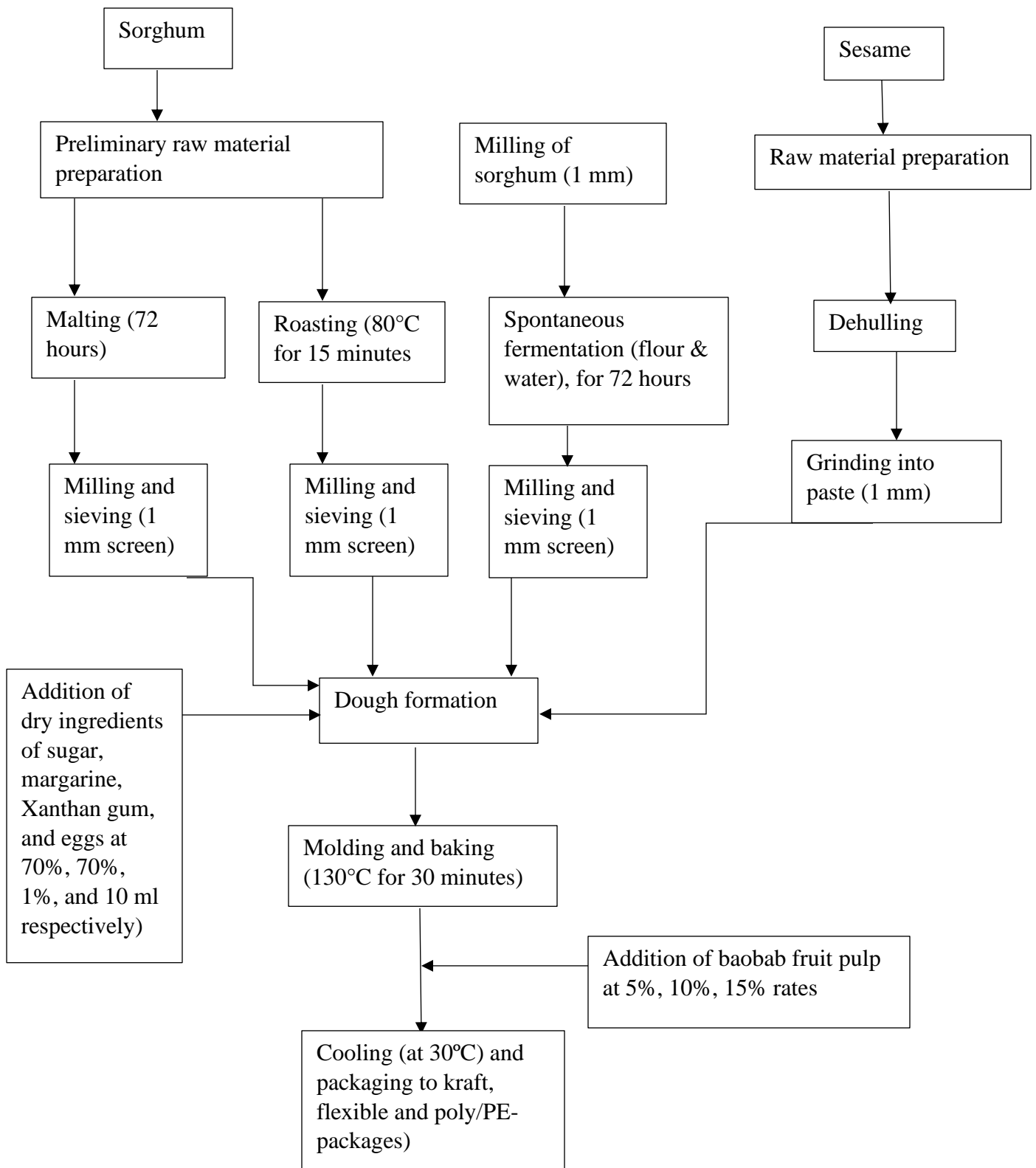
RS = Roasted sorghum, MS = Malted sorghum, FS = Fermented sorghum

The sorghum flour variations were added at different proportions with dry ingredients of sugar, hydrogenated margarine and egg white and xanthan gum were added and stirred vigorously. The ingredients were standardized for all factors of roasting, malting and fermentation. Xanthan gum was added as a binding agent at 1% level of total flour weight (Shittu *et al.*, 2009; Preichardt *et al.*, 2011). The liquid egg white functioned as emulsifying agents, while the margarine was to improve the texture of the dough, due to the rough texture of sorghum attributed to the coarse grits formed during milling which causes a sandy mouthfeel (Onyango *et al.*, 2011) while also replacing the use of water. To this mixture, sesame paste was incorporated and the mixture stirred well. The sesame paste was prepared by taking the previously dehulled roasted sesame seeds (section 3.2.1) and grinding them into a fine paste by a blender (Krupps, Model Type KB703, Mayenne - France). The dough was placed in pre-molds and baked at 130°C for 30 minutes. Baobab fruit pulp was sprinkled on the formulations, then remolded and packaged. Table 3.2 indicates the ingredient formulation for the snack.

**Table 3. 2: Basic formulation of a sorghum lunch bar**

S/NO	Ingredients	Samples			
		F1	F2	F3	F4
1	Sorghum (%)	60	70	80	100
2	Sesame (%)	25	20	15	0
3	Baobab fruit pulp powder (%)	15	10	5	0
4	Sugar (g)	70	70	70	70
5	Margarine (g)	70	70	70	70
6	Xanthan gum (%)	1	1	1	1
7	Egg (ml)	10	10	10	10

F1 = Formulation with 60% sorghum flour, 25% sesame, 15% Baobab fruit pulp powder, F2 = Formulation with 70% sorghum flour, 20% sesame, 10% Baobab fruit pulp powder, F3 = Formulation with 80% sorghum flour, 15% sesame, 5% Baobab fruit pulp powder, F4 = Formulation with 100% sorghum flour only.



**Figure 3. 1 Flow diagram for the product formulation**

### 3.2.3 Nutritional analysis

Nutritional content of the raw ingredients and resultant formulated snacks was done on dry matter basis.

Moisture content was determined by the AOAC method 930.15 (AOAC, 2005). About 2g of sample was weighed in a moisture dish and both their weights taken. These were placed in the air-oven and temperatures set at 105°C and then allowed to dry for 3 hours. They were then removed, cooled in a desiccator and weight taken. Moisture was calculated as:

$$\% \text{ Moisture} = \frac{W_1 - W_2}{S} \times 100$$

where  $W_1$  = weight of sample + dish prior to drying,  $W_2$  = weight after drying,  $S$  = sample weight  
Protein content was determined by the AOAC method 992.23 (AOAC, 2005). 0.5g of the sample was digested, neutralized, distilled and then titrated with 0.1N NaOH. Protein content was determined by:

$$\% \text{ N} = \frac{(\text{Blank} - \text{Titre}) \times N \times 14.007 \times 100}{\text{Sample weight}}$$

$$\% \text{ Crude Protein} = \% \text{ N} \times 6.25 \text{ (Conversion factor)}$$

Fiber content was determined by AOAC method 978.10 (AOAC, 2005). 4g of the sample were consecutively acid hydrolyzed with 1.25%  $\text{H}_2\text{SO}_4$  and alkaline hydrolysis by 1.25% NaOH. The extracts were filtered, placed in a crucible, weighed and placed in a muffle furnace at 600C for 3 hours. They were cooled and weight taken. Crude fiber was determined by:

$$\% \text{ Fiber} = \frac{W_1 - W_2}{S}$$

Where,  $W_1$  = weight of sample prior to ashing,  $W_2$  = weight of sample after incineration,  $S$  = weight of sample.

Crude Fat content was determined by the AOAC method 920.39 (AOAC, 2005). 4g of the sample(s) was weight and put in extraction thimbles. The samples were extracted for 8 hours with petroleum ether in a Soxhlet apparatus. Crude fat content was determined as:

$$\% \text{ fat} = \frac{W_2 - W_1}{X} \times 100$$

X

Where  $W_1$  = Weight of empty glass,  $W_2$  = weight of glass + fat residue, X = weight of sample

Carbohydrates were determined by difference method described by AOAC 2000. This was by:

$$100 \% - (\% \text{ Protein} + \% \text{ Fiber} + \% \text{ Fat} + \% \text{ Moisture}).$$

Total energy of the snacks was determined as per the formula described by Momanyi *et al.* (2020) where:

$$\text{Total energy (kcal/100g)} = [(\% \text{ Carbohydrates} \times 4) + (\% \text{ Protein} \times 4) + (\text{Fat} \times 9)].$$

### 3.2.4 Mineral analysis

Iron, Calcium, and Zinc were determined by wet digestion as described by Palma *et al.* (2015). 0.5g of sample(s) was digested by  $\text{HNO}_3:\text{HClO}_4$  (2:1) at 260°C for 3 hours. Thereafter, the samples were topped up with 50 ml distilled water. The specific minerals of Fe, Zn and Ca were determined by AAS spectrophotometry (Model 210 VGP). Standards solutions of Fe, Zn, and Ca were prepared and used to prepare a calibration curve. Fe was measured at 248nm, Zn at 213.9 nm and Ca at 422.7nm.

Concentration was calculated as:

$$\frac{(\text{Absorbance} - \text{Blank}) \times V}{10} = \text{mg/100g}$$

10 × Sample weight

Where V = is the volume of distilled water topped up to the mark.



### **3.2.5 Sensory analysis**

The sorghum snack bar was assessed by a semi-trained panel consisting of undergraduate, postgraduate students and staff from the Department of Food Science and Technology at the University of Nairobi. A 5-point hedonic scale (1= dislike extremely to 5= like extremely) was used to assess color, taste, crunchiness, aroma and overall acceptability (Appendix 1). Clean water was provided to the panelists for rinsing their mouths after evaluating each sample so as to minimize errors during the process.

### **3.2.6 Statistical Analysis**

The analysis was done in triplicates. Collected data was statistically analyzed by GenStat software version 15.0 at  $P < 0.05$  significance level. Data was subjected to a one-way ANOVA to determine the least significant difference at  $p \leq 0.05$  and post hoc mean separation and comparisons performed by Tukey's multiple range test.

### 3.3 RESULTS

#### 3.3.1 Biochemical analysis of the snack bar formulations

The biochemical composition of the raw ingredients and formulated snacks are presented in Table 3.3. Raw unprocessed samples were used as the control samples. The general nutritional composition of the snacks improved significantly ( $p < 0.05$ ) when compared to raw unprocessed samples.

The general moisture content of the snacks was higher than the individual raw samples. There were significant differences in all formulations ( $p < 0.05$ ) with values ranging from 6.39% DM to 10.29% DM. The raw unprocessed samples were 4.83%, 6.60%, 3.09% for sorghum, sesame and baobab respectively. The roasted sorghum formulations had moisture content values ranging from 6.39% DM to 8.78% DM as indicated in Table 3.3. Malted sorghum formulation moisture levels ranged between 10.29% DM to 9.21% DM while fermented sorghum formulations ranged between 6.79% DM and 9.90% DM. The moisture values in malted sorghum had slightly elevated moisture levels as compared to roasted and fermented sorghum formulations. High moisture content was recorded for malted sorghum snack, MSF3 at 10.29%, as compared to high levels in fermented sorghum snack FSF1 at 9.90% and roasted sorghum snack RSF1 at 8.31% respectively (Table 3.3).

The protein content of the raw unprocessed samples and the snack formulations are as shown in Table 3.3 for roasted, malted and fermented sorghum formulations respectively. Roasted sorghum formulations had protein content levels ranging between 13.27% to 16.74% DM, malted sorghum formulations ranged between 11.28% and 14.90% DM, whereas fermented sorghum formulations ranged between 12.31% and 15.51% DM. The unprocessed sesame had high overall protein content (19.98%) compared to sorghum (10.37%) and baobab fruit pulp (4.89%). The trend in all formulations showed a decrease in protein content with low sesame supplementation as outlined in Table 3.3. Thus, the trend indicated higher sesame substitution levels at 25% has a positive net improvement in overall protein content. Formulations RSF4, MSF4, and FSF4 all had decreased protein levels as they had no sesame in them. In addition, roasted and fermented sorghum formulations had improved protein content as compared to malted sorghum formulations which were slightly lower compared to the two.

Fiber contents of the raw unprocessed samples and the snack formulations are as indicated in Table 3.3 for roasted, malted and fermented sorghum formulations respectively. The level of fiber

content was significantly ( $p < 0.05$ ) for the raw unprocessed samples and the formulated snacks as shown in Table 3.3. The fiber levels for unprocessed samples were 6.46%, 5.61% and 5.59% for sorghum, sesame and baobab fruit pulp respectively. Roasted sorghum formulations had fibre content ranging 5.59% to 8.18%, malted sorghum formulations ranged between 6.44% and 7.90% and fermented sorghum formulations had fiber content between 8.10% and 9.46% DM. The trend expresses the fermented sorghum formulations to have improved fiber content as compared to roasted and malted sorghum formulations.

Fat content of the raw unprocessed samples and snack formulations are as shown in Tables 3.3. The fat content in the snack bar formulations was attributed to contribution by sesame seeds. The raw unprocessed sesame seeds had the highest fat content (37.65%) compared to raw sorghum (3.38%) and baobab fruit pulp (0.53%) which had the least content. Roasted sorghum formulations fat content levels ranged between 12.55% and 18.58%, malted sorghum formulations ranged between 11.46% and 19.45%, while fermented sorghum formulations fat levels were between 9.65% and 19.73%. The trend indicated increased fat content with improved sesame supplementation among the formulations with RSF1, MSF1, and FSF1 with 25% sesame supplementation recording high fat content.

**Table 3. 3: Biochemical composition of the raw unprocessed ingredients and resultant snack formulations**

Processing mode	Formulation	Parameter (Dry Matter Basis)			
		Moisture (%)	Protein (%)	Fiber (%)	Fat (%)
<u>Raw unprocessed samples</u>					
	Sorghum	4.83±0.13 <sup>b</sup>	10.37±0.12 <sup>b</sup>	6.46±0.39 <sup>a</sup>	3.38±0.11 <sup>b</sup>
	Sesame	6.60±0.10 <sup>c</sup>	19.98±0.09 <sup>h</sup>	5.61±0.03 <sup>a</sup>	37.65±1.41 <sup>h</sup>
	Baobab	3.09±0.09 <sup>a</sup>	4.89±0.10 <sup>a</sup>	5.59±0.24 <sup>a</sup>	0.53±0.16 <sup>a</sup>
<u>Roasted Sorghum</u>					
	RSF1	8.31±0.38 <sup>cd</sup>	16.74±0.34 <sup>h</sup>	8.183±0.08 <sup>b</sup>	18.58±0.39 <sup>fg</sup>
	RSF2	6.84±0.17 <sup>ab</sup>	15.20±0.63 <sup>fgh</sup>	6.26±0.04 <sup>a</sup>	15.69±0.27 <sup>cd</sup>
	RSF3	6.39±0.34 <sup>a</sup>	13.65±0.29 <sup>def</sup>	5.59±0.198 <sup>a</sup>	14.52±0.19 <sup>c</sup>
	RSF4	8.78±0.22 <sup>d</sup>	13.27±0.70 <sup>de</sup>	7.65±0.39 <sup>a</sup>	12.55±0.21 <sup>b</sup>
<u>Malted Sorghum</u>					
	MSF1	9.21±0.14 <sup>e</sup>	14.90±0.61 <sup>efg</sup>	7.90±0.03 <sup>b</sup>	19.45±0.53 <sup>g</sup>
	MSF2	9.67±0.31 <sup>e</sup>	13.67±0.39 <sup>def</sup>	6.51±0.05 <sup>a</sup>	17.42±0.39 <sup>ef</sup>
	MSF3	10.29±0.43 <sup>f</sup>	12.12±0.36 <sup>bcd</sup>	6.44±0.06 <sup>a</sup>	17.53±0.07 <sup>ef</sup>
	MSF4	9.94±0.30 <sup>e</sup>	11.28±0.57 <sup>abc</sup>	7.62±0.33 <sup>b</sup>	11.46±0.46 <sup>b</sup>
<u>Fermented Sorghum</u>					
	FSF1	9.90±0.002 <sup>e</sup>	15.51±0.29 <sup>gh</sup>	8.49±0.19 <sup>b</sup>	19.73±0.15 <sup>g</sup>
	FSF2	7.715±0.35 <sup>bcd</sup>	14.45±0.22 <sup>efg</sup>	8.10±0.07 <sup>b</sup>	16.65±0.04 <sup>de</sup>
	FSF3	6.79±0.26 <sup>ab</sup>	13.37 <sup>de</sup> ±0.33 <sup>de</sup>	8.46±0.58 <sup>b</sup>	15.67±0.52 <sup>cd</sup>
	FSF4	7.28±0.07 <sup>abc</sup>	12.31±0.37 <sup>cd</sup>	9.46±0.46 <sup>c</sup>	9.65±0.31 <sup>a</sup>

Mean values with different superscript in a column are significant at p<0.05. Means separated and compared with Tukey's test.

### **3.3.2 Carbohydrates and energy content**

The calculated carbohydrate content as outlined in table 3.5 was significantly different ( $p < 0.05$ ) with roasted sorghum formulations had carbohydrate content ranging between 48.20% and 59.85% DM, malted sorghum formulations ranged between 48.54% and 59.71% DM and fermented sorghum formulations at 46.37% and 60.31% DM. The trend shows increasing carbohydrate content as sorghum levels increases from 60% substitution levels to 100% levels. Thus, formulations RSF4, MSF4, and FSF4 had improved carbohydrate content as compared to other formulations.

The energy content expressed in kcal/100g of the formulations were significant at  $p < 0.05$ , as shown in table 3.5 with values ranging between 397 to 426.9 kcal/100g for roasted sorghum snack formulations, 387.1 to 428 kcal/100g for malted sorghum snack formulations, and 377.3 to 425.1 kcal/100g for fermented sorghum snack formulations. The trend indicates decreasing energy level from formulations RSF1, MSF1, and FSF1 which had highest energy levels and subsequent formulations with decreasing calculated energy content.

**Table 3. 4: Level of Carbohydrates and Energy content in roasted sorghum formulations**

Processing mode	Formulation	Parameter (Dry matter basis)	
		Carbohydrates	Energy (kcal/100g)
<u>Roasted Sorghum</u>			
	RSF1	48.20±0.25 <sup>b</sup>	426.9±3.14 <sup>d</sup>
	RSF2	55.82±0.83 <sup>de</sup>	425.3±1.59 <sup>d</sup>
	RSF3	57.75±0.65 <sup>f</sup>	424.7±3.11 <sup>d</sup>
	RSF4	59.85±0.06 <sup>ef</sup>	397±1.71 <sup>c</sup>
<u>Malted Sorghum</u>			
	MSF1	48.54±0.03 <sup>b</sup>	428.8±2.21 <sup>d</sup>
	MSF2	52.73±0.36 <sup>c</sup>	422.4±3.38 <sup>d</sup>
	MSF3	53.63±0.06 <sup>cd</sup>	420.7±1.82 <sup>d</sup>
	MSF4	59.71±1.66 <sup>ef</sup>	387.1±0.22 <sup>bc</sup>
<u>Fermented Sorghum</u>			
	FSF1	46.37±0.64 <sup>b</sup>	425.1±0.08 <sup>d</sup>
	FSF2	53.08±0.01 <sup>cd</sup>	420±1.29 <sup>d</sup>
	FSF3	55.71±0.65 <sup>de</sup>	417.3±5.95 <sup>d</sup>
	FSF4	60.31±0.33 <sup>f</sup>	377.3±0.02 <sup>ab</sup>

Mean values with different superscript in a column are significant at  $p < 0.05$ . Means separated and compared with Tukey's test. Energy values converted to kJ where 1kcal = 4.184 kJ.

### 3.3.3 Mineral content

The specific minerals of iron, calcium and zinc were assayed for the formulated sorghum-based snack and compared to raw unprocessed samples as outlined in Table 3.5 for raw ingredients, roasted, malted and fermented sorghum formulation. Iron content was high in raw sesame (157.76 mg/100g) as compared to sorghum and baobab fruit pulp powder. Iron content in all formulations were significant at  $p < 0.05$  with RSF1 recording highest content at 14.611 mg/100g. The roasted sorghum formulations iron content ranged between 6.393 to 14.611 mg/100g, malted sorghum formulations at 5.462 to 11.441 mg/100g, and fermented sorghum formulations were at 6.477 to 11.452 mg/100g. There was positive correlation in increase of sesame and baobab effect more.

The calcium content varied significantly ( $p < 0.05$ ) among the snacks. The trend shows improved calcium levels in the formulations. Roasted sorghum formulations had calcium content between 82 to 227.2 mg/100g, malted sorghum formulations were between 131.5 and 246.7 mg/100g,

whilst fermented sorghum ranged between 122.1 to 171.5 mg/100g. The formulation(s) MSF1 recording the highest concentration (246.7 mg/100g) and RSF4 recording the least amount at 82 mg/100g. High calcium content was realized at 15% baobab supplementation level in RSF1, MSF1 and FSF1.

The Zinc content varied significantly ( $p < 0.05$ ) among the formulations with roasted formulations ranging between 1.377 and 4.817 mg/100g, malted sorghum formulations ranged between 2.303 and 4.98 mg/100g and fermented sorghum zinc content ranged between 1.831 and 2.954 mg/100g. The processing effect on zinc content was not significant ( $p > 0.05$ ) across the formulations, but a difference with increase in sesame seeds and baobab fruit pulp.

**Table 3. 5: Mineral content in raw unprocessed samples and formulations**

Processing mode	Formulation	Parameter (Dry matter basis)		
		Iron (mg/100g)	Calcium (mg/100g)	Zinc (mg/100g)
<u>Raw unprocessed samples</u>				
	Sorghum	72.52±1.38 <sup>d</sup>	179.5±0.95 <sup>cd</sup>	25.35±1.91 <sup>b</sup>
	Sesame	157.76±3.16 <sup>f</sup>	124.49±28.16 <sup>f</sup>	119.60±2.97 <sup>d</sup>
	Baobab	138.55±4.47 <sup>e</sup>	155.96±28.90 <sup>g</sup>	52.47±2.08 <sup>c</sup>
<u>Roasted Sorghum</u>				
	RSF1	14.61±0.17 <sup>c</sup>	227.2±2.45 <sup>de</sup>	4.82±0.54 <sup>a</sup>
	RSF2	8.47±0.01 <sup>ab</sup>	161.1±3.02 <sup>c</sup>	4.24±0.99 <sup>a</sup>
	RSF3	8.38±0.23 <sup>ab</sup>	101.8±2.07 <sup>ab</sup>	2.56±0.88 <sup>a</sup>
	RSF4	6.39±0.30 <sup>ab</sup>	82±3.18 <sup>a</sup>	1.38±0.08 <sup>a</sup>
<u>Malted Sorghum</u>				
	MSF1	11.44±0.03 <sup>bc</sup>	246.7±23.96 <sup>e</sup>	4.98±0.19 <sup>a</sup>
	MSF2	6.49±0.51 <sup>ab</sup>	153.6±15.16 <sup>bc</sup>	2.95±1.33 <sup>a</sup>
	MSF3	6.79±0.03 <sup>ab</sup>	149.1±15.37 <sup>bc</sup>	2.93±0.55 <sup>a</sup>
	MSF4	5.46±0.63 <sup>a</sup>	131.5±11.25 <sup>abc</sup>	2.30±1.02 <sup>a</sup>
<u>Fermented Sorghum</u>				
	FSF1	11.45±0.32 <sup>bc</sup>	171.5±11.32 <sup>cd</sup>	2.95±0.24 <sup>a</sup>
	FSF2	11.45±0.01 <sup>bc</sup>	166.8±5.99 <sup>c</sup>	2.17±1.14 <sup>a</sup>
	FSF3	8.18±0.12 <sup>ab</sup>	145.4±6.21 <sup>bc</sup>	1.83±0.11 <sup>a</sup>
	FSF4	6.48±0.55 <sup>ab</sup>	122.1±13.12 <sup>abc</sup>	1.732±0.88 <sup>a</sup>

Mean values different superscript in a column are significant at  $p < 0.05$ .

### 3.3.4 Sensory Analysis results

There were significant differences ( $p < 0.05$ ) in the color, taste and overall acceptability (Table 3.12). There were thus varying sensory perception in color, taste and overall acceptability among the processing methods and level of sesame and baobab supplementation. There were no significant differences ( $p > 0.05$ ) in the aroma and crunchiness of the snacks.

**Table 3. 6: Sensory evaluation of the prepared lunch bar snacks**

PARAMETERS					
<sup>9</sup> Formulation(s)	Color	Aroma	Taste	Crunchiness	Overall acceptability
RSF1	3.62±0.99 <sup>ab</sup>	3.21±1.12 <sup>a</sup>	3.24±1.10 <sup>ab</sup>	3.12±1.34 <sup>a</sup>	3.35±1.07 <sup>abc</sup>
RSF2	3.41±0.82 <sup>ab</sup>	2.94±1.13 <sup>a</sup>	3.47±1.16 <sup>ab</sup>	3.15±1.33 <sup>a</sup>	3.27±0.90 <sup>abc</sup>
RSF3	3.59±1.10 <sup>ab</sup>	2.85±1.16 <sup>a</sup>	3.24±1.10 <sup>ab</sup>	3.29±1.14 <sup>a</sup>	3.12±0.91 <sup>abc</sup>
RSF4	3.59±1.13 <sup>ab</sup>	3.32±1.09 <sup>a</sup>	3.65±0.98 <sup>b</sup>	3.29±1.32 <sup>a</sup>	3.85±0.99 <sup>c</sup>
MSF1	3.50±0.96 <sup>ab</sup>	2.77±1.23 <sup>a</sup>	2.56±1.13 <sup>a</sup>	2.77±1.10 <sup>a</sup>	2.77±1.05 <sup>a</sup>
MSF2	2.91±1.22 <sup>a</sup>	2.82±1.09 <sup>a</sup>	3.0±1.28 <sup>ab</sup>	3.56±1.21 <sup>a</sup>	2.97±1.17 <sup>ab</sup>
MSF3	3.41±1.21 <sup>ab</sup>	2.91±1.26 <sup>a</sup>	2.62±1.18 <sup>a</sup>	2.88±1.09 <sup>a</sup>	3.09±1.08 <sup>abc</sup>
MSF4	3.47±1.11 <sup>ab</sup>	3.21±1.07 <sup>a</sup>	3.71±0.80 <sup>b</sup>	3.59±1.16 <sup>a</sup>	3.53±0.99 <sup>abc</sup>
FSF1	3.82±0.72 <sup>b</sup>	2.88±1.15 <sup>a</sup>	3.03±1.47 <sup>ab</sup>	3.21±1.25 <sup>a</sup>	3.38±1.21 <sup>abc</sup>
FSF2	3.91±0.79 <sup>b</sup>	3.09±1.08 <sup>a</sup>	2.97±1.17 <sup>ab</sup>	3.24±1.16 <sup>a</sup>	3.15±0.99 <sup>abc</sup>
FSF3	3.18±1.03 <sup>ab</sup>	3.00±1.10 <sup>a</sup>	2.94±1.35 <sup>ab</sup>	3.41±1.16 <sup>a</sup>	3.24±1.33 <sup>abc</sup>
FSF4	3.47±1.19 <sup>ab</sup>	3.27±1.05 <sup>a</sup>	3.35±1.35 <sup>ab</sup>	2.94±1.21 <sup>a</sup>	3.68±1.34 <sup>bc</sup>

Mean values of duplicate (n = 17) with different superscript in a column are significant at  $p < 0.05$ . Post hoc mean separation and comparison by Tukey's test.

<sup>9</sup> RSF1- Roasted sorghum formulation 1, RSF2 – Roasted sorghum formulation 2, RSF3 – Roasted sorghum formulation 3, RSF4 – Roasted Sorghum Formulation 4. MSF1 – Malted Sorghum Formulation 1, MSF2 – Malted sorghum formulation 2, MSF3 – Malted Sorghum formulation 3, MSF4 – Malted Sorghum Formulation 4, FSF1 – Fermented sorghum formulation 1, FSF2 – Fermented sorghum formulation 2, FSF3 – Fermented Sorghum formulation 3, FSF4 – Fermented sorghum formulation 4



### **3.3 DISCUSSION**

#### **3.5.1 Moisture content**

Cereal snack products are normally associated with low moisture levels primarily attributed to processing techniques involving heat treatment. Moisture content ranges of 6.39%-10.29% are in agreement with Momanyi *et al.* (2020) who reported moisture levels between 9.43% and 9.5% for a sorghum snack bar composed of popped sorghum and supplemented with baobab fruit pulp powder. The slight increase in water content could be attributed to sesame which in its composition has elevated moisture levels. For the malted sorghum formulations had slightly elevated moisture levels which could be attributed to release of metabolic water during malting and resultant drying regimes (Asuk., *et al.*, 2020). Low moisture content in cereal baked goods is essential in maintaining the microbiological integrity thus extending their shelf life (Kince *et al.*, 2017). Yeast and molds are common spoilage microorganisms in low moisture cereal products and thus water activity below 0.65 is preferable in retarding their growth.

#### **3.5.2 Protein content**

High supplementation of sesame seeds at 25% in the formulations resulted in protein quality enhancement in the snack bars recorded at 16.74%, 15.90%, and 14.90% for RSF1, FSF1, and MSF1 respectively. The trend in crude protein content in the roasted, malted and fermented sorghum snack bars decreased with decreased supplementation of roasted sesame. Sesame seeds have been profiled to contain up to 18-25% protein (Tenyang *et al.*, 2017) and rich in essential amino acids of tryptophan and methionine (Lawal *et al.*, 2019). In addition, dehulling roasting sesame seeds had no effect on protein quantity which agrees with studies by Lawal *et al.* (2019). Thus, by supplementing sesame seeds, there is overall improvement in overall protein quality which is essential in combating protein energy malnutrition.

There was observed effects of processing on crude protein among the formulations. The range of protein content has been reported to range between 11.5-12.3% (Serna-saldivar *et al.*, 2019) thus, processing techniques are essential in improving the overall protein content. Roasted sorghum formulations snacks had higher crude protein content (13.27% g/100g to 16.74% g/100g) when compared to fermented formulations (12.31% g/100g to 15.51% g/100g) and malted formulations (11.28% g/100g to 14.90% g/100g) as per table 2.3. These observations agree with Tamilselvan and Kushwaha. (2020) who recorded increase in crude protein during fermentation and net

reduction during malting of sorghum. Malted sorghum formulations had lower crude protein content which could be due to degradation of proteases of some of the amino acids present are synthesized during the germination period (Nkhata *et al.*, 2018). Roasting of sorghum disrupts the encapsulated protein in the endosperm complex thus releasing the stored sorghum (Ratnavathi, 2016). These could be attributed to the high crude protein content in roasted sorghum formulations as compared to the malted and fermented snack bars. Spontaneous fermentation by *Lactobacillus plantarum* improved overall protein content. Sorghum proteins are localized in the endosperm, germ and pericarp and fermentation has been attributed to breakdown these complexes by action of microorganisms (Tamilselvan and Kushwaha, 2020). Improved crude protein during fermentation may be attributed to breakdown of complex sorghum kafirins thus releasing peptides and amino acids particularly lysine and improving their digestibility (Nkhata *et al.*, 2018).

Thus, the combined effects of sesame supplementation and processing methods had a net effect in overall crude protein content in the snack bars.

### **3.5.3 Fiber content**

Sorghum is a rich source of dietary fiber that is associated to its pericarp and endosperm walls, usually ranging at 6%-9.3% (Stefoska-needham *et al.*, 2015; Serna-saldivar *et al.*, 2019). The fiber in sorghum is largely the insoluble type which has been associated with decrease in gastrointestinal problems, glycemic control and slow release of glucose into the bloodstream (Stefoska-Needham *et al.*, 2015).

The effect of substitution across the processing methods of roasting, malting and fermentation with sesame and baobab fruit pulp did not yield an increase in crude fiber. Sesame seeds have fiber in the range of 6-8% concentrated in their hull layers (Hegde, 2012) while baobab fruit has been reported in the 6-8 g/100g (Kinuthia *et al.*, 2017). Dehulling is done on sesame seeds to remove the hull which contains significant oxalic acid that have a bitter taste and as a consequence, most crude fiber is lost in the process. That could be a possible reason for sesame seeds not improving the overall crude fiber content in the snack bars. Nevertheless, the crude fiber content in fermented snack bars were comparatively higher when compared to roasted and malted snack bar formulations. Formulation FSF4 in particular had high crude fiber content at 9.46 g/100g. The findings agree with Mohapatra *et al.* (2019) who observed increase in fiber content from 2.76% to 3.41% in fermenting sorghum grain. However, the findings from malted sorghum snack bars

indicate lower crude fiber content relative to fermented snack bars. These are contrary to findings by Ogbonna *et al.* (2012) who reported increase in crude fiber by 72.5% by malting sorghum grist. Sprouting of sorghum could have reduced the crude fiber due to degradation of cell walls during sprouting (Taylor and Kruger, 2019). In addition, by subjecting sorghum to roasting temperatures ruptures the endosperm complex, thus degrading the starch and fiber content (Taylor and Kruger, 2019).

Thus, the result findings suggest fermentation has positive effect on the crude fiber content as compared to roasting and malting. In addition, dehulling of sesame has net negative effect on crude fiber, however, the process is necessary for reduction in the bitter oxalates. Supplementation with sesame seeds and baobab fruit reduced the proportion of processed sorghum in the ultimately, the available fiber it comes with.

#### **3.5.4 Fat content**

Sesame seeds have significant levels of oil content in the upward ranges of 48-55% (Hegde, 2012). Baobab fruit pulp is usually low in fat content, with some studies reporting contents in the average of 0.5%-2% (Sabo *et al.*, 2014; Aluko *et al.*, 2016). High fat content in the formulations was attributed to the roasted sesame seeds. Thus, as the level of sesame seeds were supplemented, there was a net positive improvement in the fat content. Snacks have a reputation for saturated fats content which renders dietary fears among potential consumers due to associated health risks such as heart diseases, hypertension and diabetes. However, sesame oil is rich in unsaturated fats of oleic and linoleic fatty acids that have beneficial health benefits of lowering of blood cholesterol levels and reducing the risk of heart related ailments (Anilakumar *et al.*, 2010).

#### **3.5.5 Carbohydrates and energy content**

Individual carbohydrate content of sorghum ranges at 72% total weight (Stefoska-Needham *et al.*, 2015b), while raw sesame seeds have 20-25% (Onsaard, 2012) and baobab fruit pulp at 74% (Oyeleke *et al.*, 2012). Processing of sorghum by fermentation reduced available carbohydrate attributed to decrease in dry matter by action of LAB (Mugula and Lyimo, 2009). The corresponding benefit is contributes to carbohydrates bioavailability encapsulated in the sorghum's endosperm (Taylor and Kruger, 2019). The malted snack bar samples had reduced total carbohydrates which could be attributed to metabolism during steeping and sprouting periods (Ogbonna *et al.*, 2012). Thus, while the snack bars had reduced carbohydrate content, the

corresponding benefit is improved protein and fat content at 14.90% and 19.45% respectively, which have important roles in cell metabolism.

Snack bars are preferred due to their nutritional density, convenience and source of energy. There is improved energy intake with increase sesame and baobab fruit supplementation. Snack bars with 25% sesame and 15% baobab have overall energy content. This could be attributed to high oil content in sesame which once metabolized by body cells, releases energy (Momanyi *et al.*, 2020).

Energy requirements are dependent on factors such as the person's age, sex, height, weight and level of physical activity. Fermented snack bars exhibited slightly higher energy content which could be attributed to the role of microorganisms in improving the starch and protein digestibility (Duodu *et al.*, 2003). For malted snack bars, there is the possibility of respiration during the germination period that depletes some of the stored starches, which ultimately has an effect on the overall energy content (Udeh *et al.*, 2018).

Two servings of the formulated snack bars will meet the total RDI for men and women >19 years old who have a moderate active lifestyle and who need minimum of 1600 kJ in women and 2000 kJ in men (USDA and HHS, 2015). The snack bar FSF4, with least energy content which recorded 1578.62 kJ will meet 98% of total RDI for children < 8 years old (USDA and HHS, 2015). For teenagers with moderate active and active lifestyles, the snack bars will adequately their total energy RDA (USDA and HHS, 2015). It should be noted that while estimates are provided, the differences in basal metabolic rates among men and women will ultimately determine needed energy content.

### **3.5.6 Mineral Content**

Iron is an important micro-nutrient in diets which is important in formation of hemoglobin in the body. The processing steps thus had the effect on mineral content in final formulations. Fermentation has the positive impact of breaking down tannins and phytates in the sorghum and this is indicated by positive iron concentration in FSF1, FSF2, and FSF3. This suggests that while sesame was dehulled, fermentation of sorghum is efficient in releasing complexed iron content (Serna-saldivar *et al.*, 2019). In contrast, the malted sorghum bars recorded lower iron content which is contrary to past observations that sprouting has a positive effect on mineral content. It is postulated by sprouting, the process reduces antinutrients present thus improving their bioaccessibility (Taylor and Kruger, 2019). It can be deduced that leaching of iron during steeping

and dehulling of sesame reduced iron contribution. Females have a higher daily requirement for iron, two servings of the snacks are able to provide more than 50% of total RDI. For females in the 19-50 age bracket, one serving of the snack bar will fulfill their Iron RDA.

Calcium is essential for bone development, cardiac and muscular contractions, transmission of nerve impulses and coagulation of the blood (FAO/WHO, 2001). The formulation(s) MSF1 recording the highest concentration (246.7 mg/100g) and RSF4 recording the least amount at 82 mg/100g. High calcium content was realized at 15% baobab supplementation level in RSF1, MSF1 and FSF1. Sesame seeds hulls have predominant calcium fractions, which once dehulled, lowers its content (Hegde, 2012). Thus, predominant calcium in the formulations were provided by baobab fruit pulp. Various authors have evaluated the baobab fruit pulp and found high calcium levels at 430 mg/100 g (Muthai *et al.*, 2017), 128 mg/100 g (Amarteifio and Mosase, 2009). The trend shows improved calcium levels in the formulations. However, snack bars RSF4, MSF4, and FSF4 had no supplementation. The malted bar, MSF4 compared better to FSF4 and RSF4 which can be attributed to processing parameters during malting such as type of water used, steeping and deculming steps. In particular, the breakdown of anti-nutrients present especially phytates contents, makes calcium more bioavailable in sorghum (Taylor and Kruger, 2019). The least calcium concentration in formulation RSF4 (82 mg/100g) could be attributed to roasting temperatures not able to breakdown the anti-nutrient elements so as to release complexed calcium content.

The Zinc content varied significantly ( $p < 0.05$ ) among the formulations with roasted formulations ranging between 1.377 and 4.817 mg/100g, malted sorghum formulations ranged between 2.303 and 4.98 mg/100g and fermented sorghum zinc content ranged between 1.831 and 2.954 mg/100g. The processing effect on zinc content was not significant ( $p > 0.05$ ) across the formulations, but a difference with increase in sesame seeds and baobab fruit pulp (Amarteifio and Mosase, 2009). Nevertheless, two servings of RSF1, RSF2 and MSF1 would meet the RDI for females >19 years and above. comparatively, males would require three servings of the same snack bars to attain their daily zinc RDI (Appendix 2). Zinc is essential for gene expression, metabolic breakdown of proteins, carbohydrates and fats and forms part of the enzyme structure and proteins (FAO/WHO, 2001).

### 3.5.7 Sensory Analysis

There were no significant differences in the aroma and crunchiness of the snack bars ( $p>0.05$ ) with average sensory scores of 3 indicative of the perception of neither liking nor dislike. These collaborate with Momanyi *et al.* (2020), the effect of beany flavor in cowpeas lowered the scores. Sesame seeds have significant oil content which is utilized in frying operations (Hwang, 2005), and roasting their seeds enhances the aroma. Furthermore, malting of sorghum which involves activation of endogenous enzymes, release of starch content, which, during baking, improves the overall flavor of the snacks. For the fermented snacks, the prevalence of residue lactic acid that could have added an acidic taste was minimal. Snack bars are appealing to consumers due to their crunchy nature. Furthermore, the fiber content in sorghum maintains its rough texture if milling or sifting is inadequate. The prepared snacks were not significant with sensory scores  $<3.5$ . Sorghum has characteristic rough nature which processing operations of malting and fermentation have a great impact in reducing its fiber content to soluble form (Taylor *et al.*, 2006; Schober *et al.*, 2005; Schober *et al.*, 2007).

The trio of roasted, malted and fermented sorghum treatments had a dark color (sensory score  $>3$ ). The prevalence of dark color was attributed to higher sesame seed supplementation which caramelizes during roasting process, and lower baobab levels. These observations agree with Momanyi *et al.* (2020), where the color of lunch bars were comparatively darker with increase in cowpea and low baobab supplementation. In addition, roasting sorghum improved the appearance due to maillard reactions of its stored starch levels (Taylor and Kruger, 2019). The palatability of the snack bars was significant ( $p<0.05$ ) among the panelists. Samples MSF4 and RSF4 with 0% sesame and baobab were most preferred with MSF1 and MSF3 least preferred. Baobab has characteristic astringency taste due to high vitamin C content. Momanyi *et al.* (2020) points that supplementation of baobab above 25% levels will consequently have a characteristic bitter taste in the final product which is not acceptable amongst most consumers. Sesame which is sweet, can be overwhelmed by the astringency in the baobab fruit, and generally, snacks with lower baobab were preferred.

In general, the snack bars had an acceptability score of between 2.76 and 3.67, ( $p<0.05$ ). The mark of good quality is a rating score of  $\geq 4$  on a 5-point hedonic scale. The skepticism among panelists regarding new product could be a factor for the low overall acceptability scores.

### **3.4 CONCLUSION**

A nutritional viable ready-to-eat snack bar at supplementation level of sorghum, sesame and baobab fruit was found acceptable at 60:25:15. This level of supplementation will address issues of protein energy malnutrition and low nutrient density associated with highly processed snacks. The sensory attributes of color, taste and acceptability were affected by processing modes of roasting, malting and fermentation and influence of sesame seeds and baobab fruit. Nevertheless, this study demonstrates the potential of underutilized crops in food product innovation.

## **CHAPTER FOUR: PROCESSING EFFECTS ON ANTI-NUTRIENT FACTORS AND PHYTOCHEMICAL COMPOSITION OF A READY TO EAT SORGHUM-BASED SNACK SUPPLEMENTED WITH SESAME AND BAOBAB FRUIT POWDER**

### **ABSTRACT**

Sorghum is among the underutilized food crop with potential to address food and nutrition security. Sorghum can be utilized in various processing technologies and can be incorporated into a range of products in therapeutics and conventional snack-based products. This study investigated the effect of roasting, malting and fermentation of sorghum (*Sorghum bicolor (L.) Moench*) and sorghum-based snack bar supplemented with sesame (*Sesamum indicum L*) and baobab (*Adansonia digitata L.*, Malvaceae) on the anti-nutrients and phytochemical components in snack bar formulations.

The anti-nutrients investigated were total phenolic content, tannin and phytates present in the formulated snack bars. The study was set in a 3 × 4 factorial design with three levels of sorghum processing modes of roasting, malting and fermentation and four blends (60:25:15; 70:20:10; 80:15:5; 100:0:0). The effectiveness of processing was analyzed with reference to raw unprocessed samples. Thus, it was observed that roasting reduced tannin content averagely by 82.71%, phytates by 53.26%. Malting had a reductive effect on tannins by 78.66%, phytates by 48.89%. Fermentation process induced an average tannin reduction by 78.71%, phytates by 51.54% respectively. The processing treatments had a much-improved tannin reduction as compared to phytate content in resulting snack bar formulation.

The results indicate that all the processing treatments improved retention of total phenolic content (TPC) in the formulations. The average retention for roasted sorghum formulations was 59.58%, malted sorghum formulations at 59.6% and fermented sorghum formulations at 58.31%. The results further indicated improved total phenolic retention with reduced supplementation with sesame and baobab fruit pulp powder.

The study results in this present study are useful in selecting the processing conditions for development and further utilization of sorghum in development of functional and innovative convenient foods for various health benefits, however, the individual bioactive components as affected by processing conditions need to be studied further.



## 4.1 INTRODUCTION

Food and nutrition security in the developing world has been defined with availability of traditional high value cereal crops that provide essential macro- and micro- nutrients to a majority of people living in the arid and semi-arid zones of Africa, Asia and Latin America (Henry *et al.*, 2016; Ramatoulaye *et al.*, 2016). These indigenous cereal grains such as sorghum and oilseeds such as sesame, albeit their underutilization, contribute to food and nutrition security to communities in marginalized areas with arid and semi-arid conditions (Nikmaram *et al.*, 2017; Kaur *et al.*, 2018; Kropff and Morell, 2019).

Whilst sorghum and sesame grow in the arid areas of SSA, their underutilization is mostly down to changing attitudes, cultural beliefs, and change in cuisines (Popkin, 2015). Sorghum has been relegated to mundane uses such as in porridge and thick gruel preparation (*Ugali*) with minimal effort to explore its use in modern convenient food products such as snack bars, flakes, breakfast cereals etc. (Njagi *et al.*, 2019). Meanwhile, sesame seeds have been largely used as roasted seed snacks, confectionery toppings, and its oil as a salad dressing (Asghar *et al.*, 2014).

Past and recent studies have shown that cereals not only provide essential nutrients but provide bioactive components that have been linked to therapeutic benefits such as low risk of disease occurrence. Phenolic compounds are secondary metabolites occurring naturally in some cereals, fruits and vegetables that provide extra nutritional quality (Oliveira *et al.*, 2014). In cereals, phenolic acids are the most abundant characterized group of polyphenols that are concentrated in the bran layers (Sidhu *et al.*, 2007). In sorghum, benzoic and cinnamic derivatives are the dominant components with ferulic acid being a major component in the composition of cereal grains phenolic acid profile (Awika, 2014). Liu. (2007) suggests that incorporating phenolic acids in diets may provide health benefits such as reducing risks of chronic diseases through their in vitro antioxidant activity. In particular, past studies have shown that oxidative stress to be involved in the etiology of many chronic diseases (Van Hung, 2016). Thus, sorghum has the potential to protect against oxidative stress (Taylor *et al.*, 2014), anti-inflammatory effects (Salazar-López *et al.*, 2018), antihypertensive properties (Dykes *et al.*, 2005), protection against cardiovascular diseases (Duodu, 2011), anti-cancer properties (Duodu & Awika, 2019), and anti-diabetic properties (Duodu, 2014).

Nevertheless, harnessing the potential of these bioactive compounds remains low, mainly due to presence of anti-nutrients factors of tannins, phytates, oxalates and trypsin inhibitors that limit starch and protein digestibility in both sorghum and sesame (Pathak *et al.*, 2014; Serna-saldivar *et al.*, 2019). Previous studies have opined the negative effects of anti-nutrients on chelating of micro-nutrients ultimately reducing their bioavailability (Galán *et al.*, 2018). Cereals contribute to almost a third of source of nutrients globally and hence therein the imperative to minimize the level of anti-nutrients (Kropff and Morell, 2019). Various cereals such as quinoa (Filho *et al.*, 2017), finger millet (Ramashia *et al.*, 2019), pearl millet (Chinenye *et al.*, 2017) sesame (Olagunju and Ifesan, 2019) in addition to sorghum (Usman *et al.*, 2018) have been shown to contain inherent levels of anti-nutrients.

Processing techniques are aimed at enhancing the phenolic content whilst reducing the antinutrients present. Processing operations such as fermentation, freezing, thermal processing and pasteurization have been shown to release bound phenolic acids, thereby improving their availability (Liu, 2007) whilst reducing the level of anti-nutrients to safe levels (Kaur *et al.*, 2015). However, Udomkun *et al.* (2019) observes that these processing methods can still pose a health risk if the original levels of anti-nutrients are high. Traditional processing techniques of malting (Duodu, 2014b), fermentation (Ojha *et al.*, 2018b), roasting (Taylor and Duodu, 2015) have been investigated with a broad agreement on their positive enhancement of some bioactive compounds and reduction of anti-nutrients present. Thus, new product development efforts are directed towards incorporating a balance of the functional properties in our daily foods at adequate levels to meet consumer's demand for healthy and convenient foods.

The present research attempts to analyze the effect of processing on total free phenolics, and level of anti-nutrients present in a sorghum-based snack bar.

## **4.2 MATERIALS AND METHODS**

### **4.2.1 Chemicals**

Hydrochloric acid, sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), Wade reagent (0.03%  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ), 0.3% sulfosalicylic acid, 70% Acetone, methanol, petroleum ether, Folin-Ciocalteu reagent, Sodium carbonate, vanillin (Merck, Darmstadt, Germany).

### **4.2.2 Snack bar Preparation**

The RTE snack bars were formulated from fermented, malted and roasted sorghum, roasted sesame and baobab fruit. The ratios of sorghum, sesame and baobab fruit pulp powder were 100:0:0, 80:15:5, 70:20:10, and 60:25:15 respectively. Dry ingredients of sugar, hydrogenated margarine and egg white and xanthan gum were added to the flour and stirred vigorously. The prepared dough was shaped in bars and placed in pre-molds and baked at 130°C for 30 minutes and then vacuum packaged for antinutrient analysis.

## **4.3 ANALYTICAL METHODS**

### **4.3.1 Total Free Phenolics determination**

The total phenolic content were assessed by use of the Folin-Ciocalteu reagent method (Singleton *et al.*, 1999). 0.5g of the sample(s) was defatted by petroleum ether and centrifuged at 13000 rpm for 3 minutes. The defatted sample was extracted sequentially for phenolics with 2ml of 80%, 50% methanol and 70% acetone acidified with 1% concentrated HCl. All the supernatants were pooled together and made to 10 ml. 3 ml of the collected supernatant were oxidized with 1.5ml Folin-Ciocalteu reagent, and 1.2 ml of 7.5% sodium carbonate used to neutralize the reaction. The resulting blue complex extracts were assayed for total phenolics at 765 nm with a uv-vis spectrophotometer (Perkin Elmer Model 6067). A standard curve was prepared with a (+)-catechin hydrate (20-100  $\mu\text{g}$ ), and amount of total phenolics in the samples expressed in mg GA equivalent/L of extract grams per 100g on dry matter basis.

$$\text{Total Phenolics (g/100g)} = \text{Constant} \times \text{Absorbance} \times \text{Dilution factor}$$

### 4.3.2 Calculation of Total phenolic retention

The % retention of the phenolic content was assayed by comparing to the raw unprocessed samples as controls and the resultant snack bar formulations.

Total retention for supplemented snack bar formulation was given by:

$$\frac{\text{TPC in the snack bar formulation}}{\text{(Sum of TPC in raw sorghum + sesame + baobab)}} \times 100 = \% \text{ TPC retained}$$

The % loss in TPC after processing = 100 – (% TPC retained)

### 4.3.3 Condensed tannins determination

Condensed tannins were estimated by the vanillin-HCl by Broadhurst & Jones. (1978) with some modifications. method of 1g of the sample(s) was defatted by petroleum ether and centrifuged. The tannins from the defatted sample(s) were extracted sequentially by centrifugation with 100%, 90%, 80% and 70% acetone solutions acidified with 1% concentrated HCl. After centrifugation, all the supernatants were pooled together to a known volume. 100 µl aliquot of the supernatant was taken in a test tube and treated with 3ml of 4% vanillin and 5ml of 70% HCl. The samples were left to stand for 12 minutes at room temperature. The samples were measured for their absorbance at 515 nm with a uv-vis spectrophotometer (Perkin Elmer Model 6067) and the amount calculated against a standard curve. The standard curve was prepared from a purified tannin which was isolated from the sample, dissolved in distilled water to give a stock solution of 1 mg ml<sup>-1</sup>. The stock solutions were made into stock solutions containing 10 to 1000 µg tannin ml<sup>-1</sup> concentration. The samples were measured for their absorbance at 515 nm and the amount calculated against a standard curve.

$$\text{Condensed tannins } (\mu\text{g}/100\text{g}) = \text{Constant} \times \text{Absorbance} \times \frac{100}{\text{Sample weight}}$$

#### **4.3.4 Determination of % residual tannin and % loss in tannins as affected by processing mode**

The effect of processing in reducing the condensed tannins contents was determined as:

$$\frac{\text{Tannic acid in the snack bar formulation}}{\text{(Sum of tannins in raw sorghum + sesame + baobab)}} \times 100\% = \% \text{ residual in tannins in snacks}$$

(Sum of tannins in raw sorghum + sesame + baobab)

% change in loss of condensed tannins in resultant snacks = 100 – (% residual tannins in snack formulations)

#### **4.3.4 Phytic Acid determination**

The total phytic acid was determined according to the method by Latta and Eskin, (1980) and Fruhbeck *et al.* (1995) with modifications. Defatted flour samples were sequentially centrifuged 3 times with 5 ml 2.4% HCl for one hour at room temperature. The supernatants were pooled together in a 50 ml volumetric flask and topped to the mark with distilled water. 1 ml of the sample was transferred to a test tube to which 5ml of distilled water and 2ml of wade reagent (0.03% FeCl<sub>3</sub>.6H<sub>2</sub>O and 1ml 0.3% sulfosalicylic acid). The mixture was centrifuged for 10 minutes and absorbance read at 500 nm with a uv-vis spectrophotometer (Perkin Elmer Model 6067). Standard solutions were prepared from phytic acid containing 5-40 µg/ml in distilled water. The content of phytic acid was calculated from the prepared calibration curve and expressed as mg/100 g on dry weight basis.

$$\text{Phytates (mg/100g)} = \text{Constant} \times \text{Absorbance} \times \text{Dilution factor}$$

#### **4.3.5 Determination of phytic acid reduction in the snack bars**

The effect of processing in reducing the phytic acid contents was determined as:

$$\frac{\text{Phytic acid in the snack bar formulation}}{\text{(Sum of phytates in raw sorghum + sesame + baobab)}} \times 100\% = \% \text{ reduction in phytates}$$

(Sum of phytates in raw sorghum + sesame + baobab)

% Change in loss of phytates in resultant snacks = 100 – (% residual phytates in snack formulations)

#### **4.4 STATISTICAL ANALYSIS**

The analysis was done in triplicates and data was statistically analyzed by R statistical software. Data was subjected to a one-way ANOVA and differences in the mean variances compared to Tukey's multiple range test at  $p \leq 0.05$ .

#### **4.5 RESULTS**

##### **4.5.1 Level of Total Phenolic Content**

The total phenolic content of the methanolic extracts of the various formulations and raw unprocessed samples are as shown in Table 4.1. The TPC ranged from 0.14 g/100g in raw sesame, to 0.30 g/100g in raw sorghum and 0.17 g/100g in raw baobab. The TPC in roasted sorghum formulations ranged from 0.15 g/100g DM to 0.21 g/100g DM, with % TPC retention decreasing with decreasing levels of sesame and baobab supplementation (Table 4.1). Formulation RSF4 and FSF4 had the least % retention in TPC, at 24.59% as they were composed 100% roasted sorghum with no supplementation of sesame or baobab fruit pulp.

The malted sorghum formulations recorded TPC content ranging between 0.18 g/100g DM and 0.24 g/100g DM. The % retention of TPC in malted formulations showed decrease with decreasing levels of sesame and baobab supplementation. High TPC loss was in formulation MSF4 at 70.49% which corresponds to low TPC detected after processing at 29.51%.

The fermented formulations as shown in Table 4.1 indicate TPC ranged between 0.15 g/100g DM and 0.251 g/100g DM. In addition, the % rate in loss of TPC as compared to the raw unprocessed ingredients increased from FSF1 to FSF3 with increasing levels of fermented sorghum, with FSF4 with 100% fermented sorghum having a 49.58% TPC retention (Table 4.1).

**Table 4. 1: Effect of roasting, malting and fermentation on total free phenolics**

Treatment	Formulation	TPC (g/100g)	% TPC retention	% Loss in TPC	Average % in TPC loss
Raw unprocessed samples	Sorghum	0.30±0.08 <sup>e</sup>			
	Sesame	0.14±0.07 <sup>a</sup>			
	Baobab	0.17±0.07 <sup>bcd</sup>			
Roasted sorghum formulations	RSF1	0.21±0.01 <sup>bcd</sup>	34.43%	65.57%	70.49%
	RSF2	0.20±0.02 <sup>bcd</sup>	32.79%	67.21%	
	RSF3	0.16±0.04 <sup>bc</sup>	26.23%	73.77%	
	RSF4	0.15±0.05 <sup>b</sup>	24.59%	75.41%	
Malted sorghum formulations	MSF1	0.24±0.09 <sup>cde</sup>	39.34%	60.66%	65.98%
	MSF2	0.22±0.05 <sup>bcd</sup>	36.07%	63.93%	
	MSF3	0.19±0.04 <sup>bcd</sup>	31.15%	68.85%	
	MSF4	0.18±0.01 <sup>bcd</sup>	29.51%	70.49%	
Fermented sorghum formulations	FSF1	0.25±0.03 <sup>de</sup>	40.98%	59.02%	69.26%
	FSF2	0.18±0.04 <sup>bcd</sup>	29.51%	70.49%	
	FSF3	0.17±0.01 <sup>bc</sup>	27.87%	72.13%	
	FSF4	0.15±0.01 <sup>b</sup>	24.59%	75.41%	

Means values with different superscript in a column are significant at p<0.05. Means separated and compared with Tukey's test at p<0.05

#### **4.5.2 Level of Tannins**

The condensed tannin content in the raw unprocessed samples and the formulations are presented in Table 4.2. The tannic content ranged from 179.4 mg/100g in raw sorghum, to 535 mg/100g in sesame and 160.7 mg/100g in baobab respectively. The condensed tannin content for the formulations and the control were significantly different ( $p < 0.05$ ) among the three processing factors of roasting, malting and fermentation as indicated in Table 4.2.

The roasted sorghum formulations had tannin levels in the range of 119.7-218.9 mg/100g with increasing levels of % condensed tannin reduction with decreasing levels of sesame and baobab supplementation (Table 4.2). Formulation RSF4 exhibited the biggest reduction in tannic content at 86.32% at 119.7 mg/100g from in the raw unprocessed sorghum flour at 179.4 (Table 4.2).

The malted sorghum formulations exhibited reducing levels of tannin content as sesame and baobab levels were reduced (RSF1 through RSF4 with 100% malted sorghum). Range of tannin levels ranged between 164.9 mg/100g and 231.2 mg/100g (Table 4.2). The % change in tannin content reduction as affected by processing method peaked at 81.16% in MSF4 from 73.58% in MSF1 which was indicative of tannin reduction with decreasing levels of sesame and baobab supplementation.

The tannin content in the fermented sorghum formulations ranged between 141.1 mg/100g and 249.9 mg/100g. A comparison of level of reduction of tannins with the raw unprocessed components showed an average reduction of 78.71%. The trend thus indicated improvement in reduction of condensed tannin levels with decreasing supplementation levels of sesame and baobab fruit pulp.



**Table 4. 2: Effect of roasting, malting and fermentation on condensed tannins**

Treatment	Formulation	Tannins (mg/100g)	% Residual tannins	% Tannin reduction	% Average tannin reduction
Raw unprocessed samples (Unprocessed)	Sorghum	179.4±1.02 <sup>ab</sup>			
	Sesame	535±9.90 <sup>d</sup>			
	Baobab	160.7±1.06 <sup>abc</sup>			
Roasted sorghum formulations	RSF1	218.9±10.456 <sup>b</sup>	25.01%	74.99%	82.71%
	RSF2	137.6±2.04 <sup>abc</sup>	15.72%	84.28%	
	RSF3	129.2±8.30 <sup>ab</sup>	14.76%	85.24%	
	RSF4	119.7±8.20 <sup>a</sup>	13.68%	86.32%	
Malted sorghum formulations	MSF1	231.2±14.39 <sup>bc</sup>	26.42%	73.58%	78.66%
	MSF2	179.4±8.25 <sup>abc</sup>	20.50%	79.50%	
	MSF3	171.4±4.11 <sup>abc</sup>	19.59%	80.41%	
	MSF4	164.9±7.25 <sup>abc</sup>	18.84%	81.16%	
Fermented sorghum formulations	FSF1	249.9±19.57 <sup>c</sup>	28.56%	71.44%	78.71%
	FSF2	189.6±9.32 <sup>abc</sup>	21.67%	78.33%	
	FSF3	164.7±21.74 <sup>bc</sup>	18.82%	81.18%	
	FSF4	141.1±11.37 <sup>abc</sup>	16.12%	83.87%	

Means values with different superscript in a column are significant at  $p < 0.05$ . Means separated and compared with Tukey's test at  $p < 0.05$ .

### 4.5.3 Level of Phytates

The level of phytate levels are presented in Table 4.3 with comparative differences in their levels for raw unprocessed samples and their subsequent formulations. Phytate levels ranged from 20.80 mg/100g in sorghum, to 11.68 mg/100g in sesame to 8.0 mg/100g in baobab.

The roasted sorghum formulations varied significantly ( $p < 0.05$ ) with phytate content ranging between 17.47 mg/100g and 20.97 mg/100g. The rate of change in reduction of phytate levels as compared to the raw ingredients averaged at 53.26%. The effect of roasting was noted in high rate of phytate reduction with decreasing supplementation of both sesame and baobab fruit pulp as indicated in Table 4.3.

The malted sorghum formulations varied significantly ( $p < 0.05$ ) in their level of phytate levels ranging between 19.94 mg/100g and 21.72 mg/100g (Table 4.3). There was decreasing levels in detected phytic acid content with decreasing supplementation of sesame and baobab fruit pulp. Table 4.3 indicates the comparative analysis of raw unprocessed components and the malted sorghum formulations revealed an average 48.89% reduction in phytate levels. Of interest, formulation MSF4 with 100% malted sorghum recorded 19.94 mg/100g phytic acid and compared with raw sorghum (20.8 mg/100g), showed slightly 50.74% reduction.

The fermented sorghum formulations also showed a slightly decreasing phytic acid levels with decreasing supplementation of sesame and baobab fruit pulp. Phytate levels ranged between 20.31 mg/100g and 18.81 mg/100g (FSF1 through FSF4) as outlined in table 4.3. Comparative assessment of the formulation's phytic levels with raw unprocessed components of sorghum, sesame and baobab fruit pulp was on average 51.54% reduction as influenced by the fermentation process.

**Table 4. 3: Effect of roasting, malting and fermentation on phytates**

Treatment	Formulation	Phytates (mg/100g)	% Phytic residue	% Phytic reduction	Average % Phytic reduction
Raw unprocessed samples	Sorghum	20.80±0.07 <sup>fg</sup>			
	Sesame	11.68±.02 <sup>b</sup>			
	Baobab	8.0±0.09 <sup>a</sup>			
Roasted sorghum formulations	RSF1	20.97±0.01 <sup>g</sup>	51.80%	48.20%	53.26%
	RSF2	19.28±0.08 <sup>d</sup>	47.63%	52.37%	
	RSF3	17.96±0.33 <sup>c</sup>	44.37%	55.63%	
	RSF4	17.47±0.23 <sup>c</sup>	43.16%	56.84%	
Malted sorghum formulations	MSF1	21.72±0.01 <sup>h</sup>	53.66%	46.34%	48.89%
	MSF2	20.70±0.05 <sup>fg</sup>	51.14%	48.86%	
	MSF3	20.39±0.01 <sup>efg</sup>	50.37%	49.63%	
	MSF4	19.94±0.11 <sup>e</sup>	49.26%	50.74%	
Fermented sorghum formulations	FSF1	20.31±0.27 <sup>ef</sup>	50.17%	49.83%	51.54%
	FSF2	20.23±0.23 <sup>ef</sup>	49.98%	50.02%	
	FSF3	19.12±0.04 <sup>d</sup>	47.23%	52.77%	
	FSF4	18.81±0.04 <sup>d</sup>	46.47%	53.53%	

Means values with different superscript in a column are significant at  $p < 0.05$ . Means separated and compared with Tukey's test at  $p < 0.05$ .

## 4.6 DISCUSSION

### 4.6.1 Effects of roasting, malting and fermentation on Total Phenolic Content

Past studies have established the importance of phenolic compounds in diets particularly in prevention of diseases such as some cancers, cardiovascular diseases among others (Xiong *et al.*, 2019). In these regard, retention of these compounds is of essence during processing of plant-based ingredients. Three processing modes of roasting, malting and fermentation were subjected on sorghum and subsequently, supplemented with roasted sesame seeds

Supplementation with sesame seeds yielded no substantial increase in TPC in resultant snack bar formulations due to dehulling. Sesame seeds have significant portion of the phenolics, lignans which are lost when dehulled (Hegde, 2012). Figure 4.2 illustrates the impact of supplementing sorghum with sesame seeds and baobab fruit pulp powder. Baobab fruit pulp while rich in vitamin C, is low in phytochemicals and its overall contribution to the snack bars is minimal (Braca *et al.*, 2018).

The study indicated malting had an improved TPC as compared to roasting and fermentation. Figure 4.2 indicates the comparative favorable retention of TPC in malted formulations compared to the raw unprocessed ingredients. This is attributed to germination of sorghum activating the endogenous sorghum enzymes that breaks down the complexed tannins holding phenolic acids. The loss in phenolic acids during malting has been attributed to their solubility characteristic which contributes to their leaching during steeping and forming insoluble complexes with proteins (Duodu, 2011; Hübner & Arendt, 2013).

Comparative TPC analysis of individual raw samples and supplementation levels indicate increasing retention in TPC except RSF4. This could be attributed to complexes between sesame lignans and sorghum phenolics in addition to release of bound sorghum phenolics through thermal pressure which degrades the phenolic-tannin complexes rendering them less extractable. These were unavailable in formulation RSF4 which had its TPC lost by either formation of Maillard products and thermal degradation of its phenolic compounds. Contrary results by Xiong *et al.* (2019) and Wu *et al.* (2013) found increased TPC, total flavonoid content and tannins during consecutive processes of steaming and roasting sorghum. However, past studies have reported either an increase or decrease in phenolics due to varying effects of thermal processing effects such as release of bound phenolics, oxidation and formation of Maillard reaction products (Taylor

and Duodu, 2015). Cardoso *et al.* (2014) found overall dry heating operations of oven, popping and microwave heating did not affect TPC and antioxidant activity in sorghum.

Higher retention in TPC were noted in formulations FSF1, FSF2 and FSF3 as illustrated in Figure 4.2, and this can be attributed supplementation with roasted sesame seeds that have some retained phenolic acids and due to the action of *Lactobacillus plantarum* spp. that are able to break down some of the bound phenolic compounds during fermentation of sorghum. Studies by Dlamini *et al.* (2007) found that fermentation led to a decrease in TPC in decorticated and whole grain sorghum when compared to unprocessed decorticated and whole grain sorghum.

#### **4.6.2 Effects of roasting, malting and fermentation on Tannins**

Sorghum and sesame are naturally high in condensed tannins as shown in table 4.3 for the non-processed samples. Raw sesame in particular recorded high levels of tannic content (535 mg/100g), due to presence of its hulls which have significant levels of oxalic acid and tannins. Their subsequent reduction was down to removal of these hulls and roasting sesame, and due to thermal pressure, contributed to more complexed tannins rupturing thus releasing them (Embaby, 2011). Similar effects were reported by (Mijena, 2017).

Tannins have been shown to have a profound effect on bioavailability of some nutrients in the body (Duodu and Awika, 2019). Past studies have shown traditional methods of malting, fermenting and roasting have been effective in reducing condensed tannins in cereals when done effectively (Duodu, 2011). The present study showed malting and fermentation had similar average reduction in tannins at 78.66% and 78.71% respectively with roasting at 82.71%. In particular, the formulations with 60:25:15 (RSF1, MSF1, FSF1) were noted with a slight lower tannin reduction compared to subsequent levels of supplementation which could be due to higher sesame levels complexing released tannins from sorghum. Roasting sorghum has the effect of rupturing tannin complexes (Dlamini *et al.*, 2007), which releases them thus lowering their levels. In malting, the tannic reduction could be attributed to leaching during steeping of sorghum and subsequent growth period which enabled the breakdown of stored tannins. Similar trend in tannin reduction was reported in studies by (Kayode *et al.*, 2013; Tamilselvan and Kushwaha, 2020). Further studies by Eburuche *et al.* (2019) on red and white sorghum varieties observed malting decreased tannins albeit at higher levels from 35.24 to 16.79 mg/kg and 27.84 to 11.47 mg/kg respectively.

In fermented formulations, the mode of tannin reduction was postulated to the action of *Lactobacillus plantarum* activating endogenous enzymes that break down the tannin complexes. Sorghum tannins are complexed with proteins, particularly kafirins (Taylor & Duodu, 2015) which are broken down during fermentation. A closer observation is a reduced in tannin levels with reduced sesame and baobab supplementation levels. Formulation FSF1 had slightly higher tannin levels than the rest which are due to inadequate breakdown by *L. plantarum* and higher sesame supplementation levels. Similar observations were observed by Sorour *et al.* (2017) where sorghum cultivars were fermented with *Saccharomyces cerevisiae* starter culture at 40°C for 12 hr with resultant 48.6% reduction in tannin levels. Study by Onuoha *et al.*(2017) observed *L. plantarum* starter culture effects on pearl millet had net reduced tannins compared to spontaneous fermentation.

The study thus indicates the traditional modes of cereal processing are adequate in reduction of condensed tannins is significant. These methods can be exploited in new product development since condensed tannins have been shown in past studies to reduce the bioavailability of some minerals and proteins.

#### **4.6.3 Effects of roasting, malting and fermentation on phytates**

Phytates are a common presence in all cereals due to their role as storage of phosphorous that is needed during germination and early seedling growth (Taylor and Kruger, 2019). However, presence of phytates in cereals has been attributed to its role in chelating minerals thereby reducing the nutritional value that results to micronutrient deficiencies (Duodu, 2014a).

Roasting, malting and fermentation had a net average reduction effect of 53.26%, 48.89% and 51.54% respectively (Table 4.4). It can be deduced that preliminary operations of soaking and steeping of sorghum prior to further processing helped leach out the phytates as they are water soluble. Further processing step of germinating may have activated the endogenous phytase and hydrolyzed some phytates present in the cereals. The present results agree with findings by Ogbonna *et al.* (2012) on sorghum grits recorded a 66% decrease in phytate content which demonstrates the effectiveness of malting. Previous studies by Eburuche *et al.* (2019) on effect of malting on red and white sorghum varieties recorded decreased phytate from 9.764 to 3.893 mg/kg and 8.849 to 3.898 mg/kg with malting period.

Fermentation of cereals is a long-held traditional processing mode in African societies. Breakdown of endogenous phytate content in the formulations is a combined effect of steeping both sorghum and sesame and subsequent fermentation of the steeped sorghum on the action of *L. plantarum*. Comparative findings by Sorour *et al.* (2017) observed fermentation by *Saccharomyces cerevisiae* on sorghum has significant effect thus indicating different strains are capable of activating phytase enzyme that ultimately breaks down phytic acid.

#### **4.7 CONCLUSION**

This study establishes that processing methods have a direct influence in resultant phytochemicals available. Supplementation of dehulled and roasted sesame seeds have a net change in TPC, tannins and phytate levels albeit in different concentrations. The average total phenolic retention is similar across the three treatments albeit with slight differences in supplementation levels. TPC is pronounced in malted sorghum formulations as compared to the roasted and fermented formulations.

Tannin reduction improved with decreased sesame and baobab fruit supplementation with roasted sorghum snack bars having a slight improvement in average reduction than malting and fermented sorghum formulations. The phytates content was similar across the treatments, but, the combined effect of steeping and thermal treatment slightly improved reduction than malting and fermenting of sorghum.

Thus, while all three processes had positive effect on phytochemicals, supplementation of sesame at 25% had improved range of TPC and reduced antinutrients.

## **CHAPTER FIVE: STORABILITY AND THE SHELF LIFE OF A SORGHUM SNACK SUPPLEMENTED WITH SESAME AND BAOBAB FRUIT POWDER**

### **ABSTRACT**

Sorghum is an underutilized cereal crop whose utilization as snack-based foods and their resultant shelf life and acceptability is not yet fully explored. This study investigated changes in microbiological and rancidity properties of a sorghum-sesame lunch bar during storage. The snacks were made from roasted, malted and fermented sorghum supplemented with sesame at 25%, 20% and 15%, and baobab fruit powder at 15%, 10% and 5% levels. The snack bars were packaged in flexible, kraft and poly/PE-coated pouches and stored at accelerated conditions of 55°C±2 °C for 6 days, each day representing one month. Quality parameters investigated were total viable count, yeasts and molds, *Staphylococcus aureus*, total coliforms, *E. coli*, *Salmonella* spp., peroxide value and free fatty acid content. The microbiological count (log cfu g<sup>-1</sup>) and the peroxide value increased with storage days and were significantly different among the packaging material. The mean count of TVC and yeast and molds for samples stored in kraft and poly/PE-coated package were highest at the second day of storage as compared to the flexible package which recorded high mean counts at day 3 of storage (p<0.05). The *S. aureus* mean counts were found to be of acceptable limits of 10<sup>2</sup> log cfu g<sup>-1</sup>. The pathogenic microorganisms were not detected in the formulations during the duration of storage. The FFA content among the snack formulations was not significantly different (p>0.05) during the accelerated shelf-life period in the three packaging materials. The peroxide values were significantly influenced (p<.001) by days of storage and the package material (p<.001). The mean peroxide value was 5.084 meq O<sub>2</sub>/kg in kraft package detected at day 2 of storage compared to poly/PE-coated package at 4.942 meq O<sub>2</sub>/kg and flexible package at 2.031 meq O<sub>2</sub>/kg at day 3 of storage. The overall microbiological and oxidative stability was exhibited with roasted sorghum snack formulations with the snack bar RSF4 being acceptable up to 4 months of storage. The Flexible packaging material showed better microbiological and oxidative rancidity stability of up to 3 months of storage. Hence, sorghum-based snack bars have the potential for commercialization.



## 5.1 INTRODUCTION

Cereal based products more so baked products form a major portion of global diets (Gebreselassie and Clifford, 2016) due to their valuable source of nutritional needs of calories and half of protein requirements (Saranraj and Geetha, 2012). Baked goods are subject to chemical spoilage, physical spoilage and microbial spoilage having an influence with their resultant shelf life (Galić *et al.*, 2009). With new innovations in RTE snack products, Galić *et al.*, (2009) espouses that factors of formulation, processing, packaging and storage conditions influence the longevity of food products. Shelf life is defined to construe the period of time from processing and packaging a food maintains its acceptable eating quality to the point when such a food product becomes unacceptable for consumption under a set of environmental conditions (Galić *et al.*, 2009; Kince *et al.*, 2017). Microbial spoilage by molds have a predominant role in baked products posing significant annualized global losses and a food safety issue thus underscoring their importance (Rico-Munoz *et al.*, 2019). Flours are generally regarded as microbiologically safe as they contain low water activity to support pathogenic microbial growth (Khanom *et al.*, 2017). In extension, baked products by their nature have inherent reduced water activity levels conducive for mold growth thus having an influence in both high-moisture and intermediate-moisture baked goods (Galić *et al.*, 2009). Heating operations during baking process destroys all yeasts and molds (Shovon Al-Fuad, 2018), but recontamination from air, equipment surfaces and handling processes during cooling and packaging operations are vulnerable points (Cook and Johnson, 2009). Furthermore, it has been shown that improper storage conditions may lead to proliferation of various microorganisms (Khanom *et al.*, 2017). Thus, it is imperative to ensure post process hygienic and aseptic conditions necessary to prevent mold growth.

The clamor for heathy nutritious snacks by blending different ingredients has increased (Vila-Real *et al.*, 2017), thereby prompting evaluation of oxidative stability. It is important to understand the role of ingredients, storage conditions on the impact of oxidative stability and secondary volatile lipid oxidation products such as hexanal in their shelf life (Jensen and Risbo, 2007). Thus, a blend of sorghum and sesame is aimed at improving the overall nutritional value of such a snack more so its protein, fat and mineral content. Incorporation of added ingredients must not adversely alter the storability of such a product that would prove not viable economically (Vasanthakumari and Jaganmohan, 2018). Closer examination of sorghum lipids has its ranges reported at 2% to 6% whose profile consist of palmitic acid (12%-15%), stearic acid (1%-3%), oleic acid (34%-37%),

linoleic acid (42%-43%), linolenic acid (1%-2%) and palmitoleic acid (1%) (Bekele *et al.*, 2020). Sesame is composed of oil content reported in the range of 48-55% (Hegde, 2012) with oleic and linoleic acids as the dominant unsaturated fatty acids (Gharby *et al.*, 2015). This suggests vulnerability to auto-oxidation under storage conditions though Hu, (2016) suggests that in low moisture products, many factors such as maillard reactions, the intrinsic interaction of proteins and carbohydrates and added ingredients may play a role in rancidity.

The ecosystem of packaging material to be used considerations should be based on its physical properties, chemical composition and physical properties (Galić *et al.*, 2009). The storability of a food is not only dependent on good manufacturing practices but should extend to the type of package necessary to retard deteriorative actions. Products incorporated with sesame have high content of unsaturated fatty acids that are susceptible to lipid oxidation. Package material are necessary in retarding the growth of molds and pathogenic bacteria during storage (Yadav and Bhatnagar, 2016).

The aim of the study was to investigate the efficacy of three packaging materials on the changes in microbial and oxidative rancidity of a sorghum lunch bar during several days of accelerated storage conditions.

## **5.2 MATERIALS AND METHODS**

### **5.2.1 Snack bars preparation**

The RTE snack bars were formulated as previously described in chapter 3, section 3.2.1 and 3.2.2.

### **5.3 Accelerated Shelf-life Analysis**

The RTE sorghum snack bars were investigated for their storability stability status in three packaging materials at accelerated conditions for six days, each day representing one month (1 day = 1 month). The RTE sorghum snack bars were packaged in three package materials of kraft paper, flexible packaging and poly/PE-coated package which were stored in an incubator (Perkin Elmer Model 6067) at accelerated conditions at temperatures of 55 °C for six days. Sampling and testing were done for both microbial and oxidative rancidity studies for six consecutive days.

Kraft paper is predominantly made from wood pulp and it is biodegradable and the material has found wide uses in packaging of flours. However, it is noted for its poor barrier mechanism to air and moisture and poor strength. The Poly/PE-coated package is composed of a layer of

polyethylene laminated on a paper for effective barrier to moisture and oxygen. The flexible packaging material is a multilayered material composed of aluminium foil and plastic film that can readily change in shape and is mostly used in packaging of confectionery such as chocolate bars (Morris, 2017).



**Figure 5. 1 Kraft package, Poly/PE-coated package and Flexible package (L-R)**

### **5.3.1 Total viable count**

The total viable count was done as per the AACC method 42-11-01. A sample of 25 g was put into 225 ml of 0.85% sodium chloride diluent and serial dilutions up to  $10^{-5}$  performed. Plate count agar was prepared as per the manufacturer's instructions. 1 ml was plated by pour plate method and plates were incubated at  $37^{\circ}\text{C}$  for 24 hours and enumerated using colony counter technique. Colonies were expressed as  $\log \text{cfu g}^{-1}$  (Ombaka, 2018).

### **5.3.2 Yeast and molds count**

The yeast and molds count were done as per the AACC method 42-50-01. 25g of the sample was placed in 225 ml of 0.85% sodium chloride diluent. Serial dilutions of up to  $10^{-5}$  were prepared. Potato dextrose agar was prepared as per the manufacturer's directions. Pour plate technique was performed aseptically, and thereafter the plates incubated upside down at  $25^{\circ}\text{C}$  for 48 hours. Enumeration was by use of a colony counter and microbial counts were expressed as  $\log \text{cfu g}^{-1}$  (Ombaka, 2018).

### **5.3.3 *Staphylococcus aureus* count**

The *Staphylococcus* count was done as per the AACC method 42-30-04. 25 g of the sample were placed in 225 ml of 0.85% sodium chloride diluent solution and serially diluted to  $10^{-5}$ . Baird-Parker agar was prepared as per the manufacturer's direction. The serially diluted samples were plated by spread method and incubated at 37 °C for 48 hours. A colony counter was used in enumeration and colonies expressed as log cfu g<sup>-1</sup>.

### **5.3.4 Total Coliforms counts**

Total coliforms were enumerated as per the ISO method ISO 4831:2006. VRBA (Violet Red Bile Agar) was transferred to distilled water and heated while stirring and brought to boiling. The medium was cooled to 45°C and transferred to sterile plates. 1 ml of the sample dilutions ( $10^{-1}$  to  $10^{-3}$ ) was transferred to the plates by pour plate method and incubated at 37°C for 24 hours. Presence of total coliforms were noted by presence of red colonies after the incubation period.

### **5.3.5 *Escherichia Coli* Counts**

The enumeration of *E. coli* was done as described in ISO 16649-1, ISO 16649-2 and ISO 16649-3 methods. Hichrome *E. Coli* selective agar was suspended in 1 litre of distilled water. The media was gently boiled to completely dissolve, and then cooled to 45°C; the molten media was then transferred to sterile plates. 1ml of each serial dilution of  $10^{-1}$  to  $10^{-3}$  was transferred by pour plate method and plates incubated at 37°C for 24hrs. A pink colony on the selective media was typical of *E.coli*.

### **5.3.6 *Salmonella* counts**

The enumeration and detection of *Salmonella* was done as described in ISO 6579-1. 56.7 g of the XLD (Xylose Lysine Deoxycholate) agar was put in 1 liter of distilled water. The mixture was heated with frequent agitation and boiled for one minute. The prepared media was cooled to 45 °C and the media transferred to sterile petri dishes. The plates were inoculated with 0.1 ml of each serial dilution  $10^{-1}$  to  $10^{-3}$  and streaked for isolation. The plates were incubated at 37°C for 24 hours. Appearance of red colonies with black centers were typical of *Salmonella* presence.

## **5.4 Oxidative rancidity**

### **5.4.1 Peroxide Value**

The peroxide value was determined by the standard AOAC method 965.33. 5 g of the sample were reacted with 30 ml of glacial acetic: chloroform (3:1, w/w) and 0.5 ml saturated potassium iodide. 30 ml distilled water and starch indicator was added and the mixture titrated against 0.01N sodium thiosulphate solution. Peroxide value was expressed as milliequivalents of peroxide oxygen per kg.

### **5.4.2 Free Fatty acid value**

The FFA were determined by the ISO method 729:1988. 2 g of the sample was weighed and 25 ml of diethyl ether:ethanol (1:1, w/w) added. Phenolphthalein indicator was added and titrated against 0.1N NaOH. FFA were expressed as g/100g % oleic acid

## **5.5 DATA ANALYSIS**

The data was analyzed in a two-way ANOVA in a randomized block design by use of R statistical software. Descriptive characteristics of the means and standard deviations of the microbial counts and oxidative rancidity were obtained. Post-hoc mean separation was done by Tukey's at significance level at  $p < 0.05$ .

## 5.6 RESULTS

### 5.6.1 Microbial quality of raw unprocessed materials

Table 5.1 shows the results of microbial quality of raw unprocessed ingredients that were used in later formulation of the snack bars as packaged in kraft, poly-coated and flexible packages. Total aerobic count showed an increase from log 3.80 cfu and 4.45 cfu to 7.88 cfu and 7.09 cfu respectively for raw sorghum stored in kraft package for the duration of the accelerated storage period from 0 to 6 days. However, raw baobab fruit pulp powder stored in kraft package showed decreases total aerobic count from log 6.76 to log 2.4 cfu across the storage period. The trends in poly/PE-coated and flexible packages showed a decrease in TVC for sorghum, sesame and baobab, ranging between log 2.52 cfu and log 7.41 cfu respectively.

**Table 5. 1: Effect of packaging material and storage period on Total Viable Count (log cfu g<sup>-1</sup>) of raw unprocessed materials**

Type of packaging	Sample	Storage Period (Days)						
		0	1	2	3	4	5	6
		<b>Total viable count</b>						
Kraft package	Sorghum	3.80±0.12 <sup>d</sup>	5.49±0.05 <sup>d</sup>	7.38±0.74 <sup>f</sup>	7.09±0.13 <sup>i</sup>	7.88±0.03 <sup>e</sup>	7.48±0.11 <sup>h</sup>	7.37±0.09 <sup>e</sup>
	Sesame	4.45±0.27 <sup>b</sup>	6.68±0.18 <sup>g</sup>	7.09±0.13 <sup>e</sup>	6.95±0.07 <sup>e</sup>	6.88±0.10 <sup>i</sup>	5.22±0.11 <sup>e</sup>	4.86±0.18 <sup>c</sup>
	Baobab	6.76±0.07 <sup>e</sup>	5.45±0.10 <sup>f</sup>	5.30±0.11 <sup>e</sup>	5.03±0.06 <sup>h</sup>	4.45±0.14 <sup>b</sup>	3.31±0.07 <sup>b</sup>	2.48±0.07 <sup>c</sup>
Poly/PE-coated package	Sorghum	7.41±0.08 <sup>g</sup>	6.95±0.07 <sup>h</sup>	6.95±0.16 <sup>f</sup>	6.95±0.13 <sup>h</sup>	6.04±0.15 <sup>ab</sup>	6.34±0.16 <sup>e</sup>	6.98±0.10 <sup>h</sup>
	Sesame	6.74±0.11 <sup>f<sup>g</sup></sup>	6.53±0.18 <sup>h</sup>	6.58±0.07 <sup>f</sup>	5.76±0.14 <sup>g</sup>	5.11±0.17 <sup>b</sup>	3.56±0.07 <sup>g</sup>	3.05±0.11 <sup>d</sup>
	Baobab	5.53±0.11 <sup>f</sup>	5.11±0.17 <sup>fg</sup>	5.11±0.06 <sup>e</sup>	4.98±0.23 <sup>fg</sup>	3.64±0.07 <sup>ab</sup>	2.88±0.04 <sup>d</sup>	1.95±0.10 <sup>ab</sup>
Flexible package	Sorghum	5.35±0.12 <sup>h</sup>	5.89±0.14 <sup>h</sup>	5.66±0.10 <sup>i</sup>	5.59±0.10 <sup>g</sup>	4.94±0.18 <sup>e</sup>	4.44±0.31 <sup>c</sup>	3.58±0.08 <sup>d</sup>
	Sesame	4.91±0.13 <sup>g</sup>	5.79±0.19 <sup>h</sup>	5.30±0.11 <sup>h</sup>	4.99±0.12 <sup>fg</sup>	4.30±0.11 <sup>e</sup>	3.55±0.05 <sup>f</sup>	3.06±0.07 <sup>cd</sup>
	Baobab	4.19±0.12 <sup>g</sup>	5.14±0.22 <sup>g</sup>	4.99±0.12 <sup>h</sup>	4.26±0.16 <sup>fg</sup>	4.19±0.17 <sup>e</sup>	3.44±0.05 <sup>ef</sup>	2.52±0.11 <sup>abc</sup>

Values with different superscript along a row are significantly different at p<0.05

Table 5.2 presents the Yeast and Mold counts for the raw samples over the accelerated six-day storage period. Yeast and molds count showed varying trends among the three-packaging material for raw samples stored in them across the accelerated shelf-life period. Raw sorghum and sesame stored in kraft package showed an increase in yeast and mold counts from log 0.82 cfu to log 6.75 cfu, whilst counts for the three samples (sorghum, sesame and baobab) stored in flexible package across the 6 days showed a decrease in yeast and mold counts ranging between log 0.13 cfu and log 5.68 cfu.

**Table 5. 2: Effect of packaging material and storage period on Yeast and Molds count (log cfu g<sup>-1</sup>) of raw unprocessed materials**

Type of packaging	Sample	Storage Period (Days)						
		0	1	2	3	4	5	6
<b>Yeast and Molds</b>								
Kraft package	Sorghum	1.04±0.0 5 <sup>a</sup>	2.49±0.09 d	3.56±0.05 cde	3.09±0.06 ab	6.75±0.0 5 <sup>c</sup>	5.92±0.06 d	2.72±0.09 <sup>d</sup>
	Sesame	0.82±0.1 3 <sup>bc</sup>	0.38±0.21 a	0.54±0.36 ab	6.66±0.12 d	5.57±0.1 0 <sup>c</sup>	4.21±0.11 c	0.63±0.04 <sup>bc</sup>
	Baobab	5.62±0.0 6 <sup>j</sup>	4.46±0.23 f	4.42±0.17 h	3.19±0.11 c	2.86±0.1 2 <sup>b</sup>	1.87±0.09 b	0.58±0.1 5 <sup>b</sup>
Poly/PE-coated package	Sorghum	0.16±0.1 4 <sup>a</sup>	0.90±0.05 cde	2.14±0.06 e	2.38±0.04 h	5.29±0.0 8 <sup>b</sup>	4.64±0.09 h	4.89±0.04 <sup>g</sup>
	Sesame	0.79±0.0 7 <sup>b</sup>	0.23±0.16 a	0.10±0.06 a	6.30±0.14 g	4.43±0.1 0 <sup>ab</sup>	3.37±0.01 g	5.42±0.05 <sup>f</sup>
	Baobab	5.40±0.1 2 <sup>k</sup>	4.24±0.14 g	4.03±0.08 j	2.99±0.04 f	1.90±0.0 3 <sup>ab</sup>	1.11±0.02 bcd	0.09±0.0 2 <sup>a</sup>
Flexible package	Sorghum	5.27±0.1 7 <sup>h</sup>	5.07±0.05 h	4.78±0.09 f	4.58±0.06 c	4.51±0.0 3 <sup>b</sup>	3.30±0.05 a	2.59±0.02 <sup>bc</sup>
	Sesame	5.39±0.0 9 <sup>h</sup>	5.55±0.09 i	4.49±0.07 f	3.44±0.15 bc	3.23±0.0 5 <sup>ab</sup>	2.76±0.01 a	0.13±0.04 <sup>a</sup>
	Baobab	5.68±0.1 5 <sup>i</sup>	4.16±0.19 g	3.89±0.12 e	3.02±0.02 abc	2.33±0.0 9 <sup>ab</sup>	1.99±0.09 a	0.40±0.0 5 <sup>a</sup>

Values with different superscript along a row are significantly different at p<0.05

Table 5.3 presents the *Staphylococcus aureus* in the raw unprocessed samples over the six-day accelerated period. The *staphylococcus aureus* counts were detected for all raw unprocessed samples with decreased counts recorded for sorghum across the three packages ranging between log 1.66 cfu and log 3.40 cfu across the accelerated storage period. Raw sesame stored in kraft and poly/PE-coated package showed increased counts ranging between log 1.99 cfu and log 3.59 cfu, however, sesame stored in flexible package showed decreasing *s. aureus* counts of between log 1.27 cfu and log 2.94 cfu. In addition, raw baobab fruit pulp powder showed increased *s. aureus* counts across the accelerated shelf-life period ranging between log 0.16 cfu and log 2.86 cfu respectively across the three packages (kraft, poly/PE-coated and flexible).

**Table 5. 3: Effect of packaging material and storage period on *Staphylococcus aureus* (log cfu g<sup>-1</sup>) of raw unprocessed materials**

Type of packaging	Sample	Storage Period (Days)						
		0	1	2	3	4	5	6
		<i>Staphylococcus aureus</i>						
Kraft package	Sorghum	3.13±0.06 <sup>g</sup>	3.32±0.05 <sup>k</sup>	2.81±0.07 <sup>d</sup>	2.79±0.10 <sup>d</sup>	2.61±0.11 <sup>c</sup>	2.90±0.05 <sup>d</sup>	1.95±0.21 <sup>b</sup>
	Sesame	2.96±0.04 <sup>g</sup>	3.01±0.11 <sup>j</sup>	2.53±0.11 <sup>c</sup>	2.63±0.03 <sup>c</sup>	2.54±0.12 <sup>b</sup>	2.45±0.04 <sup>b</sup>	2.19±0.05 <sup>c</sup>
	Baobab	0.40±0.02 <sup>b</sup>	0.76±0.02 <sup>b</sup>	1.21±0.02 <sup>g</sup>	1.29±0.01 <sup>e</sup>	2.85±0.06 <sup>d</sup>	2.58±0.04 <sup>c</sup>	1.97±0.06 <sup>bc</sup>
Poly/PE-coated package	Sorghum	3.33±0.04 <sup>j</sup>	2.96±0.09 <sup>c</sup>	2.65±0.05 <sup>c</sup>	2.78±0.07 <sup>d</sup>	2.59±0.14 <sup>c</sup>	2.85±0.04 <sup>d</sup>	2.77±0.03 <sup>d</sup>
	Sesame	2.95±0.07 <sup>i</sup>	3.59±0.56 <sup>d</sup>	2.86±0.05 <sup>d</sup>	2.53±0.06 <sup>c</sup>	1.99±0.20 <sup>b</sup>	2.26±0.05 <sup>c</sup>	2.00±0.06 <sup>c</sup>
	Baobab	0.16±0.03 <sup>b</sup>	0.44±0.04 <sup>b</sup>	1.02±0.04 <sup>b</sup>	1.06±0.03 <sup>g</sup>	2.80±0.05 <sup>c</sup>	1.53±0.09 <sup>b</sup>	1.24±0.07 <sup>b</sup>
Flexible package	Sorghum	3.40±0.07 <sup>f</sup>	2.90±0.09 <sup>g</sup>	2.51±0.09 <sup>d</sup>	2.25±0.10 <sup>c</sup>	2.02±0.08 <sup>c</sup>	1.66±0.02 <sup>c</sup>	2.53±0.04 <sup>d</sup>
	Sesame	2.94±0.11 <sup>e</sup>	2.81±0.07 <sup>g</sup>	2.30±0.10 <sup>c</sup>	1.58±0.03 <sup>b</sup>	1.44±0.02 <sup>b</sup>	2.19±0.05 <sup>d</sup>	1.27±0.10 <sup>c</sup>
	Baobab	0.22±0.03 <sup>b</sup>	1.01±0.03 <sup>e</sup>	1.16±0.04 <sup>d</sup>	2.86±0.06 <sup>d</sup>	2.20±0.07 <sup>d</sup>	1.18±0.02 <sup>b</sup>	0.90±0.00 <sup>b</sup>

Values with different superscript along a row are significantly different at p<0.05



Table 5.4 shows the pathogenic presence of raw unprocessed sorghum, sesame and baobab fruit pulp powder stored in kraft, poly/PE-coated and flexible package respectively across the six days of accelerated storage. The total coliform count in raw unprocessed sorghum stored in the three-packaging material across the accelerated storage period decreased ranging between log 0.04 cfu and log 2.57 cfu. Raw sesame recorded increased total coliform count ranging between log 0.07 cfu and log 2.97 cfu for storage in kraft and poly/PE-coated package, while raw sesame stored in flexible package recorded increased count ranging between log 0.38 cfu and log 2.39 cfu. The raw baobab fruit pulp total coliform count recorded decreasing counts across the three-packaging material ranging between log 0.41 cfu and log 3.59 cfu.

**Table 5. 4: Total Coliforms presence in the raw unprocessed material (log cfu g<sup>-1</sup>)**

Package material	Sample	Storage period (Days)						
		0	1	2	3	4	5	6
<b>Total coliforms</b>								
Kraft package	Sorghum	2.25±0.03 <sup>b</sup>	1.65±0.03 <sup>a</sup>	1.23±0.02 <sup>a</sup>	0.75±0.03 <sup>b</sup>	0.19±0.02 <sup>a</sup>	1.76±0.02 <sup>a</sup>	1.20±0.03 <sup>a</sup>
	Sesame	0.07±0.02 <sup>a</sup>	1.33±0.02 <sup>b</sup>	1.85±0.02 <sup>b</sup>	1.97±0.02 <sup>a</sup>	2.86±0.02 <sup>c</sup>	1.94±0.06 <sup>b</sup>	1.34±0.01 <sup>b</sup>
	Baobab	2.61±0.02 <sup>c</sup>	2.42±0.03 <sup>c</sup>	2.28±0.05 <sup>c</sup>	0.44±0.13 <sup>b</sup>	0.44±0.13 <sup>b</sup>	1.78±0.02 <sup>a</sup>	2.11±0.05 <sup>c</sup>
Poly/PE-coated package	Sorghum	1.98±0.01 <sup>a</sup>	1.05±0.12 <sup>a</sup>	1.14±0.01 <sup>a</sup>	0.24±0.03 <sup>a</sup>	0.04±0.04 <sup>a</sup>	2.57±0.02 <sup>c</sup>	1.59±0.05 <sup>b</sup>
	Sesame	1.94±0.02 <sup>a</sup>	2.97±0.02 <sup>c</sup>	2.57±0.03 <sup>c</sup>	2.10±0.04 <sup>c</sup>	1.56±0.06 <sup>b</sup>	1.22±0.04 <sup>b</sup>	1.97±0.01 <sup>c</sup>
	Baobab	3.50±0.03 <sup>b</sup>	2.49±0.04 <sup>b</sup>	1.90±0.04 <sup>b</sup>	1.29±0.07 <sup>b</sup>	1.95±0.01 <sup>c</sup>	1.51±0.02 <sup>b</sup>	0.72±0.05 <sup>a</sup>
Flexible package	Sorghum	2.13±0.01 <sup>b</sup>	0.73±0.03 <sup>b</sup>	0.69±0.03 <sup>b</sup>	0.14±0.03 <sup>b</sup>	1.87±0.01 <sup>d</sup>	1.47±0.02 <sup>d</sup>	0.72±0.02 <sup>c</sup>
	Sesame	2.17±0.02 <sup>b</sup>	1.43±0.02 <sup>c</sup>	2.39±0.04 <sup>d</sup>	1.87±0.04 <sup>d</sup>	0.86±0.02 <sup>b</sup>	0.38±0.02 <sup>b</sup>	1.77±0.02 <sup>d</sup>
	Baobab	3.59±0.06 <sup>c</sup>	2.29±0.03 <sup>d</sup>	1.76±0.02 <sup>c</sup>	0.93±0.14 <sup>c</sup>	1.69±0.02 <sup>c</sup>	1.33±0.03 <sup>c</sup>	0.41±0.01 <sup>b</sup>

Values with different superscript along a row are significantly different at p<0.05

Table 5.5 shows the trends in *Escherichia coli* incidences in the raw unprocessed samples in the three packaging materials over the six-day accelerated shelf-life period. The *Escherichia coli* was detected across the raw unprocessed samples with sorghum and baobab having decreased counts across the storage period for samples stored in kraft and flexible packages with ranges of log 0.26 cfu and log 1.87 cfu, and between log 0.24 cfu and log 0.94 cfu respectively while in poly/PE-coated package, *E. coli* increased during storage to between log 0.06 and log 1.96 cfu, and log 0.04 cfu and log 0.93 cfu respectively. Raw sesame recorded increase in *E. coli* counts across the 6-day accelerated storage period and the three packages with ranges of between log 0.04 cfu and log 1.98 cfu.

**Table 5. 5: *Escherichia coli* presence in the raw unprocessed material (log cfu g<sup>-1</sup>)**

Package material	Sample	Storage period (Days)						
		0	1	2	3	4	5	6
<i>Escherichia coli</i>								
Kraft package	Sorghum	1.15±0.01 <sup>c</sup>	1.09±0.02 <sup>c</sup>	0.98±0.04 <sup>b</sup>	0.26±0.01 <sup>a</sup>	0.17±0.01 <sup>a</sup>	1.87±0.01 <sup>c</sup>	1.37±0.01 <sup>c</sup>
	Sesame	0.37±0.05 <sup>a</sup>	0.15±0.02 <sup>a</sup>	1.98±0.01 <sup>c</sup>	1.73±0.01 <sup>c</sup>	1.42±0.01 <sup>c</sup>	1.06±0.04 <sup>b</sup>	0.78±0.25 <sup>b</sup>
	Baobab	0.94±0.06 <sup>b</sup>	0.84±0.07 <sup>b</sup>	0.81±0.02 <sup>a</sup>	0.66±0.03 <sup>b</sup>	0.68±0.06 <sup>b</sup>	0.41±0.04 <sup>a</sup>	0.24±0.02 <sup>a</sup>
Poly/PE-coated package	Sorghum	1.09±0.02 <sup>c</sup>	1.21±0.04 <sup>c</sup>	0.91±0.04 <sup>b</sup>	0.26±0.01 <sup>a</sup>	0.06±0.02 <sup>a</sup>	1.96±0.01 <sup>c</sup>	1.49±0.01 <sup>c</sup>
	Sesame	0.31±0.03 <sup>a</sup>	0.04±0.01 <sup>a</sup>	1.79±0.01 <sup>c</sup>	1.61±0.01 <sup>c</sup>	1.20±0.03 <sup>c</sup>	0.76±0.05 <sup>b</sup>	0.31±0.04 <sup>a</sup>
	Baobab	0.65±0.02 <sup>b</sup>	0.66±0.03 <sup>b</sup>	0.04±0.01 <sup>a</sup>	0.93±0.01 <sup>b</sup>	0.75±0.02 <sup>b</sup>	0.36±0.03 <sup>a</sup>	1.08±0.18 <sup>b</sup>
Flexible package	Sorghum	1.38±0.01 <sup>c</sup>	0.67±0.01 <sup>a</sup>	0.53±0.01 <sup>c</sup>	1.74±0.01 <sup>c</sup>	1.38±0.25 <sup>b</sup>	1.53±0.02 <sup>c</sup>	1.28±0.01 <sup>c</sup>
	Sesame	0.32±0.01 <sup>a</sup>	1.84±0.01 <sup>b</sup>	1.71±0.02 <sup>b</sup>	1.05±0.02 <sup>b</sup>	0.88±0.07 <sup>a</sup>	0.95±0.04 <sup>b</sup>	0.12±0.01 <sup>a</sup>
	Baobab	0.86±0.04 <sup>b</sup>	0.66±0.02 <sup>a</sup>	0.63±0.01 <sup>a</sup>	0.91±0.03 <sup>a</sup>	0.65±0.01 <sup>a</sup>	0.63±0.02 <sup>a</sup>	0.45±0.02 <sup>b</sup>

Values with different superscript along a row are significantly different at p<0.05

Table 5.6 presents the *Salmonella* counts across the six-day accelerated shelf-life period. The *Salmonella* counts in raw sorghum and baobab fruit pulp powder across the three-package material decreased during the accelerated storage period ranging between log 0.13 cfu and log 1.87 cfu for sorghum, and log 0.08 cfu and log 1.28 cfu for baobab respectively. Raw sesame recorded decreasing counts ranging between log 0.21-0.86 cfu and log 0.46-1.75 cfu for kraft and flexible packages respectively. However, *Salmonella* counts increased for raw sesame stored in poly/PE-coated package between log 0.09 cfu and log 1.91 cfu.

**Table 5. 6: *Salmonella* spp. presence in the raw unprocessed material (log cfu g<sup>-1</sup>)**

Package material	Sample	Storage period (Days)						
		0	1	2	3	4	5	6
<b><i>Salmonella</i> spp.</b>								
Kraft package	Sorghum	1.33±0.01 <sup>c</sup>	1.31±0.01 <sup>c</sup>	0.78±0.01 <sup>c</sup>	1.75±0.02 <sup>b</sup>	1.54±0.21 <sup>b</sup>	1.11±0.03 <sup>b</sup>	0.13±0.01 <sup>b</sup>
	Sesame	0.57±0.02 <sup>a</sup>	0.34±0.02 <sup>a</sup>	0.18±0.03 <sup>a</sup>	1.86±0.02 <sup>c</sup>	0.21±0.02 <sup>a</sup>	0.21±0.02 <sup>a</sup>	ND
	Baobab	1.09±0.04 <sup>b</sup>	0.97±0.01 <sup>b</sup>	0.48±0.11 <sup>b</sup>	0.08±0.02 <sup>a</sup>	ND	ND	ND
Poly/PE-coated package	Sorghum	1.38±0.01 <sup>c</sup>	0.99±0.01 <sup>b</sup>	0.13±0.01 <sup>a</sup>	1.47±0.03 <sup>c</sup>	0.97±0.04 <sup>c</sup>	0.19±0.01 <sup>b</sup>	ND
	Sesame	0.16±0.02 <sup>a</sup>	0.09±0.02 <sup>a</sup>	1.91±0.01 <sup>b</sup>	1.34±0.05 <sup>b</sup>	0.26±0.05 <sup>b</sup>	ND	ND
	Baobab	1.16±0.03 <sup>b</sup>	1.18±0.31 <sup>c</sup>	0.23±0.09 <sup>a</sup>	ND	ND	ND	ND
Flexible package	Sorghum	1.11±0.02 <sup>b</sup>	1.87±0.01 <sup>c</sup>	0.76±0.05 <sup>a</sup>	0.22±0.02 <sup>a</sup>	0.97±0.01 <sup>a</sup>	ND	ND
	Sesame	0.46±0.02 <sup>a</sup>	1.74±0.02 <sup>b</sup>	1.15±0.05 <sup>b</sup>	1.25±0.03 <sup>b</sup>	ND	ND	ND
	Baobab	1.28±0.04 <sup>c</sup>	1.06±0.04 <sup>a</sup>	ND	ND	ND	ND	ND

Values with different superscript along a row are significantly different at p<0.05

### 5.6.2 Microbial quality and effect of packaging material on roasted sorghum snack formulations

Table 5.7 shows the trend in total viable count for roasted sorghum snack formulations in three package materials of kraft, poly/PE-coated and flexible package during the accelerated shelf-life period of six days. The snack showed increase in TVC count in kraft package from log 0.28 cfu to log 3.84 cfu. In poly/PE-coated, the trend in TVC was from log 0.21 cfu to log, 3.75 cfu, while snacks stored in flexible showed increase from log 0.21 cfu to log 4.51 cfu. High TVC counts in kraft package material were recorded for formulation RSF3 at log 3.84 cfu/g at day 2 of storage with RSF2 recording low counts at log 2.35 cfu/g at the same period of storage. In poly/PE-coated package, high TVC counts was recorded for formulation RSF1 at log 3.75 cfu/g after two days of storage with formulation RSF3 recording low counts at log 3.43 cfu/g at the same stage of storage. Comparison to flexible package, high TVC count was recorded with formulation RSF1 at log 4.51 cfu/g after three days of storage with RSF4 recording low TVC counts recording with formulation RSF4 at log 2.73 cfu/g at day three of storage.

**Table 5. 7: Effect of packaging material and storage period on Total Viable Count (log cfu g<sup>-1</sup>) of roasted sorghum snack formulations**

Type of packaging	Sample	Storage Period (Days)						
		0	1	2	3	4	5	6
<b>Total viable count</b>								
Kraft package	RSF1	0.94±0.16 <sup>ab</sup>	0.64±0.08 <sup>a</sup>	3.43±0.14 <sup>c</sup>	0.86±0.22 <sup>ab</sup>	0.97±0.21 <sup>a</sup>	2.65±0.04 <sup>a</sup>	2.08±0.06 <sup>ab</sup>
	RSF2	0.55±0.02 <sup>a</sup>	0.65±0.57 <sup>ab</sup>	2.35±0.29 <sup>a</sup>	1.50±0.19 <sup>cd</sup>	1.81±2.55 <sup>ab</sup>	1.10±0.13 <sup>b</sup>	1.82±0.16 <sup>a</sup>
	RSF3	0.28±0.16 <sup>a</sup>	1.05±0.09 <sup>abc</sup>	3.84±0.15 <sup>d</sup>	2.14±0.25 <sup>cde</sup>	2.62±0.09 <sup>a</sup>	2.05±0.13 <sup>ab</sup>	0.83±0.53 <sup>a</sup>
	RSF4	2.57±0.11 <sup>c</sup>	3.15±0.09 <sup>e</sup>	3.78±0.24 <sup>cd</sup>	2.36±0.50 <sup>cde</sup>	2.59±0.05 <sup>abc</sup>	1.97±0.10 <sup>ab</sup>	0.67±0.50 <sup>a</sup>
Poly/PE-coated package	RSF1	0.63±0.10 <sup>a</sup>	0.46±0.05 <sup>a</sup>	3.75±0.14 <sup>d</sup>	2.77±0.02 <sup>d</sup>	2.28±0.05 <sup>cd</sup>	1.66±0.09 <sup>ab</sup>	1.15±0.32 <sup>abc</sup>
	RSF2	0.24±0.11 <sup>a</sup>	0.76±0.19 <sup>ab</sup>	3.44±0.12 <sup>c</sup>	2.93±0.34 <sup>cd</sup>	2.14±2.38 <sup>ab</sup>	2.38±0.25 <sup>d</sup>	1.84±0.09 <sup>ab</sup>
	RSF3	0.21±0.03 <sup>a</sup>	2.85±0.45 <sup>cd</sup>	3.43±0.37 <sup>c</sup>	2.39±0.38 <sup>d</sup>	1.11±0.13 <sup>ab</sup>	2.02±0.08 <sup>abc</sup>	0.64±0.08 <sup>ab</sup>
	RSF4	1.58±0.29 <sup>bc</sup>	3.39±0.09 <sup>de</sup>	3.69±0.21 <sup>c</sup>	1.90±0.04 <sup>ab</sup>	1.81±2.55 <sup>ab</sup>	2.19±0.46 <sup>cd</sup>	0.64±0.26 <sup>ab</sup>
Flexible package	RSF1	1.72±0.05 <sup>bc</sup>	1.61±0.05 <sup>cd</sup>	1.58±0.30 <sup>bc</sup>	4.51±0.16 <sup>g</sup>	3.05±0.30 <sup>e</sup>	2.80±0.04 <sup>ef</sup>	2.31±0.06 <sup>abc</sup>
	RSF2	0.96±0.06 <sup>ab</sup>	1.80±0.03 <sup>d</sup>	1.27±0.07 <sup>b</sup>	3.08±0.13 <sup>d</sup>	2.09±0.03 <sup>bcd</sup>	1.39±0.39 <sup>bc</sup>	2.64±0.05 <sup>abcd</sup>
	RSF3	0.21±0.19 <sup>a</sup>	1.47±0.07 <sup>ab</sup>	3.68±0.08 <sup>f</sup>	3.14±0.18 <sup>e</sup>	1.84±0.55 <sup>bc</sup>	2.47±0.12 <sup>abc</sup>	0.39±0.10 <sup>a</sup>
	RSF4	0.24±0.08 <sup>a</sup>	1.69±0.05 <sup>bc</sup>	2.53±0.11 <sup>de</sup>	2.73±0.05 <sup>abcd</sup>	2.40±0.18 <sup>cd</sup>	2.38±0.31 <sup>d</sup>	1.11±0.26 <sup>abc</sup>

Values with different superscript along a row are significantly different at p<0.05

The yeast and mold count of the roasted sorghum snack bars are as illustrated in Table 5.8 over the six-day accelerated period in the three package materials of kraft, poly/PE-coated and flexible. There were significant differences ( $p < 0.05$ ) in the samples stored among the three packaging material with yeast and molds counting ranging between log 0.20-3.71 cfu/g in kraft package, log 0.16-3.83 cfu/g in poly/PE-coated package, and log 0.22-4.83 cfu/g respectively. In kraft package, high yeast and molds count were recorded by formulation RSF1 on day 2 at log 3.71 cfu/g, and low counts with formulation RSF3 at log 1.83 cfu/g after day two of storage. Roasted formulation RSF2 recorded high yeast and mold counts after day one of storage at log 3.83 cfu/g and low counts in formulation RSF4 at log 2.65 cfu/g in the poly/PE-coated package. However, in flexible package, the sample RSF1 recorded the highest yeast and mold counts after day one of storage among the three packaging materials at log 4.83 cfu/g, with low counts recorded for formulation RSF4 at log 2.58 cfu/g at day four of storage.

**Table 5. 8: Effect of packaging material and storage period on Yeast and Molds (log cfu g<sup>-1</sup>) of roasted sorghum snack formulations**

Type of packaging	Sample	Storage Period (Days)						
		0	1	2	3	4	5	6
		<b>Yeast and Molds</b>						
Kraft package	RSF1	0.43±0.12 <sup>a</sup>	2.38±0.16 <sup>g</sup>	3.71±0.10 <sup>h</sup>	0.73±0.18 <sup>a</sup>	0.20±0.29 <sup>a</sup>	0.95±0.02 <sup>a</sup>	0.85±0.02 <sup>c</sup>
	RSF2	0.41±0.08 <sup>ab</sup>	2.76±0.20 <sup>g</sup>	2.82±0.13 <sup>d</sup>	0.43±0.11 <sup>a</sup>	0.23±0.09 <sup>a</sup>	0.94±0.01 <sup>a</sup>	0.82±0.01 <sup>bc</sup>
	RSF3	0.48±0.15 <sup>ab</sup>	1.03±0.08 <sup>bc</sup>	1.83±0.34 <sup>def</sup>	1.07±0.19 <sup>ab</sup>	0.27±0.27 <sup>a</sup>	0.89±0.01 <sup>a</sup>	0.78±0.02 <sup>bc</sup>
	RSF4	1.06±0.18 <sup>cd</sup>	1.27±0.05 <sup>c</sup>	2.46±0.27 <sup>fg</sup>	0.81±0.17 <sup>a</sup>	0.95±1.35 <sup>ab</sup>	0.86±0.02 <sup>a</sup>	0.72±0.02 <sup>bc</sup>
Poly/PE-coated package	RSF1	0.24±0.25 <sup>a</sup>	1.05±0.06 <sup>bc</sup>	2.91±0.04 <sup>e</sup>	1.11±0.10 <sup>bcd</sup>	1.66±0.09 <sup>ab</sup>	0.51±0.27 <sup>ab</sup>	0.28±0.09 <sup>ab</sup>
	RSF2	0.16±0.06 <sup>a</sup>	3.83±0.10 <sup>j</sup>	1.36±0.07 <sup>c</sup>	1.59±0.02 <sup>cde</sup>	2.14±2.38 <sup>ab</sup>	2.87±0.07 <sup>fg</sup>	2.21±0.12 <sup>cd</sup>
	RSF3	1.02±0.25 <sup>de</sup>	1.87±0.06 <sup>de</sup>	3.07±0.15 <sup>i</sup>	1.47±0.08 <sup>cde</sup>	1.11±0.13 <sup>ab</sup>	0.58±0.18 <sup>ab</sup>	2.89±0.02 <sup>e</sup>
	RSF4	0.88±0.14 <sup>b</sup>	2.22±0.14 <sup>gh</sup>	2.65±0.06 <sup>f</sup>	1.04±0.07 <sup>bc</sup>	1.81±2.55 <sup>ab</sup>	1.82±0.11 <sup>c</sup>	0.79±0.09 <sup>bcd</sup>
Flexible package	RSF1	0.97±0.05 <sup>c</sup>	4.83±0.08 <sup>h</sup>	3.81±0.21 <sup>e</sup>	2.90±0.05 <sup>abc</sup>	0.62±0.40 <sup>a</sup>	1.78±1.69 <sup>a</sup>	2.63±0.35 <sup>bcd</sup>
	RSF2	0.54±0.03 <sup>b</sup>	3.17±0.14 <sup>e</sup>	1.94±0.12 <sup>c</sup>	0.23±0.15 <sup>a</sup>	2.33±0.75 <sup>ab</sup>	2.52±1.32 <sup>a</sup>	1.91±0.45 <sup>bc</sup>
	RSF3	0.83±0.05 <sup>bc</sup>	3.70±0.11 <sup>f</sup>	2.62±0.23 <sup>d</sup>	0.48±0.19 <sup>a</sup>	1.39±1.62 <sup>a</sup>	2.91±0.06 <sup>a</sup>	2.52±0.56 <sup>bcd</sup>
	RSF4	0.22±0.04 <sup>a</sup>	0.77±0.04 <sup>a</sup>	1.65±0.08 <sup>bc</sup>	2.22±0.10 <sup>abc</sup>	2.58±0.43 <sup>a</sup>	1.11±1.39 <sup>a</sup>	2.20±0.03 <sup>bcd</sup>

Values with different superscript along a row are significantly different at  $p < 0.05$

The *Staphylococcus aureus* counts of the roasted sorghum snack formulations was detected on day 0 and day 1 of accelerated storage as indicated in Table 5.9. However, the incidence of Total coliforms, *E. coli* and *Salmonella* spp. during storage among the three-packaging material was not detected during the six days accelerated shelf-life period.

There was a decreasing *s. aureus* counts with no colonies detected after day 2 of storage as shown in Figure 5.4. After day 1 of storage, snacks stored in kraft package had *s. aureus* ranging between log 0.18-0.49 cfu/g, while in poly/PE-coated package ranged between log 0.01-0.31 cfu/g, and log 0.55-0.96 cfu/g for samples stored in flexible packages.

**Table 5. 9: Effect of packaging material and storage period on *S. aureus* presence (log cfu g<sup>-1</sup>) of the roasted sorghum snack formulations**

Type of packaging	Sample	Storage Period (Days)						
		0	1	2	3	4	5	6
<i>Staphylococcus aureus</i>								
Kraft package	RSF1	0.09±0.01 <sup>d</sup>	0.49±0.01 <sup>e</sup>	ND	ND	ND	ND	ND
	RSF2	0.08±0.02 <sup>a</sup>	0.18±0.01 <sup>a</sup>	ND	ND	ND	ND	ND
	RSF3	0.19±0.004 <sup>ab</sup>	0.19±0.01 <sup>ab</sup>	ND	ND	ND	ND	ND
	RSF4	0.26±0.01 <sup>b</sup>	0.21±0.01 <sup>abc</sup>	ND	ND	ND	ND	ND
Poly/PE-coated package	RSF1	0.75±0.03 <sup>e</sup>	0.31±0.01 <sup>a</sup>	ND	ND	ND	ND	ND
	RSF2	0.63±0.02 <sup>cd</sup>	0.06±0.01 <sup>a</sup>	ND	ND	ND	ND	ND
	RSF3	0.68±0.01 <sup>de</sup>	0.09±0.01 <sup>a</sup>	ND	ND	ND	ND	ND
	RSF4	0.78±0.02 <sup>e</sup>	0.01±0.002 <sup>a</sup>	ND	ND	ND	ND	ND
Flexible package	RSF1	0.63±0.02 <sup>bc</sup>	0.55±0.04 <sup>b</sup>	ND	ND	ND	ND	ND
	RSF2	0.57±0.02 <sup>bc</sup>	0.96±0.02 <sup>e</sup>	ND	ND	ND	ND	ND
	RSF3	0.65±0.03 <sup>bc</sup>	0.81±0.01 <sup>cd</sup>	ND	ND	ND	ND	ND
	RSF4	0.74±0.02 <sup>c</sup>	0.69±0.02 <sup>c</sup>	ND	ND	ND	ND	ND

Values with different superscript along a row are significantly different at p<0.05. ND = Not Detected

### 5.6.3 Microbial quality and effect of packaging material on malted sorghum snack formulations

Table 5.10 shows the trends in TVC counts of malted sorghum formulations across the three-package material during the accelerated six-day storage period. Malted snack formulations stored in kraft package ranged between log 0.21-4.67 cfu/g, for poly/PE-coated package, the range was log 0.27-5.64 cfu/g, and Flexible package was between log 0.16-4.94 cfu/g respectively. In Kraft package, the sample MSF1 recorded high TVC count at log 4.67 cfu/g, with MSF4 recording low counts at log 2.71 cfu/g at day two of storage. Comparison to poly/PE-coated package shows high counts were recorded by sample MSF1 at log 5.64 cfu/g with MSF2 recording low counts of log 2.81 cfu/g at day two of storage. However, the Flexible package showed high TVC counts after day three of storage with MSF3 at log 4.94 cfu/g with low counts recorded by MSF1 at log 2.59 at same stage of storage.

**Table 5. 10: Effect of packaging material and storage period on Total Viable Counts of malted sorghum snack formulations (log cfu g<sup>-1</sup>)**

Sample		Storage Period						
		0	1	2	3	4	5	6
		<b>Total viable count</b>						
Kraft package	MSF1	0.21±0.11 <sup>a</sup>	1.33±0.34 <sup>bc</sup>	4.67±0.25 <sup>de</sup>	1.55±0.15 <sup>bc</sup>	0.69±0.33 <sup>a</sup>	2.66±0.10 <sup>a</sup>	2.08±0.13 <sup>ab</sup>
	MSF2	0.51±0.27 <sup>a</sup>	0.76±0.11 <sup>ab</sup>	3.39±0.23 <sup>c</sup>	3.11±0.31 <sup>cd</sup>	2.39±0.09 <sup>cde</sup>	2.75±0.06 <sup>a</sup>	2.28±0.02 <sup>bc</sup>
	MSF3	0.99±0.09 <sup>ab</sup>	0.56±0.34 <sup>a</sup>	3.58±0.08 <sup>d</sup>	3.55±0.06 <sup>g</sup>	0.70±0.16 <sup>a</sup>	2.59±0.03 <sup>abc</sup>	1.99±0.04 <sup>ab</sup>
	MSF4	0.54±0.04 <sup>a</sup>	1.39±0.11 <sup>bc</sup>	2.71±0.07 <sup>a</sup>	2.07±0.22 <sup>cd</sup>	1.27±0.35 <sup>a</sup>	1.73±0.08 <sup>a</sup>	1.21±0.12 <sup>bc</sup>
Poly/PE-coated package	MSF1	4.81±0.12 <sup>d</sup>	4.68±0.27 <sup>f</sup>	5.64±0.13 <sup>e</sup>	2.72±0.38 <sup>d</sup>	1.94±0.12 <sup>ab</sup>	0.86±0.22 <sup>ab</sup>	2.62±0.07 <sup>ef</sup>
	MSF2	0.27±0.23 <sup>a</sup>	0.65±0.18 <sup>ab</sup>	2.81±0.16 <sup>d</sup>	2.72±0.18 <sup>c</sup>	2.43±0.07 <sup>de</sup>	1.47±0.26 <sup>bc</sup>	2.77±0.02 <sup>f</sup>
	MSF3	3.16±0.29 <sup>bc</sup>	4.79±0.26 <sup>fg</sup>	3.70±0.26 <sup>d</sup>	1.78±0.26 <sup>c</sup>	1.85±2.17 <sup>ab</sup>	2.63±0.04 <sup>d</sup>	2.07±0.08 <sup>abc</sup>
	MSF4	0.40±0.14 <sup>a</sup>	1.08±0.11 <sup>ab</sup>	3.19±0.18 <sup>bc</sup>	2.59±0.02 <sup>d</sup>	2.03±0.06 <sup>abc</sup>	2.68±0.07 <sup>d</sup>	2.12±0.11 <sup>bc</sup>
Flexible package	MSF1	0.97±0.21 <sup>a</sup>	0.16±0.19 <sup>a</sup>	1.57±0.07 <sup>b</sup>	2.59±0.05 <sup>abc</sup>	2.15±0.11 <sup>cd</sup>	2.59±0.05 <sup>de</sup>	1.99±0.08 <sup>ab</sup>
	MSF2	3.00±0.20 <sup>ef</sup>	4.18±0.11 <sup>f</sup>	0.24±0.05 <sup>a</sup>	2.02±0.05 <sup>cd</sup>	0.52±0.10 <sup>a</sup>	2.88±0.07 <sup>bcd</sup>	0.23±0.08 <sup>a</sup>
	MSF3	2.88±0.12 <sup>ef</sup>	1.14±0.08 <sup>bc</sup>	1.54±0.08 <sup>b</sup>	4.94±0.08 <sup>f</sup>	2.93±0.14 <sup>d</sup>	0.51±0.27 <sup>ab</sup>	2.63±0.05 <sup>abcd</sup>
	MSF4	2.41±0.40 <sup>de</sup>	1.88±0.06 <sup>cd</sup>	0.25±0.07 <sup>a</sup>	4.42±0.06 <sup>f</sup>	1.00±0.06 <sup>ab</sup>	1.68±0.56 <sup>cd</sup>	1.91±0.88 <sup>a</sup>

Values with different superscript along a row are significantly different at  $p < 0.05$

The yeast and mold count of the malted sorghum snack formulations are illustrated in Table 5.11 over the storage period. Malted snack stored in Kraft packages ranged between log 0.12-3.83 cfu/g over the six-day accelerated shelf-life storage period. In Poly/PE-coated package, the samples yeast and mold counts ranged between log 0.31-3.90 cfu/g, and in Flexible package, the range was between log 0.33-2.93 cfu/g respectively over the accelerated six-day period. High yeast and mold counts was recorded for sample MSF2 at log 3.83 cfu/g in Kraft package, and log 3.90 cfu/g in Poly/PE-coated package at day one of storage. However low counts were noted for sample MSF1 stored in kraft package at log 1.81 cfu/g, while MSF4 stored in Poly/PE-coated package recorded log 2.83 cfu/g at day two of accelerated storage period. Compared to Flexible package, the high counts were recorded at day two for sample MSF3 at log 2.89 cfu/g, and low counts for sample MSF1 at log 1.94 cfu/g at day three of storage.

**Table 5. 11: Effect of packaging material and storage period on Yeast and Mold counts of malted sorghum snack formulations (log cfu g<sup>-1</sup>)**

Type of packaging	Sample	Storage Period						
		0	1	2	3	4	5	6
<b>Yeast and Molds</b>								
Kraft package	MSF1	0.89±0.12 <sup>abc</sup>	0.29±0.17 <sup>a</sup>	1.81±0.22 <sup>f</sup>	1.41±0.16 <sup>ab</sup>	0.29±0.09 <sup>a</sup>	0.95±0.02 <sup>a</sup>	0.73±0.04 <sup>bc</sup>
	MSF2	1.29±0.10 <sup>de</sup>	3.83±0.16 <sup>e</sup>	2.34±0.10 <sup>efg</sup>	0.89±0.09 <sup>ab</sup>	0.99±1.29 <sup>ab</sup>	0.93±0.01 <sup>a</sup>	0.66±0.06 <sup>bc</sup>
	MSF3	0.26±0.11 <sup>a</sup>	2.11±0.03 <sup>fg</sup>	1.16±0.06 <sup>abcd</sup>	1.56±0.50 <sup>ab</sup>	1.54±0.21 <sup>ab</sup>	0.98±0.01 <sup>a</sup>	0.76±0.01 <sup>bc</sup>
	MSF4	0.77±0.06 <sup>abc</sup>	2.07±0.08 <sup>fg</sup>	0.74±0.40 <sup>abc</sup>	1.60±0.29 <sup>ab</sup>	0.12±0.05 <sup>a</sup>	0.98±0.01 <sup>a</sup>	0.69±0.10 <sup>bc</sup>
Poly/PE-coated package	MSF1	1.49±0.10 <sup>cde</sup>	2.95±0.03 <sup>e</sup>	2.03±0.10 <sup>e</sup>	1.42±0.09 <sup>e</sup>	1.94±0.12 <sup>ab</sup>	1.48±0.16 <sup>de</sup>	0.31±0.21 <sup>a</sup>
	MSF2	1.73±0.08 <sup>defg</sup>	3.90±0.07 <sup>g</sup>	2.52±0.09 <sup>fg</sup>	1.57±0.14 <sup>cde</sup>	1.02±0.14 <sup>ab</sup>	1.96±0.16 <sup>e</sup>	0.91±0.29 <sup>b</sup>
	MSF3	1.79±0.09 <sup>defg</sup>	2.70±0.06 <sup>fg</sup>	0.37±0.12 <sup>abc</sup>	0.60±0.23 <sup>ab</sup>	1.85±2.17 <sup>ab</sup>	1.08±0.16 <sup>bc</sup>	2.38±0.03 <sup>d</sup>
	MSF4	1.98±0.02 <sup>efgh</sup>	2.11±0.03 <sup>e</sup>	2.83±0.36 <sup>gh</sup>	1.61±0.08 <sup>de</sup>	0.31±0.21 <sup>a</sup>	0.74±0.40 <sup>abc</sup>	2.87±0.03 <sup>e</sup>
Flexible package	MSF1	0.33±0.05 <sup>a</sup>	0.69±0.04 <sup>a</sup>	1.73±0.05 <sup>de</sup>	1.94±0.10 <sup>abc</sup>	1.62±0.27 <sup>ab</sup>	1.53±1.93 <sup>a</sup>	2.54±0.42 <sup>bcd</sup>
	MSF2	1.28±0.03 <sup>b</sup>	1.31±0.03 <sup>b</sup>	1.56±0.02 <sup>d</sup>	2.79±0.15 <sup>a</sup>	2.60±0.37 <sup>ab</sup>	2.88±0.04 <sup>a</sup>	2.19±0.03 <sup>bcd</sup>
	MSF3	1.67±0.02 <sup>de</sup>	1.95±0.07 <sup>d</sup>	2.89±0.24 <sup>d</sup>	1.60±1.78 <sup>ab</sup>	1.48±0.19 <sup>a</sup>	0.62±0.43 <sup>a</sup>	2.85±0.06 <sup>cd</sup>
	MSF4	1.02±0.25 <sup>a</sup>	1.46±0.02 <sup>bc</sup>	2.16±0.05 <sup>fg</sup>	2.93±0.02 <sup>d</sup>	2.05±0.11 <sup>abc</sup>	3.14±0.08 <sup>a</sup>	1.76±0.13 <sup>b</sup>

Values with different superscript along a row are significantly different at p<0.05



The *Staphylococcus aureus* counts in Kraft package ranged between log 0.63-1.76 cfu/g, log 0.40-1.02 cfu/g in poly/PE-coated package and between log 0.94-1.77 cfu/g for malted snack samples stored in Flexible package material. The incidence of *s. aureus* after day two of accelerated shelf storage was not detected across the three-packaging material as shown in Table 5.12. The incidence of total coliforms was not detected for the malted sorghum snack formulations during the six-day accelerated shelf-life period. In addition, there was zero-incidence level of pathogenic presence across the three packaging materials for the six-day accelerated shelf-life period.

**Table 5. 12: Effect of packaging material and storage period on incidence of *S. aureus* of malted sorghum snack formulations (log cfu g<sup>-1</sup>)**

Type of packaging	Sample	Storage Period						
		0	1	2	3	4	5	6
<b>Staphylococcus aureus</b>								
Kraft package	MSF1	0.70±0.03 <sup>c</sup>	1.58±0.02 <sup>h</sup>	ND	ND	ND	ND	ND
	MSF2	0.56±0.02 <sup>c</sup>	0.63±0.03 <sup>f</sup>	ND	ND	ND	ND	ND
	MSF3	1.01±0.04 <sup>d</sup>	1.49±0.03 <sup>h</sup>	ND	ND	ND	ND	ND
	MSF4	0.55±0.02 <sup>c</sup>	1.76±0.01 <sup>i</sup>	ND	ND	ND	ND	ND
Poly/PE-coated package	MSF1	1.21±0.02 <sup>h</sup>	1.02±0.02 <sup>b</sup>	ND	ND	ND	ND	ND
	MSF2	0.74±0.01 <sup>e</sup>	0.44±0.04 <sup>ab</sup>	ND	ND	ND	ND	ND
	MSF3	0.21±0.02 <sup>a</sup>	0.43±0.01 <sup>a</sup>	ND	ND	ND	ND	ND
	MSF4	0.43±0.01 <sup>b</sup>	0.40±0.003 <sup>a</sup>	ND	ND	ND	ND	ND
Flexible package	MSF1	0.27±0.01 <sup>a</sup>	1.77±0.03 <sup>f</sup>	ND	ND	ND	ND	ND
	MSF2	0.36±0.01 <sup>a</sup>	0.98±0.01 <sup>e</sup>	ND	ND	ND	ND	ND
	MSF3	0.65±0.04 <sup>bc</sup>	0.98±0.03 <sup>e</sup>	ND	ND	ND	ND	ND
	MSF4	0.39±0.01 <sup>a</sup>	0.94±0.01 <sup>de</sup>	ND	ND	ND	ND	ND

Values with different superscript along a row are significantly different at p<0.05

### 5.6.4 Microbial quality and effect of packaging material on fermented sorghum snack formulations

The trends in total viable count of the fermented sorghum snack formulations are as presented in Table 5.13. There were significant differences ( $p < 0.05$ ) in the TVC counts with mean counts ranging between log 0.18-3.81 cfu/g in samples stored in Kraft package, log 0.21-5.69 for samples stored in poly/PE-coated package and log 0.51-4.50 cfu/g for fermented sorghum snack samples packaged in the Flexible packaging material. High TVC count was recorded for sample FSF2 stored in poly/PE-coated package after day one of storage at log 5.69 cfu/g, compared to high counts in sample FSF3 for sample stored in flexible package after day two of storage at log 4.50 cfu/g and sample FSF1 stored in Kraft package recording log 3.81 cfu/g. Low TVC counts was recorded for sample FSF4 at day two of storage at log 2.94 cfu/g compared to low counts at day two of storage in kraft package for sample FSF2 at log 3.49 cfu/g and poly/PE-coated package for sample FSF4 at log 4.33 cfu/g.

**Table 5. 13: Effect of packaging material and storage period on Total Viable Counts (log cfu g<sup>-1</sup>) of fermented sorghum snack formulations**

Type of packaging	Sample	Storage Period						
		0	1	2	3	4	5	6
		<b>Total viable count</b>						
Kraft package	FSF1	1.16±0.14 <sup>b</sup>	1.63±0.16 <sup>cd</sup>	3.81±0.12 <sup>cd</sup>	2.55±0.26 <sup>def</sup>	0.87±0.59 <sup>a</sup>	2.66±0.10 <sup>a</sup>	2.08±0.13 <sup>ab</sup>
	FSF2	0.88±0.18 <sup>ab</sup>	2.12±0.04 <sup>d</sup>	3.49±0.12 <sup>fg</sup>	1.12±0.22 <sup>ab</sup>	1.55±0.25 <sup>ab</sup>	2.75±0.06 <sup>a</sup>	2.28±0.02 <sup>bc</sup>
	FSF3	1.36±0.16 <sup>b</sup>	0.46±0.08 <sup>a</sup>	3.59±0.12 <sup>c</sup>	3.09±0.44 <sup>efg</sup>	0.38±0.30 <sup>a</sup>	2.59±0.03 <sup>abc</sup>	1.99±0.04 <sup>ab</sup>
	FSF4	0.18±0.17 <sup>a</sup>	1.66±0.15 <sup>cd</sup>	3.55±0.54 <sup>d</sup>	1.90±0.31 <sup>b</sup>	1.43±0.32 <sup>a</sup>	1.73±0.08 <sup>a</sup>	1.21±0.12 <sup>bc</sup>
Poly/PE-coated package	FSF1	0.21±0.24 <sup>a</sup>	5.48±0.18 <sup>g</sup>	3.38±0.08 <sup>a</sup>	3.99±0.20 <sup>e</sup>	2.07±0.15 <sup>ab</sup>	0.86±0.22 <sup>ab</sup>	2.62±0.07 <sup>ef</sup>
	FSF2	1.60±0.44 <sup>c</sup>	5.69±0.17 <sup>e</sup>	3.72±0.19 <sup>e</sup>	2.81±0.22 <sup>c</sup>	2.76±0.50 <sup>ab</sup>	1.47±0.26 <sup>bc</sup>	2.77±0.02 <sup>f</sup>
	FSF3	2.67±0.10 <sup>b</sup>	1.23±0.08 <sup>b</sup>	4.81±0.12 <sup>e</sup>	4.58±0.13 <sup>ef</sup>	0.72±0.56 <sup>a</sup>	2.63±0.04 <sup>d</sup>	2.07±0.08 <sup>abc</sup>
	FSF4	0.68±0.09 <sup>a</sup>	2.30±0.11 <sup>c</sup>	4.33±0.10 <sup>ef</sup>	1.78±0.52 <sup>b</sup>	1.22±0.42 <sup>ab</sup>	2.68±0.07 <sup>d</sup>	2.12±0.11 <sup>bc</sup>
Flexible package	FSF1	2.00±0.02 <sup>c</sup>	2.76±0.07 <sup>e</sup>	3.21±0.21 <sup>f</sup>	1.55±0.15 <sup>bc</sup>	0.38±0.30 <sup>a</sup>	2.59±0.05 <sup>de</sup>	1.99±0.08 <sup>ab</sup>
	FSF2	0.89±0.05 <sup>a</sup>	2.77±0.03 <sup>e</sup>	3.60±0.04 <sup>f</sup>	2.48±0.03 <sup>e</sup>	2.81±0.07 <sup>ef</sup>	2.88±0.07 <sup>bcd</sup>	0.23±0.08 <sup>a</sup>
	FSF3	2.11±0.03 <sup>cd</sup>	2.66±0.05 <sup>e</sup>	4.50±0.06 <sup>g</sup>	3.14±0.18 <sup>e</sup>	2.10±0.18 <sup>cd</sup>	0.51±0.27 <sup>ab</sup>	2.63±0.05 <sup>abcd</sup>
	FSF4	0.94±0.04 <sup>a</sup>	0.78±0.08 <sup>b</sup>	2.28±0.03 <sup>cd</sup>	2.94±0.52 <sup>d</sup>	1.08±0.03 <sup>ab</sup>	1.68±0.56 <sup>cd</sup>	1.91±0.88 <sup>a</sup>

Values with different superscript along a row are significantly different at  $p < 0.05$

The Yeast and Mold counts are as presented in Table 5.14 and shows significant differences ( $p < 0.05$ ) across the three-packaging material. The range in Yeast and mold counts for fermented sorghum snack samples stored in Kraft package was log 0.21-3.61 cfu/g, log 0.46-3.49 cfu/g for snack samples packaged in poly/PE-coated material and between log 0.12-3.94 cfu/g for snack samples stored in Flexible packaging material. The trends in Yeast and Mold counts are as presented in Figure 5.9. High counts were recorded for sample FSF1 stored in Flexible packaging material at day two of storage at log 3.94 cfu/g. This was high compared to high counts for sample FSF4 stored in Kraft package at day two at log 3.61 cfu/g and sample FSF4 stored in poly/PE-coated package at day two of storage at log 3.49 cfu/g.

**Table 5. 14: Effect of packaging material and storage period on Yeast and Mold counts (log cfu g<sup>-1</sup>) of fermented sorghum snack formulations**

Type of packaging	Sample	Storage Period						
		0	1	2	3	4	5	6
<b>Yeast and Molds</b>								
Kraft package	FSF1	0.85±0.15 <sup>bcd</sup>	0.78±0.07 <sup>abc</sup>	0.32±0.24 <sup>a</sup>	1.70±0.16 <sup>ab</sup>	0.35±0.08 <sup>a</sup>	0.95±0.02 <sup>a</sup>	0.73±0.04 <sup>bc</sup>
	FSF2	0.26±0.05 <sup>a</sup>	1.06±0.17 <sup>c</sup>	0.38±0.15 <sup>a</sup>	1.72±0.06 <sup>ef</sup>	1.66±0.10 <sup>ab</sup>	0.93±0.01 <sup>a</sup>	0.66±0.06 <sup>bc</sup>
	FSF3	0.21±0.11 <sup>a</sup>	0.98±0.08 <sup>bc</sup>	1.40±0.12 <sup>bcd</sup>	0.32±0.06 <sup>a</sup>	0.64±0.14 <sup>a</sup>	0.98±0.01 <sup>a</sup>	0.76±0.01 <sup>bc</sup>
	FSF4	0.51±0.13 <sup>ab</sup>	0.89±0.09 <sup>abc</sup>	3.61±0.03 <sup>i</sup>	2.28±0.08 <sup>bc</sup>	0.38±0.12 <sup>a</sup>	0.98±0.01 <sup>a</sup>	0.69±0.10 <sup>bc</sup>
Poly/PE-coated package	FSF1	1.68±0.23 <sup>def</sup>	2.19±0.07 <sup>e</sup>	3.26±0.09 <sup>hi</sup>	1.07±0.13 <sup>bcd</sup>	2.07±0.15 <sup>ab</sup>	1.48±0.16 <sup>de</sup>	0.31±0.21 <sup>a</sup>
	FSF2	1.39±0.12 <sup>cd</sup>	3.39±0.21 <sup>f</sup>	2.21±0.12 <sup>f</sup>	0.83±0.09 <sup>b</sup>	2.76±0.50 <sup>ab</sup>	1.96±0.16 <sup>e</sup>	0.91±0.29 <sup>b</sup>
	FSF3	0.46±0.07 <sup>abcd</sup>	1.38±0.11 <sup>c</sup>	2.97±0.03 <sup>fg</sup>	1.62±0.34 <sup>de</sup>	0.72±0.56 <sup>a</sup>	1.08±0.16 <sup>bc</sup>	2.38±0.03 <sup>d</sup>
	FSF4	2.17±0.08 <sup>fgh</sup>	1.08±0.03 <sup>bc</sup>	3.49±0.12 <sup>i</sup>	1.87±0.13 <sup>e</sup>	1.22±0.42 <sup>ab</sup>	0.74±0.40 <sup>abc</sup>	2.87±0.03 <sup>e</sup>
Flexible package	FSF1	1.79±0.09 <sup>cd</sup>	2.26±0.02 <sup>g</sup>	3.94±0.12 <sup>e</sup>	1.69±0.14 <sup>ab</sup>	0.60±0.23 <sup>a</sup>	1.53±1.93 <sup>a</sup>	2.54±0.42 <sup>bcd</sup>
	FSF2	0.77±0.07 <sup>a</sup>	1.95±0.03 <sup>ef</sup>	3.74±0.16 <sup>e</sup>	2.17±0.21 <sup>abc</sup>	0.37±0.35 <sup>a</sup>	2.88±0.04 <sup>a</sup>	2.19±0.03 <sup>bcd</sup>
	FSF3	1.48±0.07 <sup>bc</sup>	2.23±0.02 <sup>fg</sup>	3.84±0.08 <sup>e</sup>	2.41±0.06 <sup>abc</sup>	0.89±0.28 <sup>a</sup>	0.62±0.43 <sup>a</sup>	2.85±0.06 <sup>cd</sup>
	FSF4	0.12±0.07 <sup>a</sup>	1.26±0.02 <sup>b</sup>	3.22±0.07 <sup>e</sup>	1.93±0.05 <sup>abc</sup>	1.57±1.61 <sup>ab</sup>	3.14±0.08 <sup>a</sup>	1.76±0.13 <sup>b</sup>

Values with different superscript along a row are significantly different at  $p < 0.05$

The *staphylococcus aureus* counts were detected in all formulations across the three-packaging material up to the first day of accelerated shelf-life period. Table 5.15 shows the trends in *s. aureus* growth with no colonies detected from day two through day six of accelerated shelf-life storage. The range in colony counts for fermented sorghum snack samples packaged in kraft material was log 0.31-0.35 cfu/g, for poly/PE-coated package was log 0.16-0.23 cfu/g and in Flexible package between log 0.39-0.46 cfu/g respectively.

The incidence of pathogenic presence of total coliforms, *E. coli* and *Salmonella* were not detected in the fermented sorghum snack formulations during the six-day accelerated shelf-life period and in the three-packaging material of Kraft, Poly/PE-coated and Flexible material.

**Table 5. 15: Effect of packaging material and storage period on *S. aureus* counts (log cfu g<sup>-1</sup>) of fermented sorghum snack formulations**

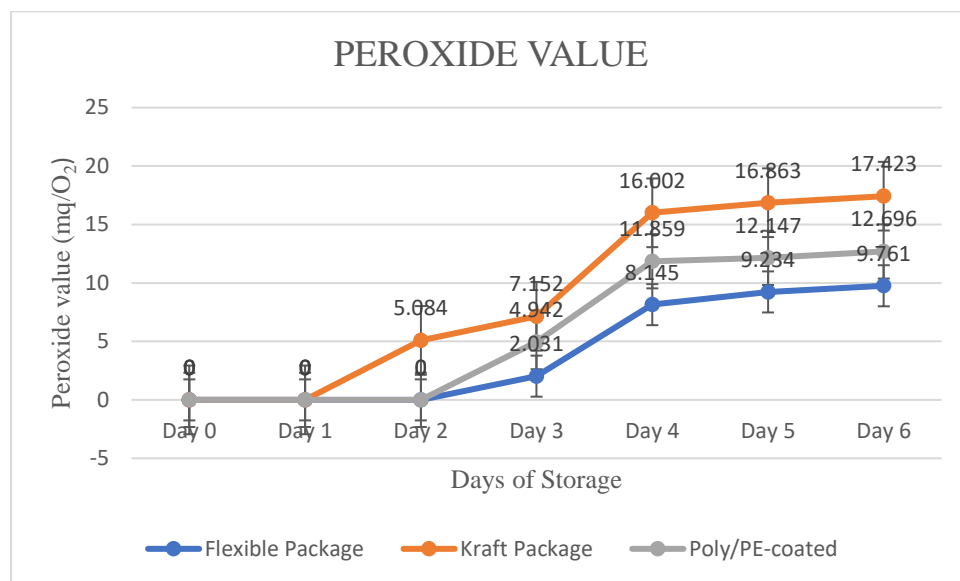
Type of packaging	Sample	Storage Period						
		0	1	2	3	4	5	6
<b>Staphylococcus aureus</b>								
Kraft package	FSF1	1.01±0.01 <sup>d</sup>	0.35±0.003 <sup>cd</sup>	ND	ND	ND	ND	ND
	FSF2	0.69±0.01 <sup>c</sup>	0.36±0.003 <sup>de</sup>	ND	ND	ND	ND	ND
	FSF3	0.21±0.08 <sup>ab</sup>	0.31±0.01 <sup>abcd</sup>	ND	ND	ND	ND	ND
	FSF4	1.85±0.11 <sup>f</sup>	0.33±0.01 <sup>bcd</sup>	ND	ND	ND	ND	ND
Poly/PE-coated package	FSF1	0.92±0.01 <sup>f</sup>	0.23±0.004 <sup>a</sup>	ND	ND	ND	ND	ND
	FSF2	0.57±0.01 <sup>c</sup>	0.16±0.01 <sup>a</sup>	ND	ND	ND	ND	ND
	FSF3	0.53±0.02 <sup>bc</sup>	0.17±0.01 <sup>a</sup>	ND	ND	ND	ND	ND
	FSF4	0.56±0.02 <sup>c</sup>	0.20±0.04 <sup>a</sup>	ND	ND	ND	ND	ND
Flexible package	FSF1	0.66±0.01 <sup>bc</sup>	0.46±0.04 <sup>ab</sup>	ND	ND	ND	ND	ND
	FSF2	0.56±0.03 <sup>bc</sup>	0.40±0.005 <sup>a</sup>	ND	ND	ND	ND	ND
	FSF3	0.38±0.05 <sup>a</sup>	0.42±0.001 <sup>ab</sup>	ND	ND	ND	ND	ND
	FSF4	0.58±0.02 <sup>bc</sup>	0.39±0.003 <sup>a</sup>	ND	ND	ND	ND	ND

Values with different superscript along a row are significantly different at p<0.05

### 5.6.5 Changes in Peroxide value and FFA of sorghum snack bars during storage

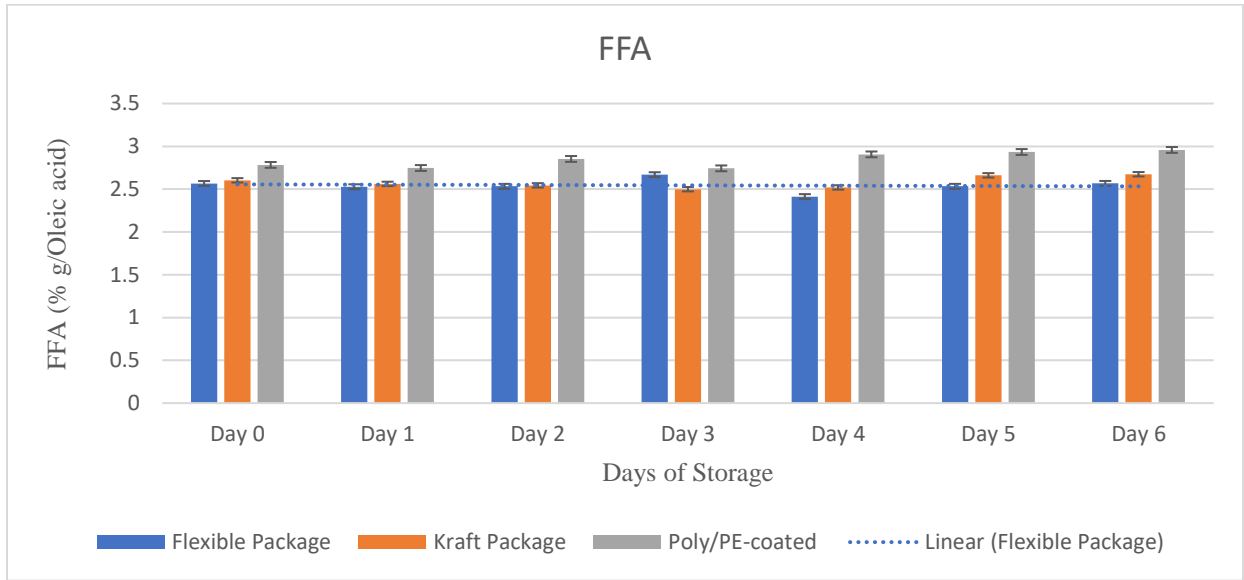
The peroxide value of the samples was significantly influenced by type of package ( $p < .001$ ) and days of storage were significant at  $p < .001$  (Appendix 3). The FFA content in the formulations was affected significantly by the type of package ( $p < .001$ ). However, the FFA content was not significantly different ( $p = 0.99$ ) affected by days of storage, thus the interactive effect between packaging material and days of storage did not significantly affect the FFA content ( $p = 0.77$ ) (Appendix 3).

Figure 5.2 shows mean peroxide values for samples stored in Kraft, poly/PE-coated and Flexible packages. Figure 5.2 indicates that snack bars stored in kraft package underwent oxidation detected at Day two of storage. This when compared to samples in poly/PE-coated and Flexible packages shows oxidation was detected at day three of accelerated shelf-life storage.



**Figure 5. 2: Influence of packaging material and storage temperatures on peroxide values of the RTE sorghum snack bars**

Figure 5.3 shows the mean trend in FFA acid content (% g/Oleic acid) in the samples among the packaging material during the six-day accelerated shelf-life storage. The samples stored in Poly/PE-coated package showed slightly higher FFA over the six-day period in comparison to those in Kraft and Flexible package respectively.



**Figure 5. 3: The incidence FFA presence in the RTE sorghum snack bars during the storage**

## 5.7 DISCUSSION

### 5.7.1 Effect of storage period and packaging materials on the microbial quality of the raw unprocessed samples and sorghum-based snack bars

The Total aerobic count, and Yeast and molds were able to grow in all the snack formulations during the storage period. The package had significant differences ( $p < 0.05$ ) in total aerobic count which was detected in all the samples on day 0 in the three package materials. These findings are contrary to those of Martins *et al.* (2020) who found no TVC growth at day 0 for a pearl millet sourdough bread stored with two packages of low density polythene and aluminum foil paper.

The TVC counts in the packaging systems of Kraft and poly/PE-coated the TVC range were within the range up to the 2<sup>nd</sup> day, and the 3<sup>rd</sup> day in Flexible package of accelerated storage which is against the recommended Kenyan standard for Total aerobic count is  $10^5$  (KS EAS 95: 2018). This may be due to the kraft and poly/PE-coated package being more permeable to air and moisture which are essential in the growth and survival of microorganisms. The low average counts of snack bars stored in flexible package is due to its structural multilayer build which provides an effective barrier to moisture and oxygen necessary to accelerate microbial growth (Morris, 2017b).

Studies have shown that baked goods have a low water activity, which inhibits the growth of yeast and molds (Johnson, 2009), and thus the necessity to maintain such levels during storage. The baseline analysis indicated presence of yeast and mold spores across all formulations in the three-packaging material. This indicated possible contamination during the processing steps which inadvertently introduced yeast and mold spores to the snack bars.

On average, the poly/PE-coated and kraft package material exhibited higher average yeast and mold counts at day 2 of storage with the flexible package having average high counts at day 3 of storage. The flexible package is shown to be an effective barrier oxygen and moisture (Morris, 2017b) which retards most yeast and mold growth. The low counts in the first three days of storage in the snacks is attributed to baking temperature which are known to destroy most microorganisms present (Galić *et al.*, 2009).

### **5.7.2 Processing conditions and the effect of storage conditions and package material on the pathogenic load of raw unprocessed samples and formulations**

Low moisture foods more so RTE cereal based snacks have less susceptible to microbial spoilage due to their low water activity (Makinde *et al.*, 2020), however hygienic controls should be ensured during production and processing steps of raw ingredients to ensure safety prior to consumption (Sánchez-Maldonado *et al.*, 2018). However, improper processing and storage conditions some pathogens may persist with *Salmonella* spp, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium* spp, have been implicated in food outbreaks in recent years (Syamaladevi *et al.*, 2016; Sánchez-Maldonado *et al.*, 2018).

The incidence of pathogenic *S. aureus*, total coliforms, *E. coli* and *Salmonella* were detected in the raw unprocessed sorghum, sesame and baobab but absent in the RTE snack bars which are as the recommended Kenyan standards (KS 2455: 2013). Mean counts were absent in the RTE snack bar due to hygienic processing conditions and exposing the snacks to temperatures of 130°C for 30 minutes. This was exemplified during storage period where the package type did not result in growth of pathogens in the RTE snacks thereby fit for consumption. Similar results were reported by (Singh *et al.*, 2020) who detected no incidence of pathogens in a popped pearl millet breakfast cereal.

The *S. aureus* was detected in the formulations up to the first day of storage, and this could be attributed to its ubiquitous nature in the air, soil, water, sewage, plant surfaces, inclusive of the food handler who is the major culprit in contamination of food (Ebert, 2018). The results showed the *s. aureus* levels of the snacks remained within the allowable safe limits of  $\log 2 \text{ cfu g}^{-1}$  ( $10^2$ ) (KS EAS 95:2017). High presence of *S. aureus* at levels of  $10^5$ - $10^6$  cfu/g (Shovon Al-Fuad, 2018) will produce enterotoxin associated with incidence of food poisoning cases. For baked goods, it involves lots of handling by the food handler in various operations, which presents the opportunity for pathogenic microbial contamination.



### 5.7.3 Changes in Peroxide value and FFA of sorghum snack bars during storage

Package material serves as an effective barrier to oxygen and moisture that accelerate formation of radicals in oil-containing foods. The present study reported influence of packaging material and storage conditions. Similar results were reported by Padmashree *et al.* (2012) who developed a cereal bar from wheat, corn and barley and stored in three package material of poly propylene, paper aluminum foil polyethylene and metallized polyester at temperatures of 37°C.

It was observed the onset of rancidity set in in snack samples stored in kraft paper package at day 2 compared to samples in poly/PE-coated and flexible package that went rancid at day 3 of storage. The Codex allowable limits for PV are up to 10 meq O<sub>2</sub>/kg (FAO, 1999), which was recorded for samples at the 6<sup>th</sup> day of accelerated storage in both kraft and poly/PE-coated packages. Similar studies by Yadav and Bhatnagar. (2016) reported increase in PV for a cereal lunch bar packaged in High Density Polyethylene (HDPE) packaging at temperatures of 23-44°C for 90 days. Dindu *et al.* (2018) reported increase in peroxide values for sorghum cookies stored for 45 days in poly propylene pouches at 38 °C at 14 meq O<sub>2</sub>/kg.

The snack bars were supplemented with sesame seeds which have been shown to contain up to 40% oil (Hegde, 2012). While this implies the susceptibility to peroxidation, studies have shown that sesame seed oil is resistant to rancidity, attributed to presence of tocopherol and lignan contents (Wan *et al.*, 2015). Studies have shown that roasting sesame seeds induces the process of peroxidation owing to breakdown of secondary products thus ensuring the storage stability is interfered with (Rizki *et al.*, 2016). During storage, the rate of auto-oxidation is influenced by the retention of oxygen, which translates to the headspace and the ability of the package material to permeate oxygen (Dindu *et al.*, 2018). Morris, (2017) reports that flexible packages are multilayered which forms an effective barrier to oxygen and light that accelerates the process of autoxidation. This could be attributed to the slow rate of samples stored in flexible packages where autoxidation was detected at day 3 of storage compared to kraft and poly/PE-coated packages where peroxidation was detected at day 2.

The FFA content in the formulations was affected significantly by the type of package ( $p < .001$ ), however the FFA content remained within range in the three packaging systems. This demonstrates that while the FFA content was influenced by the package type, there was no marked difference during the storage period. These results agree with Yadav and Bhatnagar. (2016) who reported no

significant change in FFA content for cereal-based bar packaged in High Density Polyethylene (HDPE) packaging at temperatures of 23-44°C for 90 days.

## **5.8 CONCLUSION**

It is evident that a lunch bar made using sorghum flour, sesame seeds and baobab fruit pulp powder has a shelf life of 3 months. The packaging materials and the storage period had significant differences in the microbiological and oxidative stability of the stored snacks. The flexible package was found suitable over the kraft and poly/PE-coated package(s), for packing the snack bars which retained their stability and acceptability.

## **CHAPTER SIX: GENERAL CONCLUSIONS AND RECOMMENDATIONS**

### **6.1 GENERAL CONCLUSIONS**

The processing mode has an influence on the nutritional and anti-nutritional content of the sorghum snack bar. Incorporation of sesame and baobab fruit pulp was found acceptable at 60:25:15 and there was improved protein, fat, fiber and calcium content of the snack bars. The study thus showed that use of traditionally neglected crops utilized for modern convenient snack products could address issues such as protein energy malnutrition and low nutrient density associated with highly processed snack foods.

The phytochemicals presence in the snack bars was influenced by processing mode and supplementation levels of sesame and baobab fruit pulp powder. The study found the phenolic content remained at similar levels for the three treatments of roasting, malting and fermentation. In addition, the processing modes generally lowered the level of antinutrients present while maintaining the phenolic content. The study showed that the overall tannic acid content reduced with roasting mode than in malting and fermentation treatment. The antinutrient phytic acid was showed to reduce better with a treatment combination of steeping and roasting across the snack bars. The study concludes that sesame and baobab supplementation at 25% and 15% of sesame and baobab fruit pulp powder had net improvement in Total Phenolic content retention and reduction in the antinutrients of tannins and phytic acid content.

The shelf life of the snack bars was shown to be acceptable for up to three months when stored in flexible packaging material as compared to storage in kraft and poly/PE-coated package(s). The study found that the flexible packaging material was suited in limiting the total viable counts, yeast and molds and growth of pathogenic microorganisms in addition to maintaining the oxidative rancidity stability. Thus, the sorghum-based snack bars can be commercialized for up to three months whilst maintaining their eating quality and safety for human consumption.

## **6.2 GENERAL RECOMMENDATIONS**

Traditional cereal and orphan crops should be integrated more into innovative food products that reflect current dietary patterns which will benefit the small-scale farmers, small business owners involved in food processing value chain. The small scale and medium scale processors should be trained on control measures during processing and equipped well to enable them process quality end products. Furthermore, this study shows the potential of orphan and underutilized crops have in value added products, thus future government policy should be directed towards them in enhancing their growth, processing and consumer awareness.

Future research should be directed towards traditional modes of malting, fermentation and roasting and their effects on phytochemicals and rheological properties of sorghum dough to enable further utilization.

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**APPENDIX 1**

**Sensory evaluation score sheet for a Sorghum lunch bar**

Date of evaluation.....

Name of evaluator.....

Please evaluate the food samples provided and indicate the degree of liking for color, taste, crunchiness, aroma and overall acceptability. Use numerical scores from the scoring card provided below. Enter your score under the sample in the scoring sheet. Please **DO NOT** communicate or consult with anyone while scoring.

5-Point Hedonic Scale score

QUALITY	SCORE
Dislike extremely	1
Dislike slightly	2
Neither like nor dislike	3
Like slightly	4
Like extremely	5

**Scoring card**

Sample code	RSF 1	RSF 2	RSF 3	RSF 4	MS F1	MS F2	MS F3	MS F4	FSF 1	FSF 2	FSF 3	FSF 4
Color												

Taste												
Crunchiness												
Aroma												
Overall acceptability												

## APPENDIX 2

Recommended Dietary Allowances and Adequate Intakes, Elements. Source: (Institute of Medicine, 2006)

Life-stage Group	Iron (mg/d)	Calcium (mg/d)	Zinc (mg/day)
<b>Males</b>			
14-18 y	11	1300	11
19-30y	8	1000	11
31-50y	8	1000	11
51-70y	8	1000	11
>70 y	8	1200	11
<b>Females</b>			
14-18 y	15	1300	9
19-30y	18	1300	8
31-50y	18	1000	8
51-70y	8	1200	8
>70 y	8	1200	8

### APPENDIX 3

The table indicates the peroxide value and FFA content of the snack formulations as affected by type of package and storage period.

#### Effect of packaging material and storage period on oxidative stability of Sorghum snack bars

FACTOR	PEROXIDE VALUE (mq eq/O <sub>2</sub> )		P	FREE FATTY ACIDS (% g/OLEIC ACID)		
	DF	MS		DF	MS	P
SAMPLE	11	5.182		11	2.734	
DAYS OF STORAGE	4	1824.174	* <.001	4	0.0228	0.988
PACKAGING MATERIAL	2	400.732	* <.001	2	2.7606	<.001*
PACKAGE MATERIAL	*		<.001			
STORAGE DAYS	8	83.774	* <.001	8	0.169	0.772

DF = Degree of freedom, MS = Mean of squares, P = P value. \*P<0.05. P values with an asterisk are significant at p<0.05