PRODUCT AND PROCESS DEVELOPMENT OF READY TO EAT SORGHUM-GREEN GRAM COOKIES ENRICHED WITH MANGO POWDER.

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DECLARATION

This thesis is my original work and has not been presented for an award in any other university.

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PLAGIARISM DECLARATION

PLAGIARISM DECLARATION FORM

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LIST OF ACRONYMS

ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
ASAL	Arid and Semi-Arid Lands
CLF	Cereal – Legume – Fruit
FAO	Food and Agriculture Organization
HIV	Human Immunodeficiency Virus
ISO	International Organization for Standardization
IVPD	Invitro Protein Digestibility
LSD	Least Significant Difference
NAOH	Sodium Hydroxide
NCDS	Non-Communicable Diseases
PEM	Protein Energy Malnutrition
PV	Peroxide Value
RDA	Recommended Dietary Allowance
RTE	Ready to Eat
TVC	Total Viable Count
WHO	World Health Organization

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GENERAL ABSTRACT

The triple burden of malnutrition is still a major challenge in most developing countries including Kenya. Currently, the increased dependence on convenience foods has contributed an increased consumption of energy-dense and nutrient poor snacks which have been associated with rising trend in non-communicable diseases. The utilization of healthier options for these products such as cereals and legumes can help improve the convenience food trend as well as address malnutrition problems in Kenya. Despite sorghum being a powerhouse of nutrition, it is still one of the highly underutilized cereals in Kenya with most of the sorghum products utilization being limited only at the household level. The basis of this research was therefore to develop and optimize the processing of Ready to Eat (RTE) sorghum-based products to address the underutilization of cereals and legumes through value addition and commercialization. The study employed an experimental study design with factorial arrangements of 3 treatments (whole grain, malted, fermented) sorghum at different levels of substitution with green gram flour supplemented with dried mango powder. Further, the study sought to determine the nutritional properties, consumer acceptability and shelf-storability of the developed cookies. The study also sought to determine the effects of malting and fermentation as well as addition of green gram flour to the properties analyzed. Malting, fermentation and addition of green gram flour significantly improved ($p < 0.05$) the protein contents of the cookies. Ash, protein, fat and fiber contents of the cookies increased significantly ($p<0.05$) with increased substitution with green gram flour while carbohydrates decreased significantly from 61.68 to 54.18%. Cookies from malted sorghum flour had the highest in vitro protein digestibility with values ranging from 90.05% - 86.44%. Vitamin (B1, B2, C) increased significantly ($p \leq 0.05$) with malting and fermentation of sorghum flour. Iron and zinc contents were highest in fermented cookies at 60% (2.69 ± 0.02 mg/100g), and 60%

(4.64 ± 0.08 mg/100g) while Calcium was highest in cookies from malted sorghum flour at 60% (44.48 ± 0.13 mg/100g). Malting and fermentation of sorghum significantly lowered the phytate content by 36.62% and 41.10% and tannin content by 16.16% and 25.36% respectively. Significant differences ($p < 0.05$) were observed in the phenolic contents of the cookies. Highest phenolic contents were observed in whole grain sorghum (60:30:10) - 382.3 mg/100g and fermented sorghum (60:30:10) - 427.2 mg/100g samples. Cookies from malted sorghum flour registered the lowest values of phenolic contents between 338.3 mg/100g to 363.7 mg/100g respectively. Overall acceptability of the cookies was highest in fermented cookies with a score range between 6.8 – 7.5. Most acceptable product was from fermented sorghum flour at 60:30:10 (Sorghum: Green gram: Mango powder) substitution level. Cookies from malted and whole grain sorghum were also above acceptable limits. The formulated products were packed into two different packaging materials - laminated resealable pouches and certified thermopack recyclable plastic containers- then subjected to storage under accelerated conditions (55^0C and 55% RH) where microbial load and peroxide values were monitored daily for 5 days. The microbial load and peroxide value increased significantly ($p<0.05$) in both packaging materials during the storage period however products packed in laminated resealable pouches exhibited better keeping quality. Shelf-life analysis showed a shelf stability of 3 months for the formulated cookies with no visible yeasts and mold spoilage. The study found that optimization of the malting and fermentation processes can be applied with sorghum grain processing for commercialization of baked products with improved nutritional, microbial and sensory attributes in an effort to promote food and nutrition security in Kenya.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND INFORMATION

Food security in a broader aspect addresses issues of food availability, access, utilization as well as stability for a healthy as well as active lifestyle (Bilali et al., 2018). According to Food and Agriculture Organization, food security and nutrition security go hand in hand emphasizing the health component which reflects the nutritional status of an individual (Bilali et al., 2018). Malnutrition is a major health problem globally whose cause has been attributed to inadequate food intake in terms of quantity and quality of food (Thongram et al., 2016). Research has shown the complex nature of food and nutrition security which is illustrated by outcomes of malnutrition such as obesity, underweight and overweight. Being a fundamental element of food security, nutrition security requires more consumption of nutrient-dense foods over energy- dense foods (Bilali et al., 2018)

Sorghum (*Sorghum bicolor L. Moench*) is a cereal crop that has been termed as the fifth most important cereal in the world (Okpala et al., 2013). Globally, it is a staple food that does well in most semi-arid lands. It is rich with nutrients such as carbohydrates, fiber, vitamins, minerals as well as phytochemicals (Rao et al., 2016). Presence of these nutrients play a vital role health and nutrition of human beings. Aside from being a valuable supplementation for nutrition, sorghum is also drought tolerant and can grow during drought periods. In Kenya, majority of the land is classified as arid and semi-arid receiving low rainfall annually. Being a drought tolerant crop, sorghum has the potential to contribute to food security in the country by assisting various households to be food secure (Onono, 2018). This broadly shows the potential for use of sorghum as a food for human consumption especially in development of value-added products (Onono, 2018). Despite being rich in many nutrients, sorghum flour has been reported to be lacking in terms of quality protein (Wulandari et al., 2017).

Legumes have been shown to play an important role in human nutrition most especially in dietary patterns of developing countries (Thongram et al., 2016). According to Okpala and Okoli, 2011, composite flours produced from both legumes and cereals improve nutrition and are superior in quality over those from either cereals or legumes hence the need to combine the sorghum with green grams in this research. Green grams (*Vigna radiata*) are one of the most nutritious legumes that are rich in important nutrients such as proteins, essential amino acids, crude fiber, vitamins, minerals and carbohydrates (Arun et al., 2016). Health benefits due to the slowly digestible carbohydrates in green grams is healthy for diabetic people whereas other health benefits attached to green grams such as regulation of lipid metabolism and others has been explored (Arun et al., 2016). Health promoting and nutritive effects of *Vigna radiata* have been extensively reviewed by Ganesan and Xu, 2018 which shows their potential for use in new food product development.

Cookies are baked products which have over the years become popular due to their ready to eat convenience, extended shelf-life as well as availability (Chavan et al., 2016). Baked products which are vastly consumed due to their long storage ability can be supplemented with legumes as a vehicle for providing them with proteins and essential nutrients (Thongram et al., 2016). According to Chavan et al (2016), cookies are an important snack food that appeal to many people irrespective of their ages. The fact that they are cost-effective snacks that appeal to all ages, they can thus be used as a vehicle for nutrition supplementation. Cookies are mainly made from wheat flour which through extensive research, has been proven to not only be inferior in quality but also low in terms of fiber (Chavan et al., 2016). Wheat has been and is a major burden to most of the African countries contributing to overdependence on foreign countries through spending lots of foreign exchange on wheat imports. This has therefore compelled the need to develop substitutes of wheat from root crops, tuber crops, cereals and legumes that are mainly underutilized in food processing (Okpala et al., 2013). Development of Protein-rich cookies from different sources of proteins is instrumental in curbing protein-energy malnutrition (PEM) ubiquitous in many developing countries (Kiin-Kabari and Giami, 2015).

The concept of nutrient-dense cookies in this research is in a bid to change the perception that comes with most of the snacks. According to Njike et al., (2016) most snacks are unhealthy as they are packed full of energy but very poor in terms of nutrition which is a consensus that should be changed. Consumption of these calorie-dense and energy-dense foods has led increase cases of obesity especially in young adults. Supplementation of sorghum flour with legumes is therefore necessary to increase utilization of sorghum grain as raw materials.

1.2 PROBLEM STATEMENT

Despite the many advantages of sorghum such as agronomic advantages in terms of being drought-tolerant and nutritional advantages (Koima et al., 2022)., there is still underutilization of sorghum in many parts of the country with maize being the main staple food in Kenya. With the high nutritional quality and health benefits as a result of the bioactive compounds that come with sorghum, there is still very limited information on sorghum value-added products within the country. Globally, a lot of research has been underway in increasing utilization in human diet by producing a variety of value-added products however, not much has been done in Kenya. There is limited use of sorghum in value added products especially in baked products. Sorghum is a common cereal crop in Kenya however, it is mainly grown by small scale farmers despite its high production potential. Poor market channels as compared to other cereals like wheat and maize are also major constraints that face most of the sorghum growers. To prevent this, there is need to develop new quality products from sorghum to increase sorghum utilization and productivity within the country.

Similar to sorghum, pulses such as green grams play a momentous role in nutrition due to their abundant nutritional properties are also being underutilized. Green grams and other pulses are mainly consumed as a staple food but not much has been done in terms of value addition. Since attention has not been paid to green gram production due to its underutilization in value added products, a lot of losses occur along the green grams value chain which cause massive losses to farmers in terms of produce and loss of income.

Due to the fast-paced nature of urban life, there has been increased dependence on convenience foods which include the ready to eat snack foods. Cookies being part of the ready to eat snacks appeal to many though they are predominantly made from wheat flour especially in Kenya. Research has clearly shown some of the negative effects that are associated with wheat hence the need to sought out different flour alternatives of improved nutritional value. The snack industry has been found to contribute more of energy-dense and nutrient poor snacks which are mainly very sweet and fatty. This has contributed to many health problems in terms of obesity and cardiovascular diseases especially in young adolescents who are the major consumers of cookies. Prevalence of obesity is a major health issue not only in Kenya but also worldwide. According to Mkuu et al., (2021), obesity is a clear public health challenge due to its association with several non-communicable diseases (NCDs) such as type-2 diabetes, cancer and heart deseases. As a result of the emphasize on health food options to improve the convenience food trend, the snack industry has to be redefined with more of the healthy snacks hence the aim of the research.

1.3 JUSTIFICATION

Over the years, research has clearly elaborated on the high nutritional significance of sorghum which has beneficial effects to human health. According to Llopart et al., (2017), sorghum has been recognized for the development of healthy functional foods owing to its high antioxidant activity, high polyphenolic levels as well as low starch digestibility which offer protection against chronic diseases. Minerals found in the sorghum can help reduce the micronutrient malnutrition whereas the energy obtained from the grains provide satiety value as well as reduced hunger (Rao et al., 2016). Reducing micronutrient malnutrition is in line with the National Nutrition Action Plan of 2012-2017 expected outcome.

Adeyeye, (2016) referred to sorghum as a powerhouse of nutrition as it is reported to contain essential nutrients such as vitamins, carbohydrates and minerals despite lacking in quality protein. Despite it being referred to as a powerhouse of nutrition, it is also referred to a coarse grain which describes it further as a poor people's crop that's mainly consumed by the less privileged (Chavan et al., 2016). This can however change by benefitting from the enormous benefits that are attached to sorghum especially in many of the developing countries. Aside from the nutritional aspect point of view, epidemiological studies done have also shown antimicrobial and hypoglycemic effects of sorghum grains which have been shown to provide solutions to diabetes (Olawole et al., 2018). The Global Health Observatory has linked 2.8 million deaths worldwide to obesity which is as a result of unbalanced nutrition.

Aside from obesity, Migliogzzi et al., (2015), further linked chronic diseases such as cardiovascular disease, type 2 diabetes, osteoarthritis in addition to other types of cancer to unbalanced nutrition. As a result of the increase in these life-threatening diseases, consuming healthy products is of utmost importance especially in developing countries. Migliozzi et al.,

(2015) further stressed out that solutions to such diet related non-communicable diseases should focus on diet by shifting from calorie-dense foods, overconsumption of sugars, starch and fat which have also been linked to micronutrient deficiencies. Micronutrient malnutrition and other form of malnutrition can be tackled in in various approaches one of which involves food fortification especially in developing countries (Oghbaei and Prakash, 2017). Pulses such as green grams can thus be used for food fortification since they play an important role in promoting nutritional security for many by being a major protein source (Pinto et al., 2016).

According to Oyier et al. (2016), production of sorghum in Kenya is affected by the farmers attitudes who only grow sorghum for their home consumption. Inadequate market and low prices of sorghum in the market have led to its underutilization (Oyier et al., 2016). With increased imports of wheat, efforts should be made to promote local sources of flour to reduce dependency on wheat, sorghum being one of the alternative sources (Wulandari et al., 2017). Green grams (*Vigna radiata*) are an alternative source which can be sought due to their high nutritive and health-promoting effects. Utilization of green grams in value addition to reduce postharvest losses will run along way in alleviating poverty and improved nutrition.

Development of cookies from sorghum and green grams will not only contribute to improved nutritional aspects of cookies but also positive transformations in the community through sorghum production and increased green gram utilization. The current trend in nutrition which involves consumption of functional foods is the backbone of these research. Functional foods not only provide basic nutrition but also help in preventing diseases have been advocated globally by nutrition bodies such as FAO and WHO in fighting diseases such as celiac diseases, diabetes and coronary heart diseases (Kiin-Kabari and Giami, 2015). Such advocacies have led to the need for developing healthy ready to eat functional foods. This is paramount in diversifying sorghum products as functional foods in the market aside from the traditionally known products hence boost sorghum production in the country through increased utilization of sorghum within the country.

1.4 OBJECTIVES

1.4.1 MAIN OBJECTIVE

To develop nutrient-dense ready to eat sorghum-green gram cookies enriched with mango powder through different processes.

1.4.2 SPECIFIC OBJECTIVES

I. To formulate sorghum-green gram blends for processing of ready to eat cookies.
II. To determine the physico-chemical and nutritional properties of the cookies.
III. To evaluate the effect of germination and fermentation on the bioactive compositions in the flour blends and cookies.
IV. To determine the consumer acceptability of the developed cookies.
V. To evaluate the shelf- stability of the sorghum-green grams cookies.

1.5 HYPOTHESIS

I. Blends of sorghum and green gram flours can be used to develop a healthy nutritious cookie.
II. Cookies developed from sorghum-green gram blends will not have any effect on their physico-chemical and nutritional properties
III. The use of germinated and fermented flours in the cookies will not have any effect on the antinutritional factors in the formulated cookies.
IV. The sorghum-green gram cookies will have no effect on their consumer acceptability.
V. The ready to use sorghum-green gram cookies will not have a significant shelf-life stability.

CHAPTER TWO: LITERATURE REVIEW

2.1 OVERVIEW OF FOOD AND NUTRITION SECURITY

Human health depends on proper nutrition which ample research has shown that it provides essential building blocks necessary for growth and development (Bruins et al., 2019). According to Bruins and others (2019), there is a paradigm shift that requires proper dietary management strategies which can curb the existing burden of malnutrition. Malnutrition has been termed as a global health problem that affects human performance, health as well as survival and an obstacle to the eradication of poverty (Ma et al., 2022). Research has clearly shown that malnutrition affects growth and development, reproduction and physical capacity. Malnutrition is associated with lack of enough nutrients in the body that is undernutrition or too much of the nutrients in the body (overnutrition) (Musa et al., 2014). Research has clearly shown that many countries experience the triple burden of malnutrition (Bilali et al., 2018). Developing countries are affected most by malnutrition especially undernutrition which is characterized by consumption of a diet without adequate nutrients (Ole Tankoi et al., 2016). According to Ma, 2022, undernutrition accounts for 45% of children death worldwide.

Aside from undernutrition and overnutrition that has been well documented in literature, another form of malnutrition which has been referred to hidden hunger which refers to micronutrient malnutrition has been on the rise. Hidden hunger which is as a result of micronutrient deficiencies such as iron, zinc and iodine has been reported to affect over 2 billion people its main cause being poor quality diets. According to Hwalla et al. (2016), a melange of micronutrients and macronutrients are required by humans to warrant good health as well as prevention of diseases. The World Health Organization (2016) estimated 2 billion people to be obese and overweight however this may increase to 3.3 billion by 2030, if necessary, action will not be taken. The state of obesity increase has been attributed to increase consumption of calorie-dense foods. Measures to ensure appropriate nutritious diet coupled with sanitary as well as proper health services will in the long run achieve nutrition security. According to Bilali et al. (2018), nutrition is important in achieving food security as it considers protein, energy as well as nutrient requirements for a healthy life. In terms of food security in Africa, sorghum has long been termed as an important crop (Chiremba et al., 2009).

With the triple burden of malnutrition in developing countries, measures to improve qualitative and quantitative intake of sufficient dietary protein and calories are being enforced through different food processing methods with the potential to increase different aspects of the food such as longer shelf life, nutrient bioavailability, nutrient density, safety of food, food acceptability as well as convenience (Pathak and Kochhar, 2018). Such technologies include roasting, germination, milling, baking, cooking, drying, fermenting as well as extrusion cooking (Pathak and Kochhar, 2018).

2.2 SORGHUM PRODUCTION IN KENYA

Being a genus plant belonging to the grass family, sorghum has been reported to have almost twenty-five species *Sorghum bicolor* being indigenous to most African countries (Adeyeye, 2016). Due to its versatility and diversity, sorghum in Africa is mainly grown in arid and semiarid lands (ASAL) for food security (Chepng'etich et al., 2015). Contribution of sorghum towards food security has since been seen as a move to alleviate poverty and increase income growth especially in developing countries like in Africa (Chepng'etich et al., 2015). According to Okeyo 2020, sorghum serves as a staple food for not less than 500 million people living in Arid and Semi-Arid Lands in Africa and Asia. Despite USA being a major producer of sorghum, only a small portion is used for human consumption since most of it is used for animal feed and fodder. In semi-arid tropics of Africa and India however, the situation is different since sorghum forms the staple diet for many (Chavan et al., 2016). In Africa, Sorghum *bicolor* is an important crop whose uses include being used as food that is the grains as sorghum syrup or molasses as well as animal fodder and for production of alcoholic beverages (Adeyeye, 2016).

Sorghum is an indigenous Kenyan crop which does well in areas between 500 – 1700 meters above sea level with a seasonal rainfall of above 300 mm. Being a drought-resistant crop which can survive harsh climatic conditions, it is often grown in drought prone marginal areas within Kenya (Muui et al., 2013). Its drought tolerant nature and being indigenous in Kenya, sorghum is more suitable in salvaging households from hunger and poverty (Okeyo et al., 2020). Much of the sorghum production in Kenya has been concentrated in the southwestern and south-central parts especially Eastern, Nyanza, Western and Riftvalley (Kilambya and Witwer, 2013). Being a cereal crop like maize, sorghum can therefore be used as an alternative crop in arid areas which mostly prone to drought conditions. Over the years, sorghum production in Kenya has varied a lot due to reasons such as changes in yield, area harvested, changes in weather and also political instability (Kilambya and Witwer, 2013).

In the year 2007, sorghum production was found to be most volatile contribution to lower yields. This was closely attributed to the political instability following the 2007 elections (Kilambya and Witwer, 2013). After the year 2007, sorghum production has been on the increase due the promotion of sorghum by the government as a drought resistant crop mostly in Arid and Semi-Arid lands of Kenya. Stabilizing food security in Kenya has led to renewed interest in promoting drought-tolerant crops such as sorghum. Initiative to promote sorghum production in Kenya have been concentrated in the arid and semi-arid lands such as Eastern Kenya which is mainly frequented by drought occurrences (Chepng'etich et al., 2015). Sorghum production promotion in such areas is part of the government strategy in improving food security and increasing rural income (Chepng'etich et al., 2015). Over the years, area under sorghum production has been increasing however there has been a decline in sorghums' national average (Kilambya and Witer, 2013). Since 2010, domestic yields of sorghum have been on the rise however statistics show that Kenya still imports more than one-third of the total consumption (KNBS, 2017).

The figure below shows an overview of sorghum production in Kenya over a period of years between 2010 – 2016 in tones/hectares.

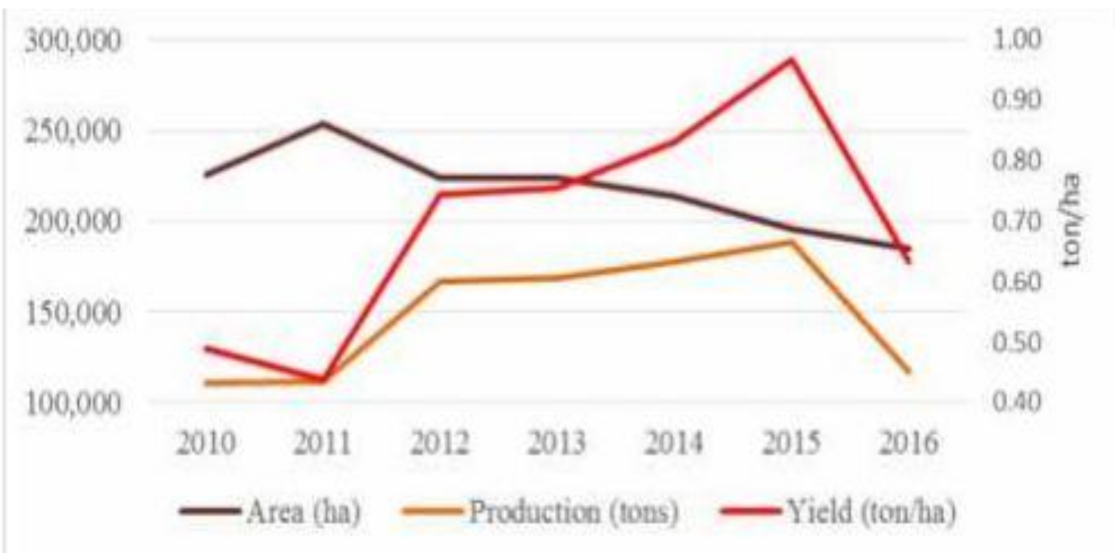

Figure 2.1: Trends in Sorghum production and yields in Kenya, 2010-2016.

Source: KNBS, 2017.

Sorghum production in Kenya is mainly by small scale farmers who face a myriad of problems such as lack of inputs, seeds, attack by pests and diseases resulting to lower yields (Muui et al., 2013). Despite sorghum grains containing many uses, in Kenya, the cereal is mainly used for animal feed and in traditional food products such as porridge, beer, ugali as well as githeri (Muui et al., 2013). Consumption of sorghum in rural households' forms much of the sorghum consumption in Kenya where sorghum is typically ground to flour to make local foods (Kilambya and Witwer, 2013). Utilization of sorghum in Kenya over the years has been divided into being consumed as a food in forms such as whole grains and flour, processed into commodities such as beer, used in the animal feed industry, in seed planting and lastly the percentage that goes to waste (Kilambya and Witwer, 2013). The figure below puts all this into perspective for utilization of sorghum in Kenya.

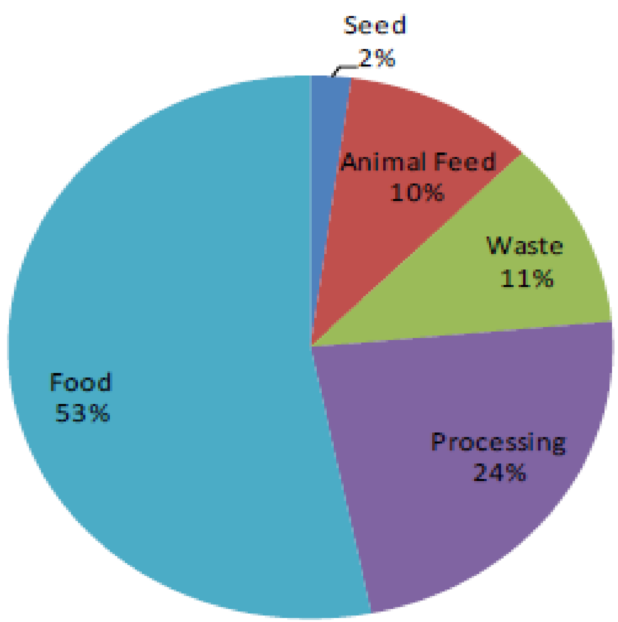

Figure 2.2: The average dispositions of Sorghum Utilization in Kenya.

Source: Kilambya and Witwer, 2013

In terms of marketing and trade, poor marketing channels and the low production which has been illustrated earlier have limited the sorghum trade in Kenya (Kilambya and Witwer, 2013). From the report by Kilambya and Witwer, 2013, it was discovered that most farmers produce just enough to sustain themselves with very little left to sell estimating that only a smaller percentage of sorghum is actually marketed. Despite the limited sorghum trade in Kenya, it has been argued that this would change over the years with increased awareness of sorghum benefits with relation to health (Kilambya and Witwer, 2013). According to these researchers, sorghum flour is likely to be the future of sorghum industry.

2.2.1 SORGHUM VALUE CHAIN IN KENYA

After understanding the production and marketing of sorghum in Kenya, it is paramount to understand the value chain and processing which are crucial to the utilization of sorghum in Kenya. Just like any other cereal crop, the key agents in sorghum value chain run from the produces to the end user. The chain involves the producers, middlemen, wholesalers, grain millers, retailers, consumers and exporters (Kilambya and Witwer, 2013). The marketed sorghum which is a small percentage is either sold directly to consumers or the middlemen and the small traders. After the direct sale, the traders bulk the sorghum in local markets after which it is transported to wholesalers who sell directly to retailers, millers and finally exporters (Kilambya and Witwer, 2013). Sorghum trade in Kenya is mainly done at individual levels rather than in organized groups.

According to the report by Kilambya and Witwer, 2013, the beer industry has come in to play an essential role in sorghum value chain in Kenya. East African Breweries has increased its demand for high quality sorghum varieties which are mainly 'gadam' and' gatiga' for production of alcohol which in turn has positively affected the sorghum value chain.

2.2.2 NUTRITION AND HEALTH CONTRIBUTION OF SORGHUM

Sorghum is a traditional indigenous that is highly underutilized though it is a major food crop for many developing countries. It is an important subsistence crop for millions of people in Africa (Proietti et al., 2015). Different studies have been done on sorghum to assess its nutritional value especially in production of new value-added products. Being the fifth most important cereal crop, sorghum has been termed as a principal source of energy, proteins as well as minerals such as zinc and iron (Ojha et al., 2017). Adeyeye (2016), assessed the quality as well as the sensory properties of sorghum-wheat flour cookies. In this research substitution of wheat flour with sorghum grains in producing cookies not only increased the crude fiber but also protein and fat content with increase in the sorghum flour. From this study, sorghum was termed as powerhouse of nutrition which can be used to add some flavor especially when used in gluten free products (Adeyeye, 2016). The nutritional content of sorghum grains (*Sorghum bicolor*) includes a total energy of 193 cal, a 52% moisture content, 7.1g of protein, 06g of fat, 38.9g of carbohydrates, 0.9g of fiber, 10mg of calcium, 3.5mg of iron and 1.7mg of niacin (Abah et al., 2020, Ajiboye et al., 2014). According to Proietti et al. (2015), sorghum has an actual nutritional value due to presence of proteins, vitamins both fat and water soluble and minerals such as iron, phosphorus and zinc.

In terms of health benefits, antioxidants found in sorghum have been reported to support cardiac health whereas the starch content in sorghum has been shown to undergo slow digestion which is very helpful to diabetic people (Adeyeye, 2016). Anti-diabetic properties of sorghum have also been reported. Karki et al. (2016), reported on high inhibitory activities of sorghum against a-glosidase as well as inhibited degradation of starch which is a positive health benefit against diabetes. Positive effects of sorghum in increasing plasma High Density Lipoprotein have also been mention (Karki et al., 2016).

Chiremba et al. (2009) further stated that the antioxidant activity of the phenolic compounds in sorghum confer other health benefits which include protection against oxidative as well as radical damage. A report by UNAIDS, 2006, showed that phenolic compounds in sorghum could contain some antiviral activity pertinent as an intervention in the control of Human Immune Deficiency

Virus (HIV) as well as Acquired Immune Deficiency Syndrome (AIDS). This however calls for extensive research to exactly determine the correlation between the phenolic compounds and the T-cells which are essential for human immunity. With relation to other cereals, the antioxidant activity of sorghum is higher making it a good source of natural antioxidant activity.

Due to its nutritional value coupled with health benefits, sorghum flour is highly being utilized especially in the United States especially in baked products (Adeyeye, 2016). Another health benefit of sorghum is being devoid of gluten hence makes it to be considered suitable for people with coeliac disease as well as gluten intolerance (Proietti et al., 2015). Being gluten free, sorghum could provide a good basis for most gluten free products thus diversifying food products for people suffering from coeliac disease. With wheat being very common in baked products, there has been an increase in wheat free baked products also known as gluten free products due to the condition known as celiac disease which is as a result of sensitivity to gluten. Celiac disease is as a result of gliadin intolerance which is contained in wheat grains. It is a chronic malabsorption disease that leads to gastrointestinal disorders in the body and remedy has been reported to be exclusion of gluten and proteins related to gluten from the diet (Wang et al., 2017).

2.3 UTILIZATION OF LEGUME CROPS FOR FOOD AND NUTRITION

Legumes which belong to the *Leguminosae* family are an important food crop after the cereal crops like maize and the likes. When reference to animal products which include meat, fish and eggs, legumes are a good source of low-cost dietary vegetable protein s as well as minerals (Olunike, 2014). According to Olunike (2014), legumes are an affordable protein source in developing countries where they are mainly consumed. Due to their protein content, many studies have since focused on their utilization in an effort to address protein malnutrition and food security issues since their protein content almost triples that of major cereals. Classification of legumes involves pulses which include peas and seeds that are of low-fat content, oilseeds which include soybeans and groundnuts, forage leguminous crops such as the winged bean and lastly the swollen root or tuberous root that are consumed as vegetables (Olunike, 2014). The chemical composition and protein richness of legumes however is affected largely by the variety, species and region.

Pulses have been referred to as edible seeds that are members of the legume family (Abdullah et al., 2017). According to Gilham et al. (2018), legumes include chickpeas, lentils, lupins, green grams and beans are known to have health benefits such as improving risk factors that have been associated with diet-related chronic disorders as well as cardiovascular diseases. Research has shown that legumes are rich in dietary fiber, protein, antioxidants, some minerals such as iron, zinc and phosphorous as well as some B-vitamins which contribute to their health benefits (Abdullah et al., 2017). Their flours have been shown to be important sources of essential nutrients which include proteins, carbohydrates, vitamins, minerals along with dietary fiber (Ouazib et al., 2016).

Albumins and globulin constitute the dominant proteins in legumes (Patil et al., 2016). The vitamins found to be contained in legumes are mainly niacin, riboflavin as well as thiamine whereas minerals such as calcium, iron, manganese and zinc have also been reported in legumes (Kohajdova et al., 2013). With legumes being good sources of minerals, the bioavailability of the minerals can therefore be improved through processing of the legumes (Olunike, 2014). Despite such findings and also the fact that pulses have been part of the human diet dating back many years ago, legumes are still highly under-consumed food groups. Most of the consumers do not really recognize nor understand the importance of legumes as reported by a school of thought (Gilham et al., 2018).

Legumes have been combined with sources of starch such as barley, oat, maize as well as wheat to improve the nutritional quality of snacks. The protein quality in the legumes was the contributing factor to the improved nutritional quality in snacks as reported by Patil et al. (2016). Consumption of products made from cereals can be increased with addition of legumes which are cheap sources of vegetable protein as well as lysine which is an essential amino acid (Koubaier et al., 2015). According to Kohajdova et al. (2013), high levels of lysine in legumes can be used in curbing lysine deficiencies especially in cereal-based diets that are lacking in essential amino acids. Due to such findings, a better amino acid balance can effectively be provided by combination of cereal and legume proteins. Aside from the nutritional benefits, medicinal properties of legumes have also been recognized. Research has brought forth the potential of legumes in reducing risks of type two diabetes, cardiovascular diseases, adrenal health issues, hypertension, osteoporosis, gastrointestinal disorders and prevention of certain types of cancers (Kohajdova et al., 2013).

The society today has been filled with lots of poor diet habits which numerous studies have shown to contribute to many diseases such as cardiovascular and obesity. Due to this fact, healthier options are being sought out and legumes have been found to be promising in combating health issues. In a research done in Canada, consumption of pulses was still low despite the Canadian Food Guide encouraging regular consumption of pulses (Abdullah et al., 2017). Abdullah et al. (2017), further showed that there is an economic burden worldwide as a result of diet-related disorders and cardiovascular diseases. Global expenditure related to such has been estimated to reach US$ 20 trillion between 2010 and 3030 hence the need to develop more healthier options. Increasing consumption of these pulses in our diet will therefore manage healthcare resources that have been associated by diet-related and cardiovascular diseases. Aside from the expenditure, WHO further estimated that the Non-communicable Diseases contributed to 1.6 billion to the global burden of diseases mainly as a result of unhealthy diets and physical inactivity.

2.3.1 NUTRITIONAL AND HEALTH CONTRIBUTION OF GREEN GRAMS

Green grams (*Vigna radiata*) are annual herb legume crops that is fully self-fertile and self-pollinated that grows to a height of 30-120cm growing extensively in all soil types (Ganesan and Xu, 2018). The legume is relatively drought resistant as it grows in arid and semi-arid conditions with a short growth cycle usually 70-90 days and belongs to the Leguminosae family (Zhu Yi-Shen et al., 2018). It is a popular common traditional food crop that has existed over many years (Ganesan and Xu, 2018). *Vigna radiata* was originally cultivated in India however over the years it has widely spread in other continents and its now being produced in Africa, South America, Australia and Southern Asia (Brishti et al., 2017). Green grams are good sources of protein, essential amino acids, soluble carbohydrates, vitamins (Vitamin B and C), minerals, enzymes, low in fat and high crude fiber content imperative to the human diet (Arun et al., 2016). The high nutritional value of *Vigna radiata* makes them an excellent and attractive option for health-conscious people (Brishti et al., 2017)

The high nutritive value of green grams consists of about 20-25% protein which is also three times that of cereal crops consist mainly of globulin and albumins as the primary storage proteins (Ganesan and Xu, 2018). Being a major source of dietary cheap protein, green grams are often referred to as “poor mans meat” (Khairnar et al., 2019). The high protein content and digestibility of Vigna radiata makes them significant in increasing quality protein intake in vegetarian diet (Zhu Yi-Shen et al., 2018). The essential amino acids contained in green grams consist of phenylalanine, leucine, isoleucine, valine, tryptophan, arginine, methionine and lysine at greater quantities due to the high protein content (Ganesan and Xu, 2018). Presence of the essential amino acids makes protein of Vigna radiata to be complete and the high lysine content in green grams makes it suitable to supplement for cereal-based diets which are mostly deficient of lysine (Ullah et al., 2014). The carbohydrate content in green grams is approximately 55-65% amounting to 630 g/kg of dry weight with starch being the principal carbohydrate (Ganesan and Xu, 2018). The mineral content of green grams also contributes to their high nutritive value whereby they consist of calcium, iron, magnesium, phosphorous as well as potassium (Khairnar et al., 2019).

In terms of their health contribution, green grams have been reported to contain some medicinal properties. Due to these properties, green grams have been used to not only treat fever bit also obesity, heart disorders and skin disorders (Arun et al., 2016). In Asian countries, green grams are used detoxification properties to reduce swelling and heat stroke and also regulation of gastrointestinal problems (Ganesan and Xu, 2018). A critical review done by Ganesan and Xu, 2018 highlighted the book Ben Cao Qui Zhen which elaborates further on Chinese medicine using green grams. In traditional Chinese medicine, sprouts of green grams are referred to *yin* which means a cooling nutritive food. The sprouts are used because of their diuretic, antiscorbutic, antipyretic, ant hypersensitive, antidote and anticancer properties (Ganesan and Xu, 2018).

Studies have shown the starch content in green grams is made up of carbohydrate that digest slowly which has been recommended for diabetic patients (Arun et al., 2016). The slow digestibility of carbohydrates also contributes to blood glycemic response in human which modifies the lipid and glucose metabolism (Arun et al., 2016). Gastrointestinal upset in humans from time to time has been regulated with green grams which do not cause flatulence unlike most other legumes. The excellent digestibility of Vigna radiata and its freedom from flatulence make them important in in developing food products that are recommended to all age groups (Dahiya et al., 2013). Sprouts of green grams have been shown to produce lower calories in comparison to other cereal hence can play a critical health role in diabetic and obese individuals (Ganesan and Xu, 2018).

Arun et al. (2016) further related the high levels of proteins, amino acids, oligosaccharides and polyphenols in green grams to their antioxidant, antimicrobial, anti-inflammatory and antitumor properties. Epidemiological studies done have clearly shown the benefits of consumption of antioxidant rich foods in this case the legumes have shown positive correlations in lowering many degenerative diseases such as diabetes, cardiovascular diseases, cancer, arthritis and Alzheimer's (Ganesan and Xu, 2018). The low glycemic index of *Vigna radiata* makes them a diabetic friendly food (Brishti et al., 2017). The protein hydrolysates and bioactive peptides that are found in green grams are important in modulating blood and heart disease as they inhibit the angiotensin converting enzyme (Brishti et al., 2017).

2.4 SORGHUM AND GREEN GRAM UTILIZATION IN FOOD PRODUCT DEVELOPMENT

There has been increased demand of novel functional foods which confer health benefits worldwide leading to utilization of various cereals and legumes (Venkatachalam and Nagarajan, 2017). Studies have further illustrated the ability of fortifying sorghum with other legumes in creating protein reach cereal foods (Njuguna et al., 2018). Different legumes have been paired with sorghum to boost the nutritional quality of sorghum products (Okpala et al., 2013). From literature, pigeon peas, soybeans are some of the legumes that have been blended with sorghum to boost the nutritional quality of the sorghum products however there is limited information on blending sorghum with green gram flour especially in Kenya to promote utilization of both the sorghum and green grams. Green grams on the hand have also been blended with other cereals such as wheat (Venkatachalam and Nagarajan, 2017; Pardeshi et al., 2013) to produce quality products of improved nutritional and sensory properties.

Despite huge benefits from sorghum and green grams as seen earlier, their utilization within the country is still limited. Studies on legume and cereal as well as their combinations have been seen to provide a good basis for gluten-free products such as baked products and snacks which are safe for consumption by with gluten intolerance (Adiamo et al., 2017).

2.4.1 BAKED PRODUCTS

Baked products which include bread, cakes, cookies, buns, biscuits and doughnuts normally depend on wheat flour due to its superior baking qualities however numerous studies have also been done to assess bakery products by use of health promoting ingredients such as pulses and tubers (Venkatachalam and Nagarajan, 2017). The bakery industry has been considered as one of the major industries in food processing (Chavan et al., 2016). However, due to the growing concern of effects of wheat on health and also the over-reliance of developing countries on imported wheat, different sources of flour are being sought sorghum and green gram flours being some of the alternatives.

According to a school of thought, wheat contains gluten which is a major component in wheat flour however it has been proven to generate inflammatory disease response hence the need to for more gluten-free products in the market (Venkatachalam and Nagarajan, 2017). Amino acid composition and fiber content in legume flours improve the nutritional value of baked products (Man and Paucean, 2013). Different studies have reported on the use of these grains in baked products for different functions altogether mainly for new product development, nutritional, sensory and improved health. A study by Karki et al. (2016) focused on the sensory quality of biscuits and cakes prepared from sorghum whereby the possibility of preparing baked products from sorghum was highly highlighted especially if the sole purpose is to promote health benefits.

Adiamo et al. (2017) did a review on the recent trends in formulation of gluten-free products from sorghum. This included sorghum gluten-free bread with and without special additional components such as starches, gums and hydrocolloids which improve texture and appearance of the gluten-free bread. Further in this review, sourdough fermented sorghum gluten free bread was also reviewed from literature. Araujo et al. (2015) focused on partial replacement of wheat flour by sweet sorghum flour in preparing bread. In this study, addition of the sweet sorghum flour improved the nutritional quality of the bread indicating that addition of sorghum flour in baked products is technically feasible with many health benefits. In this study, wheat flour was replaced with sweet sorghum flour up to 15 %.

Roti which is a traditional Indian unleavened cake has been made from sorghum other than wheat flour however the consumption of *roti* from sorghum has been on the decline in India due to urbanization and also the lack of gluten in sorghum which makes rolling the roti very difficult (Ratnavathi and Patil, 2013). Traditional bread *Injera* from Ethiopia made from cereals is made from dehulled sorghum grains. Preparation of *injera* includes making of dough with hot water which is fermented for hours before being baked (Ratnavathi and Patil, 2013). Another product native to Sudan includes *Kisra* which is a thin cake like leavened bread that is made from sorghum flour (Ratnavathi and Patil, 2013).

Legumes have also been studied as raw materials in biscuit production since they are rich in proteins, available dietary fiber and resistant starch properties (Laleg et al., 2016). A study by Ratnawati et al. (2019) in identifying a legume ingredient for wheat flour substitution in biscuits indicated that green gram flour was a better ingredient as compared to soy beans and red bean flour due to its high swelling power, peak viscosity, low setback, breakdown viscosity and pasting temperatures. Bhisale et al. (2018) studied the shelf life of a gluten free nutritious biscuit ‘triscuits’ from unique blends of amaranth flour, green gram flour, lentil flour and other ingredients. The triscuits obtained had a high fiber, protein with a favorable number of vitamins and minerals. This was done to promote use of gluten free flours in the diet owing to the benefits of gluten free diet which not only include the recovery of villi in the small intestines but also the reduction of malignant complications (Bhisale et al., 2018).

2.4.2 COMPOSITE FLOURS

Flour which is a raw material that can be obtained from different sources plays a crucial role especially in bakery products (Venkatachalan and Nagaraja, 2017). Composite flours consist of a combination of wheat and non-wheat flours for the production of baked products or just wholly non-wheat flours mainly from legumes, cereals, pulses and tubers (Menon et al., 2015). The potential for use of sorghum as a flour has been evaluated by several authors. Improved in vitro and cellular antioxidant properties of Chinese steamed bread was studied through addition of sorghum (Wu et al., 2018).

Wulandari et al. (2017) focused on the organoleptic characteristics of cookies from sorghum composite flours. Cookies were made from different blends of fermented sorghum, fermented cocoyam and germinated pigeon pea flours (Okpala et al.,2012). Ahmed et al. (2016) reported the use of decorticated sorghum and maize in production of biscuits with high nutritional value. Also, optimization of gluten free cookies from red and white sorghum flours was reported by De Petre et al. (2016). From these different studies, it clear that these grains are still being underutilized in different parts of the world despite their nutrition and health advantages.

Similar to sorghum, many studies have also been conducted on legumes to study their effect on bakery products however a few studies have focused on green gram flour. According to

Venkatachalam and Nagarajan, 2017, green gram flour is mainly used in desserts especially in Thailand. Composite flours of green grams have also been studied and excellent functional and rheological properties reported (Chandra et al., 2015). Menon et al. (2015) also investigated the use of composite flour including green gram flour in bread making.

2.4.5 SNACK FOODS AND EXTRUDED PRODUCTS

Different snack foods worldwide have been prepared from sorghum grains. *Upma* is snack food prepared from polished sorghum grains. *Upma* is native to South India and is also eaten hot with chutney from coconut and groundnuts (Ratnavathi and Patil, 2013). Blending chick pea flour with sorghum has been done to make different snack foods such as *murukku, chakkalu* and *namak* para (Ratnavathi and Patil, 2013).

A study by Venkatachalam and Nagarajan, 2017 reviewed the prospective of green gram flour as a substitute in formulating savory crackers. In this study, savory crackers were prepared at different ratios of wheat flout to green gram flour and analyzed for their physicochemical, ultrastructural, antioxidant and sensory studies. Despite the improved nutritive, functional and elevated antioxidant properties of the crackers, the consumer acceptability was however poor. The positive results in terms of health benefits have potential health benefits to consumers (Venkatachalam and Nagarajan, 2017).

Research work has shown improved nutritional quality of sorghum-based extruded products which use extrusion as a cooking method (Tadesse et al., 2019). In this study, sorghum and soymeal flour were used to produce an extruded product. Increase in moisture, crude protein, fat, ash, fiber and minerals especially calcium and iron were reported however there was decrease in the carbohydrate content. Tadesse et al. 2019 further reported on reduced anti-nutrient content as a result of the barrel temperatures during the extrusion process. The mean sensory values indicated acceptability of the sorghum-based extrudates indicating there is potential to use extrusion technology as a process in product development (Tadesse et al., 2019). Pardeshi et al. (2013), focused on the development of cold extruded ready-to-cook mung (*Vigna radiata* L.) nuggets in utilization of mung beans. Blends of green gram flour and wheat flour were used to prepare the cold extrudates which were then steamed at cooking pressure and later dried. This study highlighted the extended shelf life of the nuggets as a result of the extrusion process as well as improved sensory properties such as appreciable chewing feeling (Pardeshi et al., 2013).

2.4.6 COMPLEMENTARY FOODS

Complementary foods are important in child growth and development by providing needs which encompass nutritional as well developmental in instances where breast milk is limited for the infant (Bazaz et al., 2016). Quality of weaning food for infants has to be high and meet the WHO standards of high nutrient density, low viscosity, bulk density, high energy, protein and micronutrients to meet the needs of the infant (Bazaz et al., 2016). According to Adepeju et al. 2016, nutrients provided by complementary feeding should cover the daily requirements aside from the breast milk since infants require higher energy and protein during their development phase to meet the demand for metabolisms. Meeting these demands will come a long way in curbing child malnutrition which is very common in developing countries (Adepeju et al., 2016).

The potential of green grams in formulating a hypoallergic complementary food was investigated by a school of thought. In this study, different proportions of rice flour and green gram flour (sprouted and unsprouted) were blended with addition of other ingredients to formulate hypoallergic diets. The results obtained revealed a notable increase in the nutritional value of the weaning food especially with use of the sprouted green gram flour which was concluded that incorporation of the sprouted green gram could be used to replace milk especially in weaning food formulations thus reducing the cost of production (Bazaz et al., 2016). In the similar study, invitro protein digestibility of the weaning food was significantly enhanced due to the reduction in the antinutrients in the samples (Bazaz et al., 2016). Utilization of these legume in development of weaning food can be used as potential for green gram utilization especially in developing nations to help in addressing protein energy malnutrition and micronutrient deficiency (Bazaz et al., 2016).

The potential of sorghum in formulating complementary foods has also been investigated by Okoth et al. (2017). In this study, blends of sorghum and amaranth were used in development of nutrient-dense complementary food. Higher energy values and protein content higher than the estimated protein needs of infants was reported with these blends. Due to the processes used in preparation of the raw materials such as steeping and germination, the antinutrient content in the final product was not detected which led to high nutrient availability and digestibility according to Okoth et al., 2017. In another study, fermented sorghum was used in development of a weaning food together with germinated soybeans and defatted sesame (Adepeju et al., 2016). The results showed improved nutritional properties which led to the authors conclude that commercial production of such weaning food would go a long way to ameliorate protein energy malnutrition prevalent in most developing countries (Adepeju et al., 2016).

2.4.7 LIMITING FACTORS TO UTILIZATION OF BOTH SORGHUM AND GREEN GRAMS

Utilization of sorghum in different food products has however been limited by presence of anti-nutritional components. Some of these components include trypsin inhibitors, phytic acids as well as oligosaccharides (Hefnawy, 2011). Despite the health benefits associated with sorghum, it is still being considered to have a low nutritional value due to its poor digestibility and limited product diversification as compared to other cereals. The low nutritional value of sorghum has been attributed to the presence of anti-nutritional factors such as tannins, cyanogenic glucosides, phytic acids, trypsin inhibitors and oxalates (Ojha et al., 2017). The anti-nutritional factors in sorghum lower the protein digestibility and mineral absorption in sorghum products (Ojha et al., 2017). Insoluble complexes are formed due to the ability of the anti-nutrient factors especially phytic acid and tannins binding to proteins and divalent cations interacting negatively with bio accessibility of important elements in the digestive tract (Proietti et al., 2015).

The low digestibility issue of sorghum has been termed as a major issue with sorghum grains when compared to other cereals like maize, wheat and rice (Proietti et al., 2015). According to Proietti et al. (2015), the low digestibility of sorghum aside from being cause by presence of anti-nutrient factors, is due to high cross linking of proteins on the periphery of the protein bodies. The major protein found in sorghum are the Kafirins which are of poor nutritional quality since they contain limited amounts of essential amino acids especially lysine (Proietti et al., 2015). To improve the utilization of sorghum, various researches have focused on different processing conditions aimed at decreasing the antinutritional factors and increasing bioavailability in cereal. Ojha et al. (2017) studied effects of malting and fermentation on the anti-nutritional components in sorghum flour. Results obtained showed that fermentation of sorghum grains to obtain fermented sorghum flour did indeed reduce the anti-nutrient factors improving the functional properties.

Studies have shown that despite legumes containing high nutritive values, there are still factors that lower the nutritional quality of legumes such as the processing methods, anti-nutritional factors or toxic factors (Kavitha et al., 2015). Just like sorghum, legumes also contain several anti-nutritional factors such as amylase inhibitors, phytates, tannins, oligosaccharides and saponins which reduce bioavailability of certain minerals. Iron and zinc bioavailability in legumes are reduced by phytic acid through chelation (Kavitha et al., 2015). Absorption of vitamin B has been reported to be reduced by presence of tannins which interfere with digestive process through binding to enzymes (Kavitha et al., 2015). In terms of legumes, their hard to cook property coupled with long cooking times have also limited their utilization (Olunike, 2014).

The limited utilization of these pulses can however be improved by removal of these undesirable components which in turn will improve their nutritional quality. Presence of these anti-nutrients has however been reported to be reduced by some processing methods. Kavitha et al. (2015) studied on in vivo reduction of anti-nutrients of *Vigna radiata* using different processing methods. Sprouting, boiling, roasting, microwaving and autoclaving were the processes under study where each resulted in decreased anti-nutrient content at different levels. In this study, sprouting was seen to reduce phytic acid and tannins in the mung beans while microwaving and autoclaving reduced amylase inhibits best as compared to the sprouting (Kavitha et al., 2015). In this study, it was clear that the different methods did indeed eliminate the antinutrient factors however minerals and carbohydrate fractions were also reduced. Other methods such as dehulling process of the legumes has also been seen to reduce the anti-nutrients in most legumes (Olunike, 2014).

2.5 PROTEIN DIGESTIBILITY OF CEREALS AND LEGUMES

Proteins are such a vital part in our diet and have to be hydrolyzed into their basic building blocks of amino acids and peptides before being utilized in the body. Storage proteins have been found to contain high amino acid contents especially arginine, lysine and histidine making them more nitrogen rich (Joye, 2019). The unique amino acid sequence determines the folding conformation of proteins which hamper protein digestibility in the body. Processing of food is important as it not only ensure the safety of food, prolonged storage life but also increased digestibility. The quality of a protein source is determined by amounts of dietary essential amino acids, protein digestibility and the bioavailability of amino acids (Gilani et al., 2005).

Lower digestibility of food has been attributed to the use of less refined ingredients as well less extensive processing. According to Joye, 2019, destructive food processing triggers alterations in molecular as well as super molecular structures which improve food digestibility through easier access of nutrients by digestive enzymes. In terms of proteins, the denaturation caused by processing increases protein digestibility as the protein becomes more accessible to the hydrolytic enzymes (Joye, 2019). According to Joye, 2019, protein digestibility is often affected by internal as well as external factors. Internal factors include the protein amino acid profile and the protein folding whereas external factors include the pH, temperature and ionic strength.

Protein digestibility and absorption in human beings is an important dimension of protein quality which has been neglected (Devi et al., 2018). Functionality of dehydrated products depends on the solubility of protein which is considered as an important factor (Sashikala et al., 2015).

2.5.1 SORGHUM

The proteins in sorghum are classified as albumins, globulins, kafirins, cross-linked kafirins and glutelins where the main proteins are the kafirins with an average of 50-70% content (Needham et al., 2015). The storage proteins which consist of the prolamins and the kafirins contain low levels of amino acids particularly lysine which is a disadvantage attached to sorghum when it comes to its utilization (Needham et al., 2015). Sorghum proteins especially the kafirins form cross links when they are moist during any form of cooking which results in protease resistance which decreases their digestibility. Due to this, protein malnutrition has been an issue especially in communities that rely mostly on sorghum as staple food. This has therefore led to efforts to increase protein digestibility and amino acid availability to improve sorghum products (Needham et al., 2015).

2.5.2 GREEN GRAMS

High protein content and protein digestibility has been reported in green grams (Zhu Yi-Shen et al., 2018). Consumption of green grams in combination with other cereals has been encouraged due to their high protein content and protein digestibility. Green grams have long been used to meet the protein requirements especially in India (Sashikala et al., 2015). Heat treatment causes inactivation of trypsin inhibitors in legumes which has been attributed to the improved protein digestibility of legumes (Sahikala et al., 2015).

2.6 COOKIES AS CONVENIENCE FOODS

Cookies are referred to convenience foods whose consumption has increase greatly over the years. Cookies form part of the bakery industry whose consumption has increased due to urbanization leading to a fast-paced nature of urban life which has contributed to increase in dependence on convenience foods. According to Chavan et al. (2016), the word cookie originates from a Dutch word *koekje* which refers to a little cake. Due to their variety in taste, crispness as well as digestibility, they are an important snack food (Chavan et al., 2016). Due to their ready to eat nature, cookies have been named a better composite food in comparison to bread (Kiin-Kabari and Giami, 2015).

Over the years, different researchers have tried to produce cookies with high sensory ratings from a mixture of flours with the sole purpose of improving their nutritional compositions and improving crop utilization. This being in an effort to increase healthier and more convenient food products with premium sensory qualities that will appeal to the consumer. McWatters et al. (2003)

produced cookies from blends of wheat and soybean, Ndife et al (2014) cookies from wheat and full fat soya. Aside from wheat based composite flours, other studies have also focused on non-wheat flours with high nutritional and sensory attributes. Chinma et al. (2012) produced cookies from flour blends of plantain and defatted sesame flour, Okpala and Okoli, 2011 produced cookies from sorghum, cocoyam and pigeon pea flour blends while Agriga and Iwe, 2009 used cassava groundnut and corn starch blends.

2.7 PROCESSING TECHNOLOGIES FOR CEREALS AND LEGUMES

Different technologies from time immemorial have been used in processing of both legumes and cereals. Traditional simple methods such as precooking of grains as well as fermentation process have been well documented over the years through research. These processing technologies are mainly aimed at improving nutritive value, phenolic content as well as antioxidant properties which have been shown to have many health benefits (Salazar-lopez et al., 2017). Other processing techniques include milling, cooking, soaking, germination as well as extrusion which have also been documented to improve the nutritional value of foods.

2.7.1 EXTRUSION PROCESS

Extrusion cooking has over time become a popular process due to its automated control system which makes it very versatile with a high productivity rate coupled with low cost of production (Pathak and Kochhar, 2018). In terms of food and nutrition security, extrusion has been put in the fore front as a method which can essentially be used to eradicate poverty in developing countries by producing safe food by utilizing underutilized legumes and cereals (Pathak and Kochhar, 2018). In the process of developing a novel product, extrusion process involves several other processes such as mixing, forming, texturing as well as cooking (Pathak and Kochhar, 2018). The process essentially involves conditions of high temperature and pressure within a short period of time which changes the biochemical properties of the food. When the food material reaches its plasticizing point, shear energy is exerted by the rotating screws heating the barrel thus cooking the food material (Pathak and Kochhar, 2018).

Being a contemporary food processing technology, extrusion process is mainly used in development of snacks as well as supplemented foods. Its massive advantages include the development of ready-to-eat foods that are characterized with desired shape, size, texture with improved sensory properties at lo processing costs (Pathak and Kochhar, 2018). Plenty of extruded products such as ready to eat cereal, snacks, textured vegetable protein, pasta and meat products have been prepared depending on the ingredients or a combination of different ingredients (Pathak and Kochhar, 2018). Extrusion process has been widely accepted by the scientific community due to its convenience especially in making breakfast cereals as well as innovative modified beverages (Patil et al., 2016). Enhanced mineral bioavailability and protein digestibility has been reported in extrusion of whole grain sorghum grains (Llopart et al., 2017). Nutritional and textural quality of extruded products has also been reported (Pathak and Kochhar, 2018).

2.7.2 FERMENTATION PROCESS

Fermentation is another major process that has been used over the years on foods to significantly enrich foods with essential amino acids and fatty acids, proteins as well as vitamins. The process involves soaking of selected grains, cereals or legumes in water at room temperature over a period of time mostly 24-72 hours (Adeyeye et al., 2017) within which growth of favorable microorganisms will take place. These microorganisms are responsible for breaking down fermentable carbohydrates into the end products of fermentation which are organic acid, carbon dioxide as well as alcohol not leaving metabolites such as bacteriocins which have been shown to increase safety of food (Sanlier et al., 2017).

According to Adeyeye et al. (2017), production of acetic acid and lactic acid which are anti-bacterial compounds anti-bacterial compounds during the process of fermentation has been shown to improve digestibility and acceptability of foods. Aside from the nutritional aspect of fermentation which has been reported to increase protein and vitamins in foods, Adeyeye et al. (2017) further investigated the effect of fermentation on antinutrient properties of grains such as sorghum and soybeans and discovered that indeed this process led to hydrolysis and reduction of antinutritional factors in cookies made from sorghum and soybeans. In this study, fermentation process of sorghum and the soybeans for 24 to 72 hours elevated the thiamine, niacin as well as riboflavin content of the cookies. This was attributed to the biological and enzymatic activities that occurred during the soaking and fermentation process (Adeyeye et al., 2017).

Legumes and cereals have long been shown to contain natural toxicants such as isoflavonoids, cyanogen, metal chelates, saponins, trypsin inhibitors, phytic acid and tannins (Adeyeye et al., 2017). These substances affect the food negatively by reducing the nutritional value by interfering with the bioavailability of minerals, proteins as well as carbohydrates. Due such problems, processing methods such as fermentation and the others have been encouraged. Fermentation of cereal-based foods has been reported to improve the nutritional, sensory and functional properties of such foods by decreasing the non-nutrients and increasing synthesis of certain vitamin B complexes (Sanlier et al., 2017). In a review by Sanlier et al. (2017) on the health benefits of fermented foods, taste, appearance, nutrient digestibility, nutritional value, texture as well as shelf life of legume-based foods are improved by fermentation process.

2.7.3 GERMINATION PROCESS

The process of germination has been widely used for legumes and cereals for the sole purpose of increasing the palatability and nutritional value of the pulses and legumes through breakdown of the antinutrients contained in them. Most of the traditional sorghum products are malted for the production of weaning foods, beers and other traditional dishes. Increased nutrient bioavailability by germination is due to the triggered enzymatic activity of the sprouted grains which in turn leads to the breakdown of nutrients such as proteins, carbohydrates and lipids into their simpler forms (El-Moneim et al., 2012).

Improved sensory properties of baked products have been known to be improved by germinating the grains used in making the baking flour. Free limiting amino acids, available vitamins, protein digestibility and crude fiber have been reported to be increased by the process of germination whereas antinutrient factors are reduced (Okpala and Okoli, 2011). Effects of germination on the mineral content, antinutritional factors, dietary fibers and nutritional components have been reported by Sharma et al. (2015). Sharma et al. (2015) observed that the process of germination increased the protein content of foxtail millet which was attributed to the synthesis of new amino acids during the process. Germination process also increases the mineral content by activating the phytase which hydrolyzes it to inositol, free orthophosphate releasing minerals (Sharma et al., 2015). Decrease in antinutritional factors such as tannins and phytates has been reported as a result of increase phytase enzyme due to the hydrolytic activity of this enzyme (Sharma et al., 2015). to Leandro de MoraisCardoso et al. (2015) reported on reduction of protein digestibility of sorghum once any form of heat was involved however processes such as fermentation and germination were seen to increase the protein digestibility.

2.8 FRUIT POWDERS

Fruits are produced in large amounts in different parts of the country however they are seldom processed for value addition in new products or even existing products (Thivani et al., 2016). Fruits are important to the human diet due to their nutritional value which studies have shown they can be used to provide nutritional security as well as contribute to food security (Rajeshwari et al., 2018). High metabolic activity of fruits is an added advantage over plant derived foods such as tubers. Due to the high metabolic activities of fruits, fruits are highly perishable as their metabolic activities continues even after harvesting period (Thivani et al., 2016). Due to this, diversification of fruit utilization needs to be at the forefront. Fruits have been utilized in different processes such as in juices, jams, concentrates and jellies however their utilization as fruit powder is a novel way that is growing much attention (Thivani et al., 2016).

2.8.1 MANGO FRUIT POWDER

Mango (*Mangifera indica L*.) which belongs to the genus *Mangifera* is one of the most important fruits characterized with a good taste, bioactive compounds, good source of sucrose, fibers, fat, protein, beta carotene. calcium as well as vitamins (B and C) (Agustini, 2018). Due the rich source of nutrients, health benefits such as antioxidant capacity and ability to overcome oxidative stress has been reported (Acham et al.., 2018). According to Lauricella et al. (2017), the mango fruit is known as the ‘king of fruits’ due to its popularity especially in tropical regions. It is a seasonal fruit that is perishable and its quality has been reported to be mostly damaged during handling, harvesting, distribution, transportation and storage (Agustini, 2018). Being a good source of sucrose, the deterioration level is quite high due to the high sugar and moisture content (Agustini, 2018).

Processing mango into juice or powder has however been seen to be advantageous in preventing post-harvest losses and increasing their utilization (Agustini, 2018). Processing of mango into fruit powder has found many uses as flavor enhancer in different types of foods and beverage. The process of converting perishable fruits like mangoes into shelf stable products has been seen to be economically advantageous (Acham et al., 2018). Sun drying, oven drying, freeze drying, vacuum drying, spray drying and vacuum drying are some of the technologies used in preparation of various fruit powders (Agustini, 2018).

2.9 GAPS IN KNOWLEDGE

Composite flours of sorghum with other legumes such as soybeans, pigeon peas and other legumes have been extensively studied however there is no information in composite flour of sorghum and green grams and their utilization in product development. Green grams are class of legumes whose nutritional and health benefits have also been extensively studied over time however much of their information with relation to Kenya is limited therefore still being underutilized in value addition.

In terms of the nutritional properties of mostly products made from sorghum and green gram flours, not much has been done on determining the actual protein that is being consumed which is important to the human body. Most studies just focus on the protein from proximate composition as representative of the protein consumed. Sorghum being lower in protein and green grams being high in protein content, it is paramount to determine the actual protein that will be consumed in the cookies that will be developed despite the different processes that will be used to come up with the end product.

This study aims to develop cookies from sorghum and green gram blends enriched with mango powder to increase utilization of cereal-pulse-fruit combinations in developing food products of high nutritional, sensory quality that are shelf stable.

CHAPTER THREE: IN-VITRO PROTEIN DIGESTIBILITY AND NUTRITIONAL QUALITY OF READY TO EAT SORGHUM-GREEN GRAM COOKIES SUPPLEMENTED WITH MANGO POWDER

3.1 ABSTRACT

The aim of this research was to develop Ready to Eat (RTE) sorghum-based products to address underutilization of cereals and legumes. This study evaluated the physico-chemical and nutritional properties of cookies from sorghum-green grams supplemented with mango powder. Whole untreated grain (WS), malted (MS) and fermented (FS) sorghum flour was used to formulate blended flours at 100%, 80%, 70% and 60%. Green gram flour (GG) was incorporated at 10%, 20%, 30% and dried mango powder (MP) at 10%. Protein, fat, fiber and ash increased significantly ($p < 0.05$) with increase in green gram flour substitution. Significant differences ($p < 0.05$) in protein content were found in cookies ranging from 9.52% to 13.60%. Fiber increased significantly ($p < 0.05$) from 9.40% to 10.90%. In vitro protein digestibility ranged from 67.75 ± 0.01% to 90.05 ± 0.10 %. Vitamins increased with addition of green gram flour. Thiamine content ranged from 0.22±0.02 to 0.61±0.02 mg/100g, riboflavin from 0.09±0.00 to 1.39±0.04 mg/100g and ascorbic acid from 13.87±0.79 to 19.31±0.94 mg/100g. Value addition of under-utilized crops like sorghum and green grams can play a vital role in development of high nutritional quality RTE products.

Keywords: **In-Vitro Protein Digestibility, Physico-chemical, Nutritional Quality, Ready-To-Eat, Cookies**

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3.2 INTRODUCTION

Food and Nutrition Security is still a big challenge particularly to underprivileged population groups leading to the Food and Agriculture Organization estimating 800 million people are still food insecure (Baldermann et al., 2016). Preliminary assessment by the FAO has shown an additional 83 to 132 million people to the total number of undernourished people globally in 2020 especially due to the COVID-19 pandemic which has caused food supply disruptions (FAO, 2020). According to the Hunger Map 2019, more than 1 in 9 people of the estimated 821 million people do not get enough food to eat leading to the high prevalence of malnutrition especially undernourishment in majority of the population (FAO, 2019). Of the estimated 800 million people in the world, more than 30% of the population that is undernourished is from Africa (FAO, 2019). With these statistics, the Zero Hunger target in the Sustainable Development Goals is far from being achieved.

Malnutrition has been described in many forms which all trickle down to malnutrition being the deficiency of nutrients as a result of not ingesting the proper nutrients or consumption of nutrient-poor food with regards to the daily nutritional requirements (Baldermann et al., 2016). It is an atrocious dilemma which is affecting the masses whose diet is comprised of cereal based, starchy foods. Diseases such as iron deficiency anemia, mental impairments, vitamin A deficiency, blindness, chronic diseases like cancer, cardiovascular diseases as well as diabetes have been associated with malnutrition due to unbalanced diet (Baldermann et al., 2016). With diet-related non communicable diseases such as overweight and obesity being on the rise, major shifts in the production and consumption of food to improve diet quality and reduce food insecurity need to be explored further to address these other forms of malnutrition.

Cookies are ready-to-eat convenient foods categorized as snacks which appeal to a wide range of population due to their taste, longer shelf-life as compared to bread and their relatively low cost (Yusufu et al., 2016). According to various studies, snack consumption has been on the rise as a result of urbanization, cookies representing the largest category among baked food products (Onwurafor et al., 2019). Cookies being cereal-based are a good source of carbohydrate and are predominantly made from wheat. They are however lacking in other essential nutrients such as proteins and vitamins hence the many attempts to improve their nutritive value through modifying their nutritional composition. Improving their nutritive value has led to the use of non-wheat flour or composite flour from local underutilized crops with high protein content to overcome the dependence on wheat importation especially in developing countries (Yusufu et al*.,* 2016).

Sorghum (*Sorghum bicolor (L) Monench*) has long been a staple food in many parts of the continent (Rao et al., 2016). Besides wheat, rice, maize and barley, sorghum is the fifth most significant cereal crop sustainable in arid and semi-arid lands (Wu *et al*., 2018). Sorghum is known for its nutritional quality which has been extensively studied and explored by Ratnavathi and Patil et al. 2013. Aside from the nutritional quality of sorghum, protective health effects against certain chronic diseases have also been explored through food products that incorporate sorghum (Wu et al., 2018). Phenolic compounds and antioxidant activity of sorghum which are both correlated to each other offer these protective health effects. Earlier studies have shown the antioxidant activity and anticarcinogenic effects of sorghum grains which can help mitigate risks of cardiovascular diseases (De Petre et al., 2016). For centuries, sorghum has been utilized as food especially in Africa and Asia due to its many health benefits.

Legume grains are important sources of feed and food protein. Reduction in proinflammatory status and improvement of certain metabolic features has long been linked to consumption of legumes (Yousaf et al., 2012). Green grams (*Vigna radiata*) are legumes with a high nutritional quality including being rich in proteins, vitamins (A, B1, B2, niacin) and minerals such as phosphorus, potassium, and calcium (Nanyen et al., 2016). Due to their nutritive composition, green grams can be utilized in baked products to offer healthy nutritious products. Legumes have been used in food products to improve the quality of cereal protein by supplementing with limiting amino acids in cereals (Nanyen et al., 2016).

The development of cookies from cereal-legume-fruit flour blend in this research could provide healthy nutritious and gluten free products which can provide solutions to food insecurity and malnutrition problems through diversification of under-utilized crops. The use of sorghum and green gram flour as ingredients in common baked products is not common in Kenya hence the need for this research to improve the usefulness of sorghum and green grams in food processing to substitute wheat in baked products. The present study therefore aimed to develop gluten free cookies from sorghum and green grams supplemented with mango powder acceptable to consumers.

3.3 MATERIALS AND METHODS.

3.3.1 Sample Collection

Sorghum (*Sorghum bicolor (L) Monench*), green grams (*Vigna radiata*) and ripe mangoes (*Mangifera indica L*.) were purchased from a local open-air market in Kangemi, Nairobi-Kenya. All the other ingredients (margarine, sugar, skimmed milk powder, vanilla essence, baking powder, salt) used in the baking of the cookies were purchased from the local retail outlets. All materials were transported to the department of Food Science, Nutrition and Technology at the University of Nairobi, Kenya where the study was conducted.

3.3.2 Malting of Sorghum

Sorghum (Sorghum bicolor (L) Monench) was manually cleaned to remove damaged grains, husks and stones. To suppress mold growth, the grains were washed in 5% (w/v) sodium chloride solution after which they were steeped in tap water in ratio 1:2 (w/v) at room temperature (32 ± 20C) for 12 hours. After steeping, water was drained and the grains were allowed to germinate between two damp cloths at ambient temperature for 72 hours as described by Ouazib et al. (2016). After every 12-hour interval, water was sprinkled to facilitate the germination process. The germination time and the temperature chosen for this study were chosen from previous studies (Okpala et al., 2013, El-Moneim et al., 2017, Bello et al., 2020, Olamiti et al., 2020). At the end of the germinating period, the grains were dried in the oven (Schutzart DIN 40050 – IP20) at 65^0C for 9 hours followed by removal of the dried sprouts on the grains manually (Siddiqua et al., 2019). The germinated sorghum grains were milled into flour using a hammer miller and passed through a 1mm sieve to obtain fine sorghum flour of uniform particle size free from clumps. The obtained flour was stored in an airtight container stored at room temperature for further analysis.

3.3.3 Fermentation of Sorghum

Sorghum (*Sorghum bicolor (L) Monench*) was sorted manually to remove damaged seeds and stones. The grains were then washed and dried then milled into flour using a hammer mill. The milled flour was subjected to spontaneous fermentation by microflora (lactic acid bacteria) naturally present in the grains. Sorghum flour was fermented according to the method described by Elkhalifa et al. (2017). Flour was mixed with water (1:2 w/v) to form a slurry which was incubated at 370C for 48hrs in sterile covered flask. After the fermentation period, excess water was removed and the fermented slurry was oven (Schutzart DIN 40050 – IP20) dried at 65^0C for

8hrs and then ground into flour. The fermented ground flour was passed in a 0.4mm sieve and stored in an airtight container until needed for analysis.

3.3.4 Preparation of Whole Grain Sorghum Flour

Sorghum (*Sorghum bicolor (L) Monench*) was sorted out to remove excess dirt and thoroughly washed as per the method reported by Adeyeye, 2016. The cleaned grains were oven (Schutzart DIN 40050 – IP20) dried at 65^0C for 8 hours then milled using a commercial hammer mill into fine flour. The dried flour was packaged in a clean airtight container for further use.

3.3.5 Preparation of Green Gram Flour

Green grams (*Vigna radiata*) flour was produced using the method of Dabels et al. (2016). The grains were manually cleaned to remove all the dirt then washed with clean water. The clean seeds were then spread on tray and placed in an oven (Schutzart DIN 40050 – IP20) dried at 65^0C to dry for 4 hours. The dried green grams were then milled into fine flour using a commercial hammer miller (GM25- TEFC). The flour was then passed through a 1mm sieve and packed in a clean airtight container for further use.

3.3.6 Drying of Mango (*Mangifera indica L.*) Fruits

In preparation of the dried mango powder, the method of Sengev et al. (2015) was adopted with slight modifications. Twenty large-sized ripe mangoes bought from the market were sorted out, washed, peeled and the mesocarp was sliced manually to a thickness of 2.0mm. The thin slices were spread on a tray lined with aluminum foil and oven (Schutzart DIN 40050 – IP20) dried at 65^0C for 24 hours. The dried slices were then ground (350W- 220V-AK 300, Nunix 2 in 1 Blender) into powder using a grinder and sieved through a 0.5mm sieve to obtain the dried mango powder that was stored in an airtight container stored at room temperature.

3.3.7 Experimental Design and Sample Formulation

The experiment was laid out as a 3×4 factorial design with three levels of sorghum flour (whole grain, malted, fermented) and four levels of green gram flour and dried mango powder substitution (100:0.0, 80:10:10, 70:20:10, 60:30:10) for each of the treatments as represented in **Table 3.2**. The ratio of the flour blends was labelled CLF (Cereal: Legume: Fruit) to represent the sorghum: green gram: mango powder ratio in the formulations **(Table 3.2).** Sample CLF1 with 100% whole grain sorghum flour served as the control sample. Formulation (**Table 3.1)** was done based on the recommended minimum levels of protein, fiber, fat by FAO/WHO which were achieved using NutriSurvey Linear Programming Package 2004 version (Amegovu et al., 2013).

Table 3.1: Formulation of Ingredients Used in the Sorghum-Green Gram Cookies

Ingredients	Amount (grams)
Flour *	200
Sugar	50
Margarine	50
Salt	0.35
1 Whole Egg	30
Skimmed Milk Powder	8
Baking Powder	1.25
Vanilla Essence	1.25

***Sorghum flour, green gram flour and dried mango powder**

Table 3.2: Ratio of the Flour Blends

Flour Blends	Whole-Grain Sorghum Flour (%)	Malted Sorghum Flour (%)	Fermented Sorghum Flour (%)	Green Gram Flour (%)	Dried Mango Powder (%)
CLF 1	100	0	0	0	0
CLF 2	80	0	0	10	10
CLF 3	70	0	0	20	10
CLF 4	60	0	0	30	10
CLF 5	0	100	0	0	0
CLF 6	0	80	0	10	10
CLF 7	0	70	0	20	10
CLF 8	0	60	0	30	10
CLF 9	0	0	100	0	0
CLF 10	0	0	80	10	10
CLF 11	0	0	70	20	10
CLF 12	0	0	60	30	10

Key: CLF = Cereal: Legume: Fruit Blend

3.3.8 Formulation of the RTE Cookies

The ingredients used in the sorghum-green gram cookie preparation included the different ratios of the sorghum flour (whole grain, malted and fermented), green gram flour and mango fruit powder. The cookies were prepared according to the American Association of Cereal Chemists (AACC, 10-50D) with slight modifications. For each of the experiment, 200g of the blended flour (CLF – Cereal: Legume: Fruit) was used. Other ingredients included sugar, margarine, skimmed milk powder, baking powder, eggs, vanilla essence and cinnamon powder were used in the cookie recipe. Sugar was creamed with the margarine to a fluffy consistency using a hand mixer (Geepas Hand Mixer – GM6127). Whole egg and vanilla essence were added to the creamed mixture and whisked to a soft texture. Flour and all the dry ingredients were sieved together and mixed gently with the creamed mixture to obtain a stiff paste. The dough obtained was rolled out on a board and cookie cutters used to shape out the cookies. Cut-out pieces of cookies were placed on a tray lined with parchment paper and baked at 180^0 C for 15-20 minutes. Cookies were cooled and stored in 500g plastic containers at ambient temperature (30 ± 2^0C) for further analysis.

3.4 ANALYTICAL METHODS

3.4.1 Physical Analysis of Cookies

Physical parameters of the cookies such as weight, thickness, diameter and the spread ratio (diameter/thickness) were estimated for all the 12 samples of the cookies as described by Kaur et al. (2019). Cookie diameter and thickness were determined using a Vernier caliper while the weights were determined using an electronic weighing balance. The average values obtained for the cookie sample replicates were recorded.

3.4.2 Color Analysis of Cookies

Color measurement of the cookie samples was carried out using a Colorimeter (Model NO PCE-CSM 1) fitted with an optical sensor. Samples were analyzed in triplicate and the mean values obtained within 24 hours after baking of the cookie samples. Color measurement was on the basis of CIE *L**, *a**, *b** system where the *L** values measure black to white (0-100), *a** values measure the redness when positive while the *b** values measure the yellowness when positive.

3.4.3 Chemical Analysis

Proximate composition of the cookie samples was analyzed according to AOAC (2012) methods. in terms of moisture content (method 925.10) and ash in a muffle furnace at 550^0C (method 923.03). Fat was extracted with petroleum ether by Soxhlet (method 922.06C). Protein determination was by the Kjeldahl method (method 920.87), crude fiber (method 920.86) and carbohydrate content (by difference method). Samples were ground into fine particles before each of the analysis.

3.4.4 In Vitro Protein Digestibility

In Vitro protein digestibility was done according to the method by Afify et al. (2012) with reference to ISO-16634-1 International Standard. One gram of the sample was added to 15ml (0.1M) HCl containing 1.5mg pepsin and incubated for 3 hours at 37^0C. 7.5 ml (0.2M) NaOH was then used to neutralize the obtained suspension which was then treated with 4 mg of pancreatin in 7.5 ml (0.2M) phosphate buffer (P^H 8.0). Toluene was added to prevent microbial growth and mixture was shaken and incubated for another 24 hours. After the incubation period, 10 ml of 10% TCA was added and centrifuged for 20 minutes to remove the undigested protein and larger peptides from the sample. Kjeldahl method (AOAC, 2012) was used to estimate the protein in the supernatant. Nitrogen in a blank sample was also estimated. Protein digestibility was calculated by the following formula;

$$Protein\ Digestibility\ \% = \frac{Nitrogen\ in\ Supernant - Nitrogen\ in\ Blank}{Nitrogen\ in\ Sample} \times 100$$

3.4.5 Vitamin C Determination

Estimation of Vitamin C in the samples was by the modified method of Barakat et al. (1955) which involved action of N-Bromosuccinimide on ascorbic acid. The procedure involved extraction of vitamin C from a known amount of solid sample with 10% TCA solution to obtain the extract or filtrate. In 5ml of the filtrate, 5ml of 4% KI solution and some drops of starch solution indicator were added. The mixture was then titrated against 0.1g/liter of N-Bromosuccinimide solution. Starch indicator solution was prepared by mixing 1g of soluble starch in 10ml of hot distilled water which was then cooled and made up to 100ml with saturated NaCl solution. N-Bromosuccinimide solution was prepared by dissolving 10mg of N-Bromosuccinimide in hot distilled water, cooling and topping up the solution to 100ml then stored at 40^0C.

3.4.6 Vitamin B_1 Determination

Vitamin B_1 in the samples was analyzed according to the AOAC method (953.17). 1.5 g of the sample was added to 100ml of 0.1N HCL and placed in a water bath at 100^0 C for 30 minutes. The cooled solution was topped up with HCL then filtered and centrifuged to obtain separate layers. Absolute alcohol was mixed with a solution of 5ml of potassium ferric-cyanide in sodium hydroxide. 10ml of toluene was added in the solution and centrifuged. Thiamine standard was also be prepared using the same process. The standard solution and the sample solution were read at 530 nm wavelength using a spectrophotometer.

$$Thiamine\ (^{mg}/_{100g}) = \frac{Absorbance\ of\ sample}{Absorbance\ of\ Standard} \times \frac{Weight\ of\ standard\ (mg)}{Weight\ of\ sample\ (g)} \times 100$$

3.4.7 Vitamin B_2 Determination

Vitamin B_2 was analyzed as per the method illustrated by Adeyeye et al. (2017). 1.5g of each of the cookie sample weighed and mixed with equal ratios of acetic acid and water (50:50) then heated in a 100^0 C hot water bath for 30 minutes. The mixture was cooled to 20^0 C and topped up with a solution of acetic acid mixed with water. Stirring of the mixture was done before being filtered in the dark. A standard riboflavin solution was also be prepared and both solutions will be read using the spectrophotometer according to the AOAC method.

$$Riboflavin\ (^{mg}/_{100g}) = \frac{Absorbance\ of\ sample}{Absorbance\ of\ Standard} \times \frac{Weight\ of\ standard\ (mg)}{Weight\ of\ sample\ (g)} \times 100$$

3.4.8 Statistical Analysis

Analysis was done in triplicate and all the data expressed as mean ± SD for each of the samples and data collected was subjected to analysis of variance (ANOVA) to determine the significant differences between the treatments. Mean values were compared by Tukey's LSD test ($p < 0.05$) using GenStat statistics software package version 15.

3.5 RESULTS

3.5.1 Physical Properties of the RTE cookies

The physical properties (weight, diameter, thickness and spread ratio) of the cookies are presented in **Table 3.3**. Weight of the cookies made from whole grain sorghum flour decreased significantly ($p \leq 0.05$) from 9.96 to 8.26. Weight of cookies from malted sorghum flour ranged from 10.40 g the highest to 8.97g while those from fermented sorghum flour decreased from 11.43g to 9.36g respectively. Spread ratio ranged from 9.82 (cookies with fermented sorghum flour and 30% green gram flour) to 13.68 (100% sorghum flour cookies which was the control) presenting a decreasing trajectory.

Table 3.3: Physical Properties of RTE Cookies from Sorghum-Green Gram Blends

Cookie Samples	Weight (g)	Diameter (mm)	Thickness (mm)	Spread Ratio
CLF 1 - Control	9.96 ± 0.15^{ef}	44.09 ± 0.07^{a}	3.22 ± 0.02^{a}	13.68 ± 0.07^{h}
CLF 2	9.76 ± 0.15^{de}	44.34 ± 0.10^{ab}	3.36 ± 0.01^{b}	13.18 ± 0.03^{g}
CLF 3	8.63 ± 0.15^{b}	44.67 ± 0.07^{cde}	3.56 ± 0.07^{c}	12.47 ± 0.28^{f}
CLF 4	8.26 ± 0.15^{a}	45.48 ± 0.05^{f}	3.89 ± 0.02^{ef}	11.67 ± 0.08^{e}
CLF 5	10.40 ± 0.10^{g}	44.26 ± 0.07^{ab}	3.79 ± 0.01^{d}	11.66 ± 0.06^{e}
CLF 6	9.69 ± 0.08^{cde}	44.34 ± 0.03^{ab}	3.84 ± 0.01^{de}	11.52 ± 0.04^{de}
CLF 7	9.49 ± 0.04^{cd}	44.29 ± 0.24^{ab}	3.92 ± 0.00^{ef}	11.28 ± 0.05^{d}
CLF 8	8.97 ± 0.13^{b}	44.63 ± 0.03^{cd}	3.97 ± 0.00^{f}	11.23 ± 0.02^{d}
CLF 9	11.43 ± 0.09^{h}	44.32 ± 0.02^{ab}	4.13 ± 0.01^{g}	10.72 ± 0.03^{c}
CLF 10	10.53 ± 0.04^{g}	44.44 ± 0.03^{bc}	4.24 ± 0.03^{h}	10.47 ± 0.09^{bc}
CLF 11	10.31 ± 0.06^{fg}	44.82 ± 0.02^{de}	4.35 ± 0.06^{i}	10.30 ± 0.13^{b}
CLF 12	9.36 ± 0.18^{c}	44.093 ± 0.02^{e}	4.57 ± 0.01^{j}	9.82 ± 0.01^{a}

Notes: Values represent mean ± standard deviation of (n=3) replications. Different superscripts in a column are significantly different ($p \leq 0.05$). CLF: Cereal-Legume-Fruit blend. WSF- Whole Grain Sorghum Flour, MSF- Malted Sorghum Flour, FSF- Fermented Sorghum Flour, GGF- Green Gram Flour, DMP-Dried Mango Powder.

Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF 4= 60%WSF:30%GGF:10%DMP, CLF5 =100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

3.5.2 Color Analysis of the Cookies

Tri-stimulus attributes L*, a* and b* values were used to express color of the cookie samples is presented in **Table 3.4**. Color affects the quality of the final product thus an important criterion to be considered. Significant differences were found in L* and b* values of the twelve cookie samples. No significant differences ($p > 0.05$) were found in a* values however, the values increased in each treatment corresponding to CLF 1- CLF 4 (from 8.24-8.81), CLF 5 – CLF 8 (from 9.27-9.46) and CLF 9 – CLF 12 (7.27-8.19) respectively. The L* (lightness) values decreased significantly ($p < 0.05$) ranging from 52.44-48.60 in cookies with whole grain sorghum flour, 54.91-50.07 in cookies with malted sorghum flour and 50.77-48.24 in cookies with fermented sorghum flour with increasing levels of green gram flour indicating darker products.

Table 3.4: Color Analysis of the RTE Cookies

Type of Cookie Sample	*L**	a*	b*
CLF 1 (Control -100%)	52.44 ± 0.01^{cd}	8.28 ± 0.01^{a}	8.13 ± 0.97^{ab}
CLF 2	51.12 ± 0.15^{c}	8.72 ± 0.82^{a}	9.66 ± 0.53^{abc}
CLF 3	50.43 ± 0.01^{abc}	8.73 ± 0.04^{a}	11.69 ± 0.75^{c}
CLF 4	48.60 ± 0.01^{ab}	8.81 ± 0.50^{a}	11.91 ± 0.67^{c}
CLF 5	54.91 ± 0.67^{e}	9.27 ± 0.57^{a}	9.59 ± 0.12^{abc}
CLF 6	$53.54 \pm 0.14d^{e}$	9.40 ± 1.29^{a}	9.78 ± 0.95^{abc}
CLF 7	51.02 ± 0.78^{c}	9.44 ± 0.21^{a}	10.34 ± 1.88^{bc}
CLF 8	50.07 ± 0.72^{abc}	9.46 ± 0.24^{a}	11.59 ± 1.14^{c}
CLF 9	50.77 ± 0.23^{bc}	7.27 ± 0.55^{a}	6.48 ± 0.50^{a}
CLF 10	48.56 ± 0.14^{ab}	7.41 ± 0.24^{a}	6.56 ± 0.59^{a}
CLF 11	48.36 ± 0.16^{ab}	7.61 ± 0.09^{a}	7.28 ± 0.07^{ab}
CLF 12	48.24 ± 1.64^{a}	8.19 ± 1.15^{a}	8.70 ± 0.20^{abc}

Notes: Values represent mean ± standard deviation of (n=3) replications. Different superscripts in a column vary significantly ($p \leq 0.05$). CLF: Cereal-Legume-Fruit blend. WSF- Whole Grain Sorghum Flour, MSF- Malted Sorghum Flour, FSF- Fermented Sorghum Flour, GGF- Green Gram Flour, DMP-Dried Mango Powder.

Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF 4= 60%WSF:30%GGF:10%DMP, CLF5 =100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

3.5.3 Effect of green gram flour addition on chemical composition of the cookies

The chemical composition of the different cookie samples produced from the different blends of sorghum flour, green gram flour and dried mango powder is presented in **Table 3.5.** Moisture content of the cookies was less than 10% which is generally low and therefore less likely to cause any adverse effects on the quality attributes of the end products thus prolonging their shelf life. Ash, protein, fat and fiber contents of the cookies increased with increased substitution with green gram flour while carbohydrates decreased significantly from 61.68 to 54.18%. Significant differences ($p<0.05$) were obtained in the protein content of the cookies which ranged from 9.52 to 13.60 %. Cookies from malted and fermented sorghum flour registered high protein contents as compared to whole grain sorghum which suggests that malting and fermentation of sorghum grains had a positive effect on the cookies. There were significant differences ($p<0.05$) in the fiber content which increased with increase in green gram flour and ranged from 9.40 to 10.90 %. According to **Table 3.5**, no significant differences were obtained in the fat content however it increased from 9.89 to 12.77% having the control sample CLF 1 (100% whole grain sorghum flour) registering the lowest fat content.

Table 3.5: Chemical Composition of the formulated RTE cookies

Cookie Sample	Moisture(%)	Ash (%)	Fat (%)	Protein (%)	Fiber (%)	Carbohydrate (%)
CLF 1	7.93±0.68[a]	1.56±0.40[a]	9.89±0.80[a]	9.52±0.11[a]	9.40±0.00[a]	61.68±0.36[b]
CLF 2	7.50±0.60[a]	1.90±0.10[ab]	12.26±1.27[a]	11.22±0.43[b]	9.56±0.11[a]	57.49±1.51[ab]
CLF 3	7.76±0.35[a]	1.80±0.10[ab]	11.33±0.72[a]	12.13±0.32[d]	9.63±0.05[a]	57.40±1.04[ab]
CLF 4	7.90±0.20[a]	1.88±0.07[ab]	11.51±1.04[a]	12.90±0.04[ef]	9.66±0.05[ab]	56.14±1.22[a]
CLF 5	7.76±0.05[a]	1.91±0.04[ab]	12.18±1.72[a]	10.93±0.53[b]	9.71±0.12[abc]	57.50±1.36[ab]
CLF 6	7.40±0.10[a]	1.92±0.02[ab]	12.77±2.22[a]	12.10±0.12[cd]	9.81±0.02[abc]	55.99±2.10[a]
CLF 7	7.63±0.20[a]	1.88±0.02[ab]	11.45±0.87[a]	12.59±0.03[de]	10.02±0.63[abc]	56.42±1.63[a]
CLF 8	7.63±0.21[a]	1.93±0.05[ab]	12.38±2.31[a]	13.01±0.21[ef]	10.87±1.18[bc]	54.18±2.48[a]
CLF 9	7.41±0.17[a]	1.83±0.05[ab]	11.22±0.79[a]	11.41±0.05[bc]	9.60±0.05[a]	58.52±0.63[ab]
CLF10	7.66±0.20[a]	1.99±0.10[b]	10.32±0.45[a]	12.49±0.02[de]	9.71±0.80[abc]	57.82±0.79[ab]
CLF11	7.60±0.10[a]	1.92±0.06[ab]	11.79±1.35[a]	12.79±0.07[de]	9.76±0.05[abc]	56.13±1.24[a]
CLF12	7.60±0.20[a]	2.01±0.47[b]	11.16±1.48[a]	13.60±0.16[f]	10.90±0.42[c]	57.75±2.05[ab]

Values with the same superscript(s) within the same column are not significantly different at 5% probability level. Values are mean ± standard deviation of triplicate determinations on a wet weight basis. CLF: Cereal-Legume-Fruit blend. WSF- Whole Grain Sorghum Flour, MSF- Malted Sorghum Flour, FSF- Fermented Sorghum Flour, GGF- Green Gram Flour, DMP-Dried Mango Powder.

Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF 4= 60%WSF:30%GGF:10%DMP, CLF5 =100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

3.5.4 Effect of malting and fermentation on *In Vitro* Protein Digestibility of cookies

The In Vitro Protein Digestibility (IVPD) of cookies from the different blends of cereal, legume and fruit powder flour are shown in **Figure 3.2**. In vitro protein digestibility of the cookies ranged from 67.75 ± 0.010 to 90.05 ± 0.10 %. In Vitro Protein Digestibility decreased significantly $p \leq 0.05$ in cookies from whole grain sorghum with the control sample (CLF 1) having 82.68% and

CLF 4 having 67.75%. Significant differences ($p \leq 0.05$) were obtained in cookies made from malted sorghum flour and fermented sorghum flour. Cookies from malted sorghum flour had the highest in vitro protein digestibility with values ranging from 90.05 ± 0.05% (CLF 5) to 86.44 ± 0.00% (CLF 8). Samples CLF 9, CLF 10, CLF 11 and CLF 12 from fermented sorghum flour registered values of 89.29 ± 0.29%, 88.40 ± 0.11%, 85.76 ± 0.18% and 84.99±0.22 % respectively.

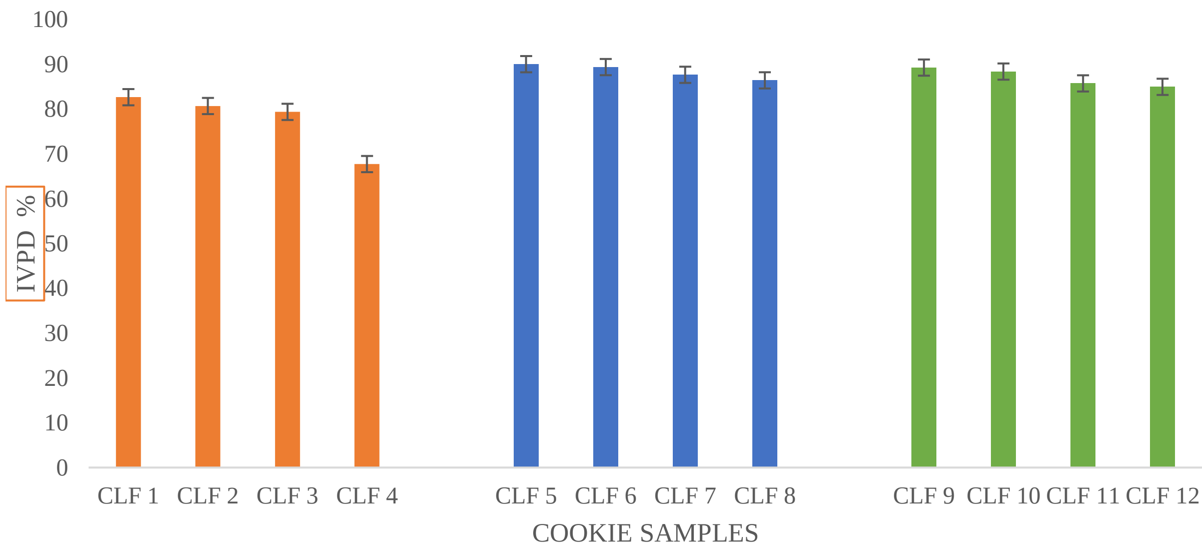


Figure 3.1: *In-Vitro* Protein Digestibility of cookies formulated. Values are means of three replicates (n=3) and error bars indicate standard deviations of the replicates. **WSF- Whole Grain Sorghum Flour, MSF- Malted Sorghum Flour, FSF- Fermented Sorghum Flour, GGF- Green Gram Flour, DMP-Dried Mango Powder.**

Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF 4= 60%WSF:30%GGF:10%DMP, CLF5 =100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

3.5.5 Vitamin Analysis

Table 3.6 shows the composition of vitamins mainly B_1 (Thiamine), B_2 (Riboflavin) and Vitamin C (*L*-Ascorbic Acid) of the cookies. Cookies made from whole grain sorghum (CLF 1, CLF 2, CLF 3, CLF 4) had a thiamine content that ranged from 0.22±0.02 to 0.41±0.01 mg/100g, riboflavin content ranging from 0.09±0.00 to 0.24±0.02 mg/100g while ascorbic acid ranged from 13.87±0.79 to 18.99±0.33 mg/100g respectively. L-Ascorbic Acid was however not detected in CLF 1 which contained 100% sorghum flour similar to CLF 5 (100% malted sorghum flour) and CLF 9 (100% fermented sorghum flour). Cookies made from malted sorghum flour (CLF 5, CLF 6, CLF 7, CLF 8) had a thiamine content that ranged from 0.34±0.00 to 0.52±0.00 mg/100g, riboflavin content ranging from 0.32±0.00 to 0.92±0.01 mg/100g while ascorbic acid ranged from 14.56±0.15 to 19.06±0.24 mg/100g. Finally, cookies from fermented sorghum flour (CLF 9, CLF 10, CLF 11, CLF 12) had thiamine contents ranged from 0.38±0.01 to 0.61±0.04 mg/100g, riboflavin from 0.36±0.00 to 1.39±0.04 mg/100g and ascorbic acid from 14.67±0.31 to 19.31±0.94 mg/100g respectively.

Table 3.6: Vitamin content of the sorghum-green gram RTE cookies

Type of Cookie Sample	Vitamin B1 (mg/100g)	Vitamin B2 (mg/100g)	Vitamin C (mg/100g)
CLF 1	0.22 ± 0.02^{a}	0.09 ± 0.00^{a}	ND
CLF 2	0.39 ± 0.02^{bc}	0.14 ± 0.00^{ab}	13.87 ± 0.79^{b}
CLF 3	0.40 ± 0.02^{bc}	0.19 ± 0.00^{bc}	17.77 ± 0.14^{cd}
CLF 4	0.41 ± 0.01^{bc}	0.24 ± 0.02^{c}	18.99 ± 0.33^{d}
CLF 5	0.34 ± 0.00^{ab}	0.32 ± 0.00^{d}	ND
CLF 6	0.40 ± 0.02^{bc}	0.49 ± 0.02^{e}	14.56 ± 0.15^{b}
CLF 7	0.46 ± 0.01^{bc}	0.73 ± 0.02^{f}	17.17 ± 0.39^{c}
CLF 8	0.52 ± 0.00^{cd}	0.92 ± 0.01^{g}	19.06 ± 0.24^{d}
CLF 9	0.38 ± 0.01^{bc}	0.36 ± 0.00^{d}	ND
CLF 10	0.46 ± 0.02^{bc}	0.72 ± 0.00^{f}	14.67 ± 0.31^{b}
CLF 11	0.52 ± 0.01^{cd}	0.92 ± 0.01^{g}	17.21 ± 0.33^{c}
CLF 12	0.61 ± 0.02^{d}	1.39 ± 0.04^{h}	19.31 ± 0.94^{d}

Values are mean ± standard deviation of duplicate determinations on a wet weight basis. Means on the same column with different sets of superscripts are statistically different ($p \leq 0.05$). Note: ND = Not Detected, WSF- Whole Grain Sorghum Flour, MSF- Malted Sorghum Flour, FSF- Fermented Sorghum Flour, GGF- Green Gram Flour, DMP-Dried Mango Powder.

Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF 4= 60%WSF:30%GGF:10%DMP, CLF5 =100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

3.6 DISCUSSION

3.6.1 Physical Properties of the RTE cookies

The decreasing trend in weight of the cookies was attributed to the increasing fiber content in the cookies as a result of sorghum and green gram flour blend. Diameter and thickness of the cookies increased significantly with each addition of the green gram flour in each treatment. Spread ratio decreased with increasing substitution of green gram flour in the cookies. A similar trend of decreased spread ratio with addition of green gram flour was reported by Rajiv and Soumya (2015) in biscuits and by Yousaf et al. (2012) in cookies. Decrease in spread ratio and increase in thickness is due to attrition of high protein and bran sources in cookies and biscuits has been reported by different schools of thought (Okpala et al., 2013, Chinma and Gernah, 2007, Thongram et al., 2016). Hydrophilic nature of the flour which increased dough viscosity limiting spread in cookies and increasing thickness (Shukla et al., 2016). Increased viscosity is due to the protein network which on heating during the baking process undergoes glass transitioning thus gaining mobility which allows the proteins to interrelated forming a web (Thongram et al., 2016).

3.6.2 Color Analysis of the Cookies

Decrease in L* values with increase in green gram flour was as result of the higher protein content in green grams as presented in **Table 3.4**. Previous reports have shown a similar result of decreased L* values with increased protein content (Bazaz et al., 2016), (Bhise and Kaur, 2013). Protein content is correlated negatively with lightness in a baked product due to Maillard reaction which plays a paramount role in color formation due to the interaction of protein and sugar during baking influencing the brown color and organoleptic properties in baked products (Ostermann-Parcel et al., 2017). The redness value (a*) and yellowness values (b*) increased with higher substitution of green gram flour. Increase in similar values with addition of green gram flour was also reported by Rajiv et al. (2013).

3.6.3 Effect of green gram flour addition on chemical composition of the cookies

Microbial spoilage has been linked to high moisture which could lead to food spoilage. There were no significant differences ($p>0.05$) in moisture contents of cookies made from whole grain, malted and fermented sorghum flour substituted with green gram flour and fruit powder ranging from 7.40 to 7.93 %. Similar ranges of moisture have been reported other researchers (Onwurafor et al., 2019) in cookies incorporated with unripe plantain and mung bean malt flours. Protein results were similar to those of Rajiv et al. (2012) where protein content in green gram cookies ranged between 10-14%. On average, the Recommended Dietary Allowance (RDA) for protein is 0.8g/kg body weight/day regardless of the age bracket however, infants and children require much more (2-3g/kg body weight/day) for growth and development (Yousaf et al., 2012).

Green gram flour can easily be used to increase the protein content of different types of food products (Tsen et al., 2006). Increased ash content was attributed to high mineral content in green grams such as potassium, phosphorus, magnesium, calcium, sodium and iron (Bazaz et al., 2016). Ash content is an indication of minerals in a food sample. Cereals contain low levels of ash which is in contrast to legumes which are good sources of ash (Dabels et al., 2016). Similar increase in ash and protein contents has been reported in blends with increasing amounts of legumes such as green grams (Rajiv and Soumya, 2015), pigeon peas (Okpala et al., 2013), soybeans and kidney beans (Ratnawati et al., 2019). Substitution with green gram flour increased fiber content in cookies. Green grams contain high levels of fiber which makes green grams important in diet and weight management.

According to Chavan and Patil (2013), 15g of fiber is provided by a one cup serving of boiled green grams which is over 60% of the daily minimum recommended amount. Increased fiber content due to incorporation of sorghum flour in bread and cookies was reported by Chavan et al. (2016). Dietary fiber plays significant roles in human nutrition with psychological effects such as blood glucose attenuation, blood cholesterol attenuation and laxation and prevention of obesity (Slavin, 2005). Increasing trend in fat with addition of green gram flour has also been reported by Bazaz et al. (2016), Hussain and Uddin (2011). Carbohydrates results show that green gram flour is low in carbohydrate and the same is reported by Dabels et al. (2016). Low carbohydrate cookies in this study is paramount to enhanced health for obese and overweight people.

3.6.4 Effect of malting and fermentation on *In Vitro* Protein Digestibility of cookies

Figure 3.2 shows fermentation and malting processes improved the IVPD of the cookies as compared to cookies made from whole grain sorghum flour despite substitution with green gram flour. Similar improvements in IVPD were observed after fermentation of sorghum flour during processing of injera (Mohammed et al., 2011). Increase in in vitro protein digestibility after germination of sorghum varieties was also reported by Afify et al., (2012). Low protein digestibility in sorghum is due to anti-nutrients which inhibit protein digestion and resistance of the seed storage protein known as kafirins to protease digestion. Germination of sorghum causes activation of intrinsic proteases, phytases, amylases as well as fiber-degrading enzymes leading to increased nutrient digestibility which increases in vitro protein digestibility (Correira et al., 2010). Enzymatic activity triggered during malting leads to breakdown of proteins, lipids and carbohydrates into simpler forms.

Fermentation eliminates antinutrients improving functional, physicochemical and nutritional properties of sorghum flour (Rahayu et al., 2019). Cookies with highest protein content had the least in vitro protein digestibility while those with the least protein had high protein digestibility. High protein content therefore does not necessarily imply high protein digestibility as reported in various studies (Kiin-Kabari and Giami, 2015). Addition of green gram flour decreased the protein digestibility due to various antinutrients such as phytic acid and tannins as explained by Mubarak, 2005. Similar decreasing trend in in vitro protein digestibility has also been reported by Bazaz et al. (2016) in hypoallergic complementary food from rice incorporated with sprouted green gram flour.

3.6.5 Vitamin Analysis

Thiamine in green grams ranges between 0.12-0.7 mg/100g with an average of 0.5mg/100g and a riboflavin average of 0.3mg/100g ranging between 0.23-0.47 mg/100g (Dahiya et al., 2015). Green gram substitution led to increased L-Ascorbic acid content which ranged between 13.87±0.79 – 19.31±0.94 mg/100g as represented in **Table 3.6.** According to Dahiya et al. (2015), ascorbic acid in green grams has a maximum of 10 mg/100g with an average of 3.1 mg/100g. Samples CLF 1, 5 and 9 did not record any trace of ascorbic acid since sorghum has been reported to contain no ascorbic acid. The high L-Ascorbic Acid contents could also be attributed to the enrichment of the cookies with dried mango powder. Mango fruit (*Mangifera indica*) is rich in L-Ascorbic Acid which ranges from 7.8 to 172 mg/100g (Caparino et al., 2017).

Fermentation has been reported to influence the production of vitamin B-complex by different schools of thought (Obadina et al., 2013). Results of fermented cookies are almost similar to those of Adeyeye et al. (2017). General functions of thiamine are in assisting the body to produce energy as well as protect the mucous membrane and nervous system while riboflavin maintains a healthy digestive system, healthy red blood cells, skin and vision (Duru et al., 2012, Ndung'u et al., 2015). Vitamin C contents are however lower than those reported by Nilugin et al. (2015) in cookies substituted with 25% mango flour. High amounts of sugar, considerable amounts of vitamin C and provitamin A in mango makes it a fruit of high commercial value with added health benefits such as prevention of scurvy (Fenech et al., 2019). Reduces risks of cardiovascular diseases and certain cancers has been linked to increased consumption of mango fruit (Adepoju and Osunde, 2017).

3.7 CONCLUSION

Cost effective methods such as fermentation and malting of sorghum grains can be used to improve the nutrient composition in cookies in an effort to develop quality functional foods. Addition of green gram flour to baked products can be used to boost the protein and vitamin contents of the products. *In Vitro* Protein Digestibility is an important parameter in product development as it measures the quality of protein depending on the amino acids present. Malting and fermentation significantly improve Protein Digestibility and thus can be used to produce quality food products. Combination of a cereal-legume -fruit flour blend will increase utilization of neglected crops such as sorghum and green grams as well as ensure healthy quality snacks such as cookies which due to the abundance of nutrients can help in countering protein energy malnutrition especially in developing countries.

CHAPTER FOUR: BIOCHEMICAL AND ANTI-NUTRIENT COMPOSITION OF SORGHUM-GREEN GRAM READY TO EAT COOKIES SUPPLEMENTED WITH MANGO POWDER

4.1 ABSTRACT

Chemical compositional analysis of cereal-based products is mainly focused on their proximate compositions with limited reports on the phytochemicals and bioactive compounds including anti-nutrients and their health promoting properties. With increased interest on better health over the years, sorghum is growing prominence due to its nutritional and health benefits. Therefore, the present study determined the mineral composition, total phenolic contents and anti-nutrients (phytic acid and tannins) in sorghum-green gram cookies enriched with dried mango powder. Whole sorghum grains, malted and fermented sorghum flour and the effect of malting and fermentation processes on the bioactive compounds in the cookies were incorporated in the experimental designs and tested. The results showed significant differences ($p < 0.05$) in mineral contents of the cookies from whole grain, malted and fermented sorghum flour. Zinc and Iron mineral contents were highest in cookies from fermented sorghum flour than at whole grain and malted sorghum flour at 4.64 mg/100g and 2.69 mg/100g. Calcium mineral content was highest in the cookies from malted sorghum flour at 44.48 mg/100g. Malting and fermentation of sorghum significantly ($p < 0.05$) lowered the phytate content by 36.62% and 41.10% and tannin content by 16.16% and 25.36% respectively. In malted cookies, phytates ranged between 24.63 – 25.4 mg/100g and tannins between 150.5 – 152.5 mg/100g. Phytate content ranged between 22.89-23.77mg/100g whereas that of tannins ranged between 133.9 - 135.1 mg/100g in fermented cookies. Significant differences ($p < 0.05$) were given by phenolic content of the cookies increasing significantly from 338.3 mg/100g to 427.2 mg/100g. Cookies from fermented sorghum flour registered the highest values of (406.9 – 427.2 mg/100g). The study findings indicate that malting and fermentation as processing techniques significantly improve the antioxidant properties and lowers the anti-nutrients in baked products to produce healthy nutritional cookies.

Keywords: Sorghum-based Product**,** Minerals, Anti-nutrients, Total Phenolic, Cookies.

4.2 INTRODUCTION

Sorghum (*Sorghum bicolor (L.) Moench*) is the second most important cereal crop after maize (Rao et al., 2016). Sorghum is an excellent source of minerals such as iron, zinc and B-complex vitamins which play a vital role in energy metabolism. According to Rao et al. (2016), mineral bioavailability in sorghum ranges from less than 1% to more than 90% hence the richness in minerals. It has numerous properties such as the absence of gluten and a relatively low glycemic index (Adebo, 2020) making it attractive to modern day consumers. These properties are crucial to patients with diabetic and celiac health issues. Infante et al., 2017, Momanyi et al., 2020 and Istianah et al., 2018 have emphasized the nutritional and functional benefits of sorghum as the presence of bioactive compounds.

As compared to other cereals, sorghum has a wide range of health benefits due to presence of the polyphenols (Girard and Awika, 2018). Flavonoids, phenolic acids and condensed tannins are among the phenolic compounds found in sorghum (Adebo, 2020). These bioactive compounds promote beneficial changes with relation to non-communicable diseases (NCDs) such as hypertension, diabetes, obesity, cancer, cardiovascular diseases and dyslipidemia (Cardoso et al., 2015). Epidemiological studies have associated sorghum consumption with health benefits such as reduction of gastrointestinal cancer (Stefoska-Needham et al., 2015) and in the control of diabetes as a result of reduction of glucose and inulin responses (Poquette et al., 2014). Phenolics in sorghum have antioxidant activities that are superior to other cereals such as millet, wheat, rye and barley (Chiremba et al., 2009). Antioxidant compounds provide protection against life-threatening diseases by scavenging for free radicals in the human body (Siddiq and Prakash, 2015). Despite some being beneficial, tannins are also the major anti-nutrients in sorghum which reduce starch and protein digestibility however, various processing methods such as fermentation have been reported to reduce the anti-nutrients releasing bound nutrients (Adebo and Medina-Meza, 2020, Girard and Awika, 2018).

Legumes provide great nutritional benefit from their carbohydrates, proteins, vitamins and minerals contents and are a rich source of calcium and iron (Thongram et al., 2016). Thongram et al., (2016) reported that despite the high nutritional quality of these legumes, most of the locally available legumes such as green grams, pigeon peas, cowpeas and chickpeas are still underutilized. Relatively high amounts of minerals such as calcium, copper, iron, zinc, potassium are present in green grams (Chandra and Samsher, 2013). Anti-nutritional factors such as such as tannins, phytic acid, hemagglutinins, polyphenols and trypsin inhibitors are found in green grams according to various literature reviewed (Dahiya et al., 2015, Kamboj and Nanda, 2017, Dhole and Reddy, 2015).

Food fortification and different processing techniques are necessary approaches to improve nutritional quality of food and overcome micronutrient malnutrition (Oghbaei and Prakash, 2017). Processing of cereals and legumes uses different techniques such as malting and fermentation which are common practices in African households (Nkhata et al., 2018). Malting changes the biochemical modification of grains improving their bioactive and nutritional quality (Dahiya et al., 2018). Fermentation process improves color, flavor, nutritional value and subsequently preservation of food (Chineye et al., 2017). Despite the numerous health benefits of malting and fermentation, there are still limited baked products in the market that contain malted or fermented flours. Adoption of these techniques in baked products can only be done if aspects of nutritional quality and consumer acceptability are achieved. Therefore, the objective of this work was to produce high nutritional quality cookies from sorghum flour, green grams and dried mango powder and determine the effect of malting and fermentation process on mineral and bioactive compounds in the final products. The outcome of this study may provide opportunities to advocate for the use of cereal-legume-fruit incorporated cookies especially to vulnerable populations like malnourished children towards addressing food and nutritional security.

4.3 MATERIALS AND METHODS

4.3.1 Sample Collection

Sorghum (*Sorghum bicolor (L) Monench*), green grams (*Vigna radiata*) and ripe mangoes (*Mangifera indica L*.) were purchased from a local open-air market in Kangemi, Nairobi-Kenya. All the other ingredients (margarine, sugar, skimmed milk powder, vanilla essence, baking powder, salt) used in the baking of the cookies were purchased from the local retail outlets. All materials were transported to the department of Food Science, Nutrition and Technology at the University of Nairobi, Kenya where the study was conducted.

4.3.2 Malting of Sorghum

Sorghum (*Sorghum bicolor (L) Monench*) was manually cleaned to remove damaged grains, husks and stones. To suppress mold growth, the grains were washed in 5% (w/v) sodium chloride solution after which they were steeped in tap water in ratio 1:2 (w/v) at room temperature (32 ± 2^0C) for 12 hours. After steeping, water was drained and the grains were allowed to germinate between two damp cloths at ambient temperature for 72 hours as described by Ouazib et al. (2016). After every 12-hour interval, water was sprinkled to facilitate the germination process. The germination time and the temperature chosen for this study were chosen from previous studies (Okpala et al., 2013, El-Moneim et al., 2017, Bello et al., 2020, Olamiti et al., 2020). At the end of the germinating period, the grains were dried in the oven (Schutzart DIN 40050 – IP20) at 65^0C for 9 hours followed by removal of the dried sprouts on the grains manually (Siddiqua et al., 2019). The germinated sorghum grains were milled into flour using a hammer miller and passed through a 1mm sieve to obtain fine sorghum flour of uniform particle size free from clumps. The obtained flour was stored in an airtight container stored at room temperature for further analysis.

4.3.3 Fermentation of Sorghum

Sorghum (*Sorghum bicolor (L) Monench*) was sorted manually to remove damaged seeds and stones. The grains were then washed and dried then milled into flour using a hammer mill. The milled flour was subjected to spontaneous fermentation by microflora (lactic acid bacteria) naturally present in the grains. Sorghum flour was fermented according to the method described by Elkhalifa et al. (2017). Flour was mixed with water (1:2 w/v) to form a slurry which was incubated at 37^0C for 48hrs in sterile covered flask. After the fermentation period, excess water was removed and the fermented slurry was oven (Schutzart DIN 40050 – IP20) dried at 65^0C for 8hrs and then ground into flour. The fermented ground flour was passed in a 0.4mm sieve and stored in an airtight container until needed for analysis.

4.3.4 Preparation of Whole Grain Sorghum Flour

Sorghum (*Sorghum bicolor (L) Monench*) was sorted out to remove excess dirt and thoroughly washed as per the method reported by Adeyeye, 2016. The cleaned grains were oven (Schutzart DIN 40050 – IP20) dried at 65^0C for 8 hours then milled using a commercial hammer mill into fine flour. The dried flour was packaged in a clean airtight container for further use.

4.3.5 Preparation of Green Gram Flour

Green grams (*Vigna radiata*) flour was produced using the method of Dabels et al. (2016). The grains were manually cleaned to remove all the dirt then washed with clean water. The clean seeds were then spread on tray and placed in an oven (Schutzart DIN 40050 – IP20) dried at 65^0C to dry for 4 hours. The dried green grams were then milled into fine flour using a commercial hammer miller (GM25- TEFC). The flour was then passed through a 1mm sieve and packed in a clean airtight container for further use.

4.3.6 Drying of Mango (*Mangifera indica L.*) Fruits

In preparation of the dried mango powder, the method of Sengev et al. (2015) was adopted with slight modifications. Twenty large-sized ripe mangoes bought from the market were sorted out, washed, peeled and the mesocarp was sliced manually to a thickness of 2.0mm. The thin slices were spread on a tray lined with aluminum foil and oven (Schutzart DIN 40050 – IP20) dried at 65^0C for 24 hours. The dried slices were then ground (350W- 220V-AK 300, Nunix 2 in 1 Blender) into powder using a grinder and sieved through a 0.5mm sieve to obtain the dried mango powder that was stored in an airtight container stored at room temperature.

4.3.7 Experimental Design and Sample Formulation

The experiment was laid out as a 3×4 factorial design with three levels of sorghum flour (whole grain, malted, fermented) and four levels of green gram flour and dried mango powder substitution (100:0.0, 80:10:10, 70:20:10, 60:30:10) for each of the treatments as represented in **Table 4.2**. The ratio of the flour blends was labelled CLF (Cereal: Legume: Fruit) to represent the sorghum: green gram: mango powder ratio in the formulations **(Table 4.2).** Sample CLF1 with 100% whole grain sorghum flour served as the control sample. Formulation (**Table 4.1)** was done based on the recommended minimum levels of protein, fiber, fat by FAO/WHO which were achieved using NutriSurvey Linear Programming Package 2004 version (Amegovu et al., 2013).

Table 4.1: Formulation of Ingredients Used in the Sorghum-Green Gram Cookies

Ingredients	**Amount (grams)**
Flour *	200
Sugar	50
Margarine	50
Salt	0.35
1 Whole Egg	30
Skimmed Milk Powder	8
Baking Powder	1.25
Vanilla Essence	1.25

*Sorghum flour, green gram flour and dried mango powder

Table 4.2: Ratio of the Flour Blends

Flour Blends	**Whole-Grain Sorghum Flour (%)**	**Malted Sorghum Flour (%)**	**Fermented Sorghum Flour (%)**	**Green Gram Flour (%)**	**Dried Mango Powder (%)**
CLF 1	100	0	0	0	0
CLF 2	80	0	0	10	10
CLF 3	70	0	0	20	10
CLF 4	60	0	0	30	10
CLF 5	0	100	0	0	0
CLF 6	0	80	0	10	10
CLF 7	0	70	0	20	10
CLF 8	0	60	0	30	10
CLF 9	0	0	100	0	0
CLF 10	0	0	80	10	10
CLF 11	0	0	70	20	10
CLF 12	0	0	60	30	10

Key: CLF = Cereal: Legume: Fruit Blend

4.3.8 Formulation of the RTE Cookies

The ingredients used in the sorghum-green gram cookie preparation included the different ratios of the sorghum flour (whole grain, malted and fermented), green gram flour and mango fruit powder. The cookies were prepared according to the American Association of Cereal Chemists (AACC, 10-50D) with slight modifications. For each of the experiment, 200g of the blended flour (CLF – Cereal: Legume: Fruit) was used. Other ingredients included sugar, margarine, skimmed milk powder, baking powder, eggs, vanilla essence and cinnamon powder were used in the cookie recipe. Sugar was creamed with the margarine to a fluffy consistency using a hand mixer (Geepas Hand Mixer – GM6127). Whole egg and vanilla essence were added to the creamed mixture and whisked to a soft texture. Flour and all the dry ingredients were sieved together and mixed gently with the creamed mixture to obtain a stiff paste. The dough obtained was rolled out on a board and cookie cutters used to shape out the cookies. Cut-out pieces of cookies were placed on a tray lined with parchment paper and baked at 180^0 C for 15-20 minutes. Cookies were cooled and stored in 500g plastic containers at ambient temperature (30 ± 2^0C) for further analysis.

4.4 ANALYTICAL METHODS

4.4.1 Mineral Content Analysis

The cookie samples were analyzed for iron, zinc and calcium contents using the Atomic Absorption Spectrophotometer according to the AOAC (2006) method 984.27. About 2g of the sample was dry ashed for 10 hours at 550^0 C in a muffle furnace as described by Paucean et al. (2018) and the ash dissolved using 10ml of 20% HCL. The solution was dispensed into a volumetric flask and the volume made up to 50ml and the macro elements were determined by the Atomic Absorption Spectrophotometer.

4.4.2 Determination of Phytates

Phytate content was analyzed as per the method described by Latta and Eskin (1980) with some slight modifications. 1 g of the sample was defatted by adding 10ml of petroleum ether and left to stand for 30 minutes then centrifuged at 13,000 rpm for 5 minutes. The supernatant was then discarded and residue air dried. The residue was extracted with 10ml of 2.4% Hydrochloric Acid and centrifuged for 10 minutes. The residue was further re-extracted thrice and all the supernatants (40ml) were pooled together and made up to a 100 ml volume with distilled water. 2ml of the extract was diluted with 6ml of distilled water and 2ml Wade reagent (mixture of 0.03% Iron chloride and 0.3% Sulfosalicylic acid) was added. The contents were vortexed (Vortex-Genie) for 5 minutes and absorbance of supernatant measured at 500nm using UV/VIS spectrophotometer (Perkin Elmer UV/VIS Spectrometer Lambda 2). Phytate content was expressed in mg/100g using phytic acid curve prepared and described by Latta and Eskin, (1980).

4.4.3 Determination of Tannins

Tannins were determined using Folin Denis reagent method as described by Makkar et al. 1993. Folin-Denis reagent was prepared as per Ferreira et al. (2004). 0.5g of sample was extracted with 50ml of distilled water, vortexed for 5 minutes and left to decant. 2ml of Folins-Denis reagent was added to 50ml of distilled water in a volumetric flask. 2ml of the sample solution and 5ml of concentrated sodium carbonate solution were added and topped up with distilled water. Mixture was properly mixed and allowed to stand for 40 minutes after which the absorbance was determined at 725nm using UV/VIS spectrophotometer (Perkin Elmer UV/VIS Spectrometer Lambda 2).

4.4.4 Determination of Total Free Phenolics

Phenolic content was analyzed according to the method outlined by Singleton et al. (1999) which involves use of Folin-Ciocalteu reagent. 0.2g of sample was treated with 2ml of petroleum ether in ultra-sonic bath for 30 minutes for defatting then centrifuged at 13,000 rpm for 3 minutes and supernatant was discarded. Defatted pellets were extracted with 2ml of 80% methanol, 2ml of 50% methanol and 2ml of 70% acetone acidified with 1% concentrated Hydrochloric acid in ultra-sonic bath for 30 minutes. Supernatants were pooled and made to a known volume of 10ml by topping up with 4ml distilled water. 3 ml of the supernatant was added to 1.5 ml of Folin-Ciocalteus phenol reagent (10% v/v) and allowed to react for 5 minutes. 1.2 ml of sodium carbonate was added to the mixture and incubated for 30 minutes. The resulting blue complex was measured at 765 nm and amount of total phenolics in the extract was calculated based on the standard curve prepared with gallic acid and expressed in mg/100g sample on dry matter basis.

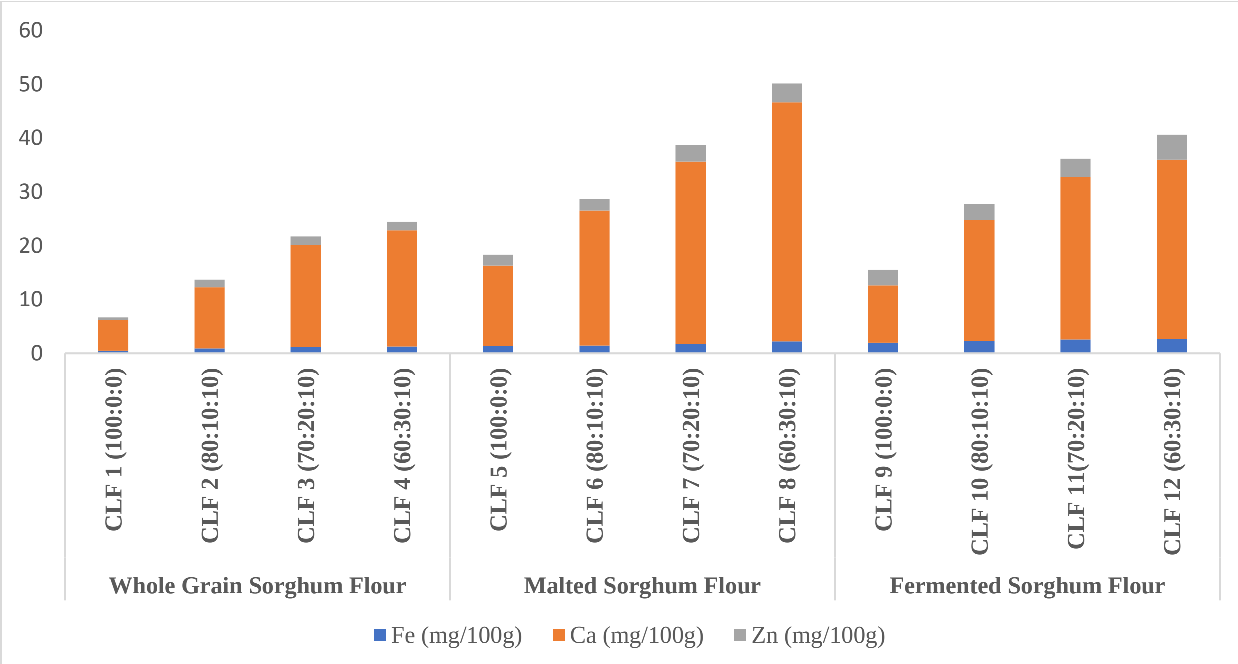
4.4.5 Statistical Analysis

All experiments in this study were carried out in triplicate and expressed as mean ± standard deviation. Statistical analysis of the data was by analysis of variance (ANOVA) at 5% significance level using the Genstat Version 15 and multiple comparisons done using the Tukeys' Honest Significance Difference post hoc test.

4.5 RESULTS

4.5.1 Mineral Contents of the RTE Sorghum-Green Gram Cookies

The iron, calcium and zinc contents of the cookies from blends of sorghum flour (whole grain, malted and fermented), green gram flour and dried mango powder are shown in **Figure 4.1.** on a dry weight basis**.** Significant differences ($p < 0.05$) were obtained in the mineral contents of the cookies prepared. The iron contents of the RTE cookies varied from 0.5 mg/100g in the control sample with 100% whole grain sorghum flour to 2.69 mg/100g in sample CLF 12 with 60% of fermented sorghum flour and 30% green gram flour. This was the lowest detected mineral in the formulated cookies as compared to the other minerals. The calcium and zinc contents of the cookies varied significantly from 5.71 mg/100g to 33.31 mg/100g and from 0.5 mg/100g to 4.64 mg/100g respectively. Iron and zinc contents were highest in fermented cookies at 60% (2.69 ± 0.02 mg/100g), and 60% (4.64 ± 0.08 mg/100g) respectively. Calcium content was highest in cookies made from malted sorghum flour at 60% (44.48 ± 0.13 mg/100g).



CLF: Cereal-Legume-Fruit blend.

Figure 4.1: **Mineral Contents of RTE Sorghum-Green Gram Cookies**

[a]CLF: Cereal-Legume-Fruit blend. [b]WSF- Whole Grain Sorghum Flour, MSF- Malted Sorghum Flour, FSF- Fermented Sorghum Flour, GGF- Green Gram Flour, DMP-Dried Mango Powder.

Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF 4= 60%WSF:30%GGF:10%DMP, CLF5 =100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

4.5.2 Anti-nutritional Factors of RTE Sorghum-Green Gram Cookies

The results of anti-nutritional factors in the developed sorghum-green gram cookies are presented in **Table 4.3.** The results show a significant decrease in the phytates and tannins content of the developed cookies. Phytate content ranged from 22.89 mg/100g in cookies with 60% fermented sorghum flour to 28.70 mg/100 in cookies with 100% whole grain sorghum flour. Highest values in phytate content were seen in cookies from whole grain sorghum flour whereas decreased levels were seen in cookies from both malted and fermented sorghum flour with those from fermented sorghum flour having the lowest values. Significant differences ($p < 0.05$) were obtained in phytate contents of cookies from each treatment. Tannin content in the cookies ranged from 133.9 mg/100g to 16.49 mg/100g. Cookies from malted and fermented sorghum flour had lower tannin values as compared to those from whole grain sorghum flour.

Table 4.3: Anti-nutritional Factors of RTE Sorghum-Green Gram Cookies

SAMPLES[a]	Phytates (mg/100g)	Tannins (mg/100g)
A (100% Raw Sorghum Flour)	38.86 ± 0.19^{k}	179.4 ± 0.02^{f}
B (100% Raw Green Gram Flour)	6.24 ± 0.04^{b}	100.9 ± 0.07^{b}
C (100% Dried Mango Powder)	1.45 ± 0.01^{a}	1.24 ± 0.05^{a}
1. Whole Grain Sorghum		
CLF 1 (100:0:0)	28.70 ± 0.03^{j} (26.15)	169.4 ± 1.41^{e} (5.57)
CLF 2 (80:10;10)	27.94 ± 0.09^{i} (28.10)	168.5 ± 2.12^{e} (6.08)
CLF 3 (70:20:10)	27.41 ± 0.09^{h} (29.46)	167.2 ± 1.88^{e} (6.80)
CLF 4 (60:30:10)	26.98 ± 0.03^{h} (30.57)	166.5 ± 0.70^{e} (7.19)
2. Malted Sorghum		
CLF 5 (100:0:0)	25.40 ± 0.11^{g} (34.64)	152.5 ± 0.00^{d} (14.99)
CLF 6 (80:10:10)	25.27 ± 0.38^{g} (34.97)	152 ± 0.33^{d} (15.27)
CLF 7 (70:20:10)	24.98 ± 0.02^{fg} (35.72)	150.9 ± 0.02^{d} (15.89)
CLF 8 (60:30:10)	24.63 ± 0.05^{f} (36.62)	150.4 ± 0.02^{d} (16.16)
3. Fermented Sorghum		
CLF 9 (100:0:0)	23.77 ± 0.02^{e} (38.83)	135.1 ± 0.16^{c} (24.69)
CLF 10 (80:10:10)	23.50 ± 0.13^{e} (39.53)	134.8 ± 0.14^{c} (24.86)
CLF 11 (70:20:10)	23.09 ± 0.13^{cd} (40.58)	134.1 ± 0.12^{c} (25.25)
CLF 12 (60:30:10)	22.89 ± 0.00^{c} (41.10)	133.9 ± 0.02^{c} (25.36)

Values represent mean ± standard deviation of (n=3) replications. Different superscripts in a column are significantly different ($p \leq 0.05$). Values in parenthesis indicate percentage change of phytates and tannins due to processing. [a]CLF: Cereal-Legume-Fruit blend. [b]WSF- Whole Grain Sorghum Flour, MSF- Malted Sorghum Flour, FSF- Fermented Sorghum Flour, GGF- Green Gram Flour, DMP-Dried Mango Powder.

Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF 4= 60%WSF:30%GGF:10%DMP, CLF5 =100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

4.5.3 Effect of malting and fermentation on antinutrient content in formulated cookies

Figure 4.2 shows the percentage change in antinutrients as a result of processing. From the graph, the percentage decrease in antinutrients was highest in fermented cookies compared to malted and whole grain cookies. Tannin content decreased by 25.36% as phytate content decreased by 41.10%. Highest decrease in malted cookies was by 36.62 %(phytate) and 16.16%(tannin). Cookies made from wholegrain sorghum flour registered lower values of 30.57%(phytate) and 7.19%(tannin) changes respectively.

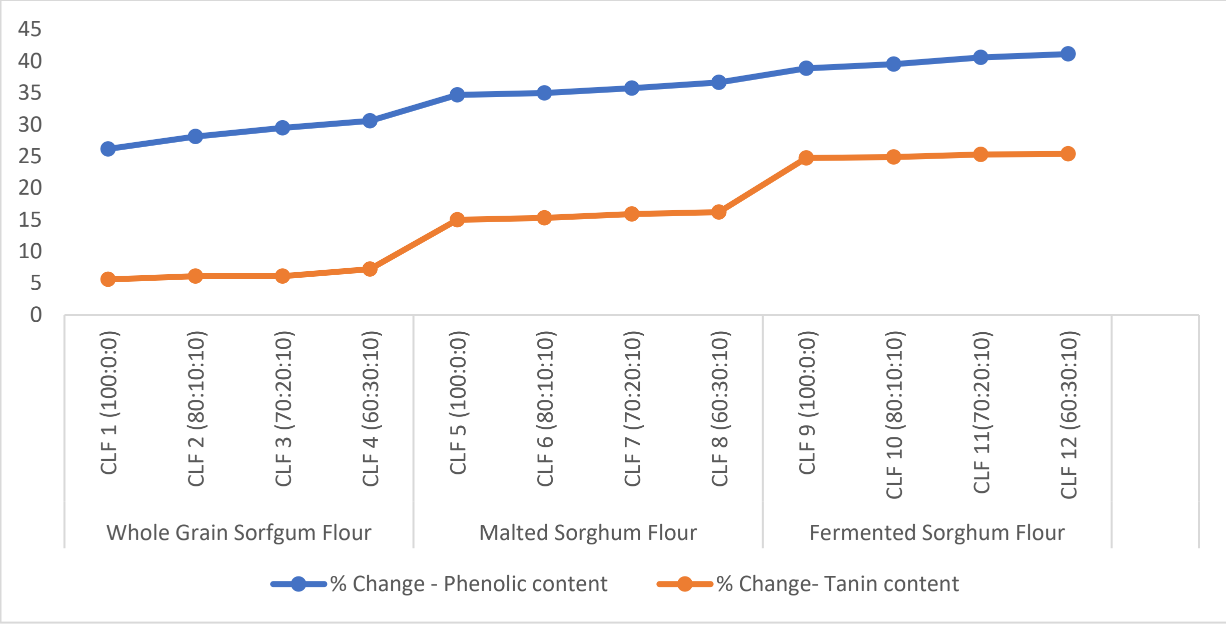


Figure 4.2 Effect of processing on antinutrient content in cookies

[a]CLF: Cereal-Legume-Fruit blend. [b]WSF- Whole Grain Sorghum Flour, MSF- Malted Sorghum Flour, FSF- Fermented Sorghum Flour, GGF- Green Gram Flour, DMP-Dried Mango Powder..

Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF 4= 60%WSF:30%GGF:10%DMP, CLF5 =100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

4.5.4 Phenolic Contents in the RTE Sorghum-Green Gram Cookies

Table 4.4 shows the phenolic contents in the developed cookies. A significant increase ($p < 0.05$) can be seen in the phenolic content of the cookies where the values increased significantly from 338.3 mg/100g to 427.2 mg/100g. Phenolic content in cookies from fermented sorghum flour registered highest values ranging from 406.9 mg/100g to 427.2 mg/100g. Cookies from malted sorghum flour registered the lowest values of between 338.3 mg/100g to 363.7 mg/100g. Values of 355.4 mg/100g – 382.3 mg/100g were found in cookies from whole grain sorghum flour. Significant differences ($p < 0.05$) were observed in the individual cookies prepared. Highest phenolic contents were observed in samples whole grain sorghum (60:30:10) and fermented sorghum (60:30:10). Figure 4.3 showed the phenolic content trend upon different processing methods.

Table 4.4: Phenolic Contents in the RTE Sorghum-Green Gram Cookies (mg/100g)

Formulation	Whole Grain Sorghum	Malted Sorghum	Fermented Sorghum
100:0:0	355.4 ± 0.49^{d}	338.3 ± 0.31^{b}	406.9 ± 0.84^{j}
80:10;10	362.5 ± 0.01^{g}	347.8 ± 0.04^{c}	415.7 ± 0.00^{k}
70:20:10	371.8 ± 0.23^{h}	356.9 ± 0.00^{e}	419.9 ± 0.85l
60:30:10	382.3 ± 0.04^{i}	363.7 ± 0.43^{g}	427.2 ± 0.16^{m}

Values (mg/100g) represent mean ± standard deviation of (n=3) replications on dry matter basis. Different superscripts in a row are significantly different ($p \leq 0.05$).

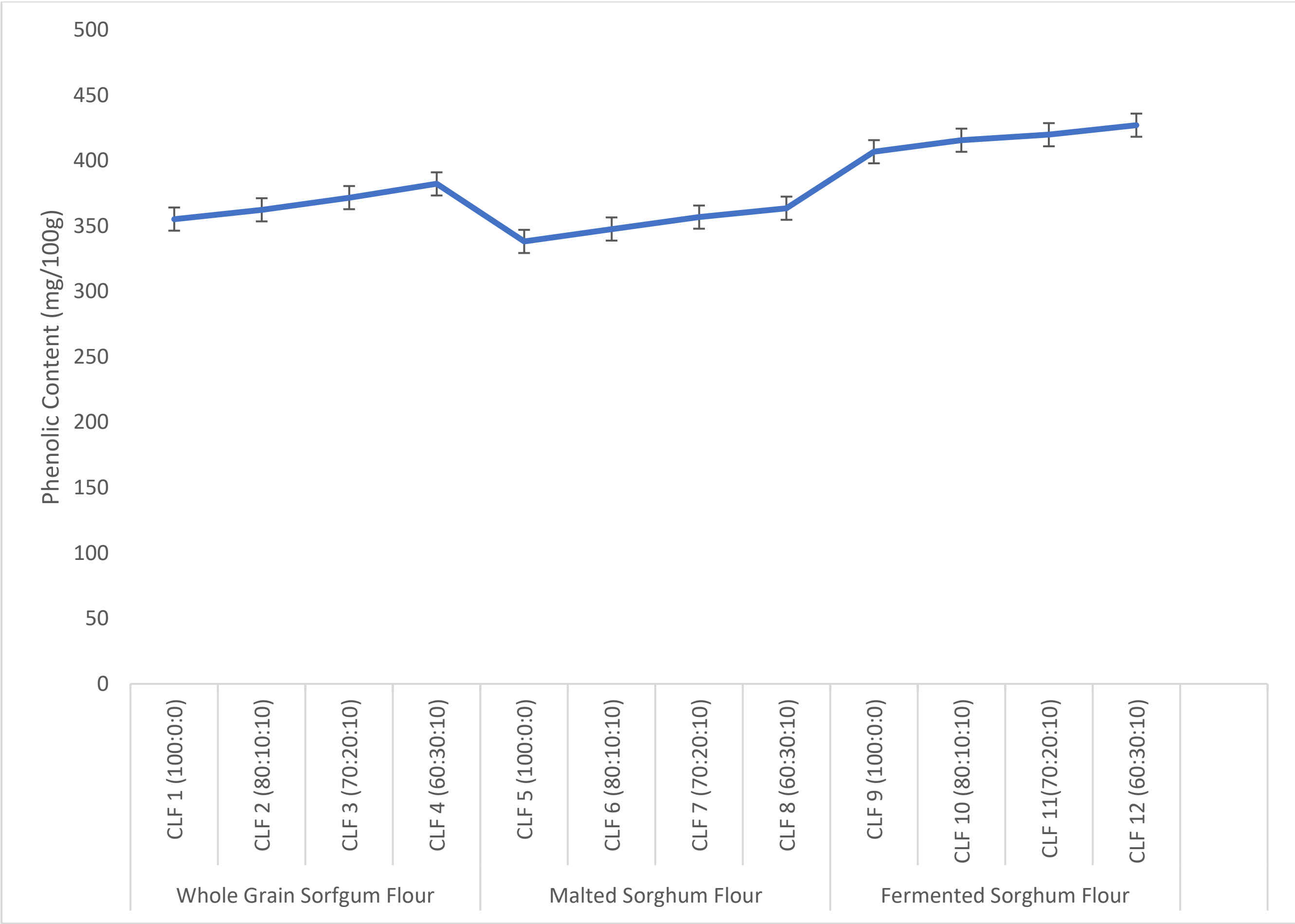


Figure 4.3: Effect of processing on the phenolic content of the cookies

Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF 4= 60%WSF:30%GGF:10%DMP, CLF5 =100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

4.6 DISCUSSION

4.6.1 Mineral Contents of the RTE Sorghum-Green Gram Cookies

Increasing the quantity of the green gram flour in the cookies samples was seen to increase the mineral contents of the cookies as compared to the control sample. Similar results of increment in mineral contents with increased green gram flour substitution have been reported by Onwurafor et al. (2019). Calcium, Iron and Zinc contents increased with corresponding increase levels of mung bean flour in composite bread from wheat, acha and mung beans (Dabels et al., 2016). Dahiya et al. (2015) showed that green gram is relatively high in minerals such as calcium, iron, zinc, sodium, copper, magnesium, manganese and phosphorus however the most important elements are iron, zinc and calcium due to their physiological functions. Green grams contain on average 5.9 mg/100g iron, 113.4 mg/100g calcium and 2.7 mg/100g zinc contents (Dahiya et al., 2015).

Besides from the increasing green gram substitution which led to the increase in mineral contents, the increment could also be attributed to malting and fermentation of the sorghum flour. The cookies made from malted sorghum flour and fermented sorghum flour had higher mineral content values as compared to cookies made from whole grain sorghum flour. Fermentation and malting processes have been previously reported to improve nutritional value of cereals and legumes through activation of various endogenous enzymes (Nkhata et al., 2018) which may explain the increased mineral content in the cookies. Asuk et al (2020) reported improved mineral content in malted sorghum-soy flour was a result of decrease in tannin and phytate contents degraded by tannase and phytase enzymes.

Iron is one of the most crucial minerals in all living organisms. It is a core element in many metabolic processes such as synthesis of hemoglobin, oxygen transport, DNA synthesis and electron transport (Rao et al., 2018). Being an essential component of myoglobin and hemoglobin, iron is required for blood formation (Asuk et al., 2020). Deficiency in iron has been reported to cause anemia and also lead to mental disorder in infants, elderly people and pregnant women (Abbaspour et al., 2014). Calcium several roles including development of strong teeth and bones, nerve impulse transmission, regulation of heart beat and muscle contraction (Pravina et al., 2013). Zinc is associated with growth and repair of tissues and boosting the immune system (Dabels et al., 2016). The results obtained below suggest that consumption of the formulated cookies would tackle calcium deficiency problems as well as reduce zinc deficiency by contributing significantly to the recommended dietary allowance (RDA) for calcium, iron and zinc.

4.6.2 Anti-nutritional Factors of RTE Sorghum-Green Gram Cookies

Sorghum-based foods have been reported to contain an impressive array of nutrients however anti-nutritional factors such as tannins and phytic acids have made then organoleptically inferior (Adeyemo et al., 2016). Tannins affect palatability by imparting astringent taste (Omoikhoje and Obasoyo, 2018). According to Ogbonna et al. (2012), presence of these anti-nutrients limit digestibility of proteins and carbohydrates which in turn inhibits their respective amylolytic and proteolytic enzymes. Tannins bind to non-heme iron forming complexes with proteins which inhibit enzymes of the digestive system (Melaku et al., 2005) while Phytic acids binds to proteins to chelate metal ions forming a protein-mineral-phytate complex that restricts the bioavailability of the minerals (Hariprasanna, et al., 2015).

In the results, anti-nutrients in cookies from malted and fermented sorghum flours were much lower than those in cookies from whole grain sorghum flour. Phytate contents (22.89 – 28.70 mg/100g) are much lower than those of Adeyeye et al. (2017) though tannin content was within the reported range. Malting and fermentation process helped in elimination of anti-nutrients present in the cookies. These results are in agreement with those of Ogbonna et al. (2012) who reported efficiency of malting technique in reducing anti-nutritional factors in sorghum grist aside from enhanced nutritional quality and Nwosu (2010) who reported soaking helped in reduction of anti-nutrients like tannins. Percentage change in phytate content of the cookies ranged between 26.15 – 41.10% while tannins was 5.57 – 25.36%. The greatest change was observed in fermented cookies followed by malted cookies. Fermentation led to a mean decrease of 40% phytates and 25.04% tannins in the cookies. Malting resulted in a mean decrease of 35.55 phytates and 15.65 tannins in the cookies. The extent of the reduction was thus dependent on the treatment done as observed in the results. The reduction in antinutrients differed significantly in the baked cookies. During the germination process, leaching of ant-nutrients during steeping process contributed to the decrease in phytates and tannins (Afify et al., 2012). Adhikari and Acharya, 2015 also reported decreased phytate content in biscuits from malted sorghum flour. Endogenous enzyme phytase is activated during germination which degrades phytate into inorganic phosphorus and inositol causing the reduction of phytate (Idris et al., 2005). Despite the baking process and the other treatments, the tannin values were still on the higher side which was also reflected in the percentage change. This could be attributed to presence of tannins in the green gram flour (100.9 mg/100g).

Natural fermentation of the sorghum flour broke down the tannin-enzyme and protein-tannin complexes leading to the decreased tannin levels as also reported by Tamilselvan and Kushwaha, 2020. The decreasing effect of phytic acid in the developed cookies as a result of fermentation are similar to those of Makokha et al. (2002) who reported that fermentation resulted in a decrease in phytic acid. Decrease in phytate content of cookies from fermented sorghum flour may be due to the increased microbial activities that produced phytase leading to enzymatic hydrolysis of phytic acid during the spontaneous fermentation process.

4.6.3 Phenolic Contents in the RTE Sorghum-Green Gram Cookies

Phenolic compounds are plant secondary metabolites present in cereals and legumes (Kunyanga et al., 2012). There has been a growing interest in phenolic compounds over the years due to their health protective effects such as antioxidant activity, cholesterol lowering potential, anti-microbial property, antimutagenic and anti-hypertensive properties (Hariprasanna et al., 2015, Kunyanga et al., 2012). All sorghum varieties contain phenolic compounds (Afify et al., 2012) shown to contain antioxidant activity paving way for use of sorghum as a cheap source of natural antioxidants. According to Affify et al. (2012), phenolic compounds possess structural features suitable for metal chelation and radical scavenging making them effective antioxidants contributing to health benefits such as protection against cardiovascular diseases and cancer.

From the results displayed in **Table 4.2**, fermentation as well as addition of green gram flour and dried mango powder to the cookies improved their phenolic contents. In comparison to cookies from whole grain sorghum flour, cookies from fermented sorghum flour registered the highest values. Microorganisms present in the spontaneous fermentation process broke down the sorghum matrix leading to release of bound phytochemicals hence the increased phenolic content. Improved bio-accessibility and bio-availability of phenolic compounds as a result of fermentation process has been reported (Balli et al., 2020, Sorour et al., 2017, Katina et al., 2014). **Figure 4.3** shows the effect of malting and fermentation process on the phenolic content. Fermentation was seen to improve the phenolic content while malting led to decreased values. Cookies produced from malted sorghum flour registered lower phenolic values the highest being 363.7 mg/100g which is lower compared to 382.3 mg/100g in cookies from whole grain sorghum flour and 427.2 mg/100g in cookies from fermented sorghum flour. The reduction in total phenolics in cookies from malted sorghum flour is attributed to the leaching of phenols into the soaking water during the steeping process before germination. These results are in agreement with Khoddami et al. (2017) who reported that malting process decreased the total phenolic content in sorghum.

Different authors (Sorour et al., 2017, Nwosu, 2010, Kayode et al., 2007) have reported a decrease in phenolic content after germination similar to this study. In contrast, Singh et al. (2019) reported an increase in total phenolic content and antioxidant activity in sorghum whereas Aruona et al. (2020) did not detect any effect on total phenolic compounds following sorghum germination. Beside from the processing effects, phenolic contents in the cookies could also be attributed to the substitution with dried mango powder and green gram flour. Mango powder is considered an excellent cost-effective source of phenolic compound with antioxidant and antimicrobial potential (Mutua et al., 2017, Gumte et al., 2018). High levels of phenolics have been reported in green grams as compared to other legumes (Shi et al., 2016). From the results displayed, phenolic content of raw green gram flour was 501.7 mg/100g which was higher than that of raw sorghum flour (360.4 mg/100g).

4.7 CONCLUSION

This study has shown that blends of flour from malted and fermented sorghum produce healthy cookies of improved antioxidant and lowered anti-nutrients contents. Addition of green gram flour and dried mango powder improved the phenolic contents of cookies. Placing attention on the health benefits associated with antioxidants such as prevention of oxidative stress, cardiovascular diseases and obesity, green gram flour and dried mango powder have the potential to be used as an ingredient in product development alongside sorghum as a valuable source of natural antioxidants. The results further indicate that simple processing techniques can be incorporated in sorghum-based products to improve their nutritional status and produce healthy products. Processing is likely to alter the functionality of different compounds by either increasing or decreasing the phenolic contents. The results reported here indicate that the output of a sorghum/legume-based food product supplemented with mango powder, is influenced by the processing techniques used and hence in a commercial setup, consideration should be given to the processing methods in order to produce a widely marketable product.

CHAPTER FIVE: CONSUMER ASSESSMENT AND SHELF STABILITY OF READY TO EAT SORGHUM-GREEN GRAM COOKIES SUPLLEMENTED WITH MANGO POWDER

5.1 ABSTRACT

The study evaluated the acceptability and the keeping quality of ready-to-eat snack made from sorghum-green gram blended cookies supplemented with mango powder. Sensory analysis using trained panelists was used to examine different quality attributes of the formulated cookies. Whole untreated grain, malted and fermented sorghum flour was used to formulate blended flours at 100%, 80%, 70% and 60%. Green gram flour was incorporated at 10%, 20%, 30% and dried mango powder at 10%. Cookie samples from fermented sorghum flour registered significantly ($p < 0.05$) high scores with an overall acceptability ranging from 6.8 – 7.51. Color, odor, texture, taste and crunchiness mean scores ranged between 6.81 – 7.46, 6.48 – 6.68, 6.70 – 6.93, 6.46 – 6.66 and 5.75 – 7.51 respectively. The formulated sorghum-green gram cookies were packed into two different packaging materials including - laminated resealable pouches and certified thermopak recyclable plastic containers- then subjected to storage under accelerated shelf-life conditions (55^0C and 55% RH) where microbial load and peroxide values were monitored monthly for five months. During the storage period, changes in total viable counts, yeast and molds and peroxide values was determined. Results showed an increment in microbial load throughout the storage period in both packaging materials showing potential contamination of the developed product during storage. Total viable counts in laminated resealable pouches ranged from log 2.13 to log 3.70 while in plastic containers the range was between log 2.29 to 3.86. Yeast and mold of cookies in laminated resealable pouches ranged from log 2.24 to log 2.62 and log 1.80 to log 2.73 in plastic containers. The peroxide value increased significantly ($p<0.05$) in both pacakaging materials during the storage period indicating possibilities of rancidity over prolonged storage periods. Peroxide value was not detected in day of storage. Peroxide value was ranged between 1 meq/kg to 17.06 meq/kg in laminated resealbale pouches and 4 meq/kg to 20.52 meq/kg in plastic containers. Peroxide values of cookies packed in laminated resealable pouches were lower than those packed in the plastic containers indicating that cookies packed in the laminated resealbable pouches had a longer keeping quality. The results of this study suggest that fermentation process can be used to produce quality cookies of high acceptability which can be stored in laminated resealbale pouches to improve their shelf-life.

Keywords: Sorghum-based Snacks**,** Shelf life, Microbial load, Sensory analysis, Peroxide value.

5.2 INTRODUCTION

Currently, diet concerns are defined by fast-faced lifestyles as a result of change in food consumption patterns. This is attributed to the availability of nutritious, palatable, affordable and easy to consume convenience foods such as snacks (Mattes, 2018). Consumption of snacks has been for a long time associated with developed countries in the West however over the recent years there has been a constant growth of snack sales in developing countries in Africa, Latin America and Asian Pacific (Momanyi et al., 2020). Snacking has however been associated with negative effects such as high body mass index and poor diet quality as a result of high sugar, saturated fats and sodium contents (Hess et al., 2017). Despite the negative effects, snacking can be practiced in a healthy manner through consumption of much more acceptable healthier snacks.

Consumption of healthier snacks such as cookies can very well be improved by development of cookies with high consumer acceptability despite being healthier. Reports by various authors show that most consumers will highly unlikely compromise on the taste of functional foods for eventual health benefits (Pestoric et al., 2017, Verbeke, 2006). Attempts are being made to not only improve the nutritive qualities but also the sensory qualities of cookies by modifying their nutritive composition (Yusufu et al., 2016). As a result of this, there is promotion of non-wheat flour such as sorghum flour in product development overcoming the challenge of continuous dependance on wheat importation which could contribute to food insecurity.

Shelf-life properties are a paramount property of any type of food and it is of great interest to not only the producers but also the consumers. Physicochemical changes during storage of food can result to loss of shelf-life leading to deterioration of food quality (Chowdhury et al.,2012). Consumer acceptability tests in the form of appropriate sensory analysis is an integral part of shelf-life evaluation for any product (Chowdhury et al., 2012). Successful product development is dependent on understanding the perception of individual attribute of the food by the consumers (Palczak et al., 2019). Properties such as texture, flavor, taste, appearance and smell of a product or food are examined in sensory analysis through senses such as sight, smell, taste, touch and feel of the panelists for the purposes of accepting or rejecting food products (Ruiz-Capillas and Herrero, 2021).

According to Momamyi et al. (2020), a variety of ready-to-eat snacks have been prepared from wheat, corn and rice but not sorghum. The use of sorghum in preparation of snacks such as cookies for commercial purposes has not been adequately explored yet it is generally easier for most people to consume snack foods compared to other complimentary foods. In addition, composite flours are better utilized in production of cookies as compared to bread due to their relatively prolonged shelf-life, ready-to-eat from, wide consumption and good eating quality (Ikuomola et al., 2017).

Sorghum cereal can complement leguminous protein sources such as green grams to prepare a nutrient diversified cereal formulation (Pradeep et al., 2014). Addition of fruit powders such as mango powder to such nutrient diversified cereal formulations can help provide nutritional security in addition to food security as they supplement carbohydrates, vitamins and minerals required for good health (Rajeswari et al., 2018). The present work was undertaken to evaluate the sensory profile and shelf-life quality of sorghum-green gram cookies supplemented with mango powder.

5.3 MATERIALS AND METHODS

5.3.1 Sample Collection

Sorghum (*Sorghum bicolor (L) Monench*), green grams (*Vigna radiata*) and ripe mangoes (*Mangifera indica L*.) were purchased from a local open-air market in Kangemi, Nairobi-Kenya. All the other ingredients (margarine, sugar, skimmed milk powder, vanilla essence, baking powder, salt) used in the baking of the cookies were purchased from the local retail outlets. All materials were transported to the department of Food Science, Nutrition and Technology at the University of Nairobi, Kenya where the study was conducted.

5.3.2 Malting of Sorghum

Sorghum (*Sorghum bicolor (L) Monench*) was manually cleaned to remove damaged grains, husks and stones. To suppress mold growth, the grains were washed in 5% (w/v) sodium chloride solution after which they were steeped in tap water in ratio 1:2 (w/v) at room temperature (32 ± 2^0C) for 12 hours. After steeping, water was drained and the grains were allowed to germinate between two damp cloths at ambient temperature for 72 hours as described by Ouazib et al. (2016). After every 12-hour interval, water was sprinkled to facilitate the germination process. The germination time and the temperature chosen for this study were chosen from previous studies (Okpala et al., 2013, El-Moneim et al., 2017, Bello et al., 2020, Olamiti et al., 2020). At the end of the germinating period, the grains were dried in the oven (Schutzart DIN 40050 – IP20) at 65^0C for 9 hours followed by removal of the dried sprouts on the grains manually (Siddiqua et al., 2019). The germinated sorghum grains were milled into flour using a hammer miller and passed through a 1mm sieve to obtain fine sorghum flour of uniform particle size free from clumps. The obtained flour was stored in an airtight container stored at room temperature for further analysis.

5.3.3 Fermentation of Sorghum

Sorghum (*Sorghum bicolor (L) Monench*) was sorted manually to remove damaged seeds and stones. The grains were then washed and dried then milled into flour using a hammer mill. The milled flour was subjected to spontaneous fermentation by microflora (lactic acid bacteria) naturally present in the grains. Sorghum flour was fermented according to the method described by Elkhalifa et al. (2017). Flour was mixed with water (1:2 w/v) to form a slurry which was incubated at 37^{0}C for 48hrs in sterile covered flask. After the fermentation period, excess water was removed and the fermented slurry was oven (Schutzart DIN 40050 – IP20) dried at 65^{0}C for 8hrs and then ground into flour. The fermented ground flour was passed in a 0.4mm sieve and stored in an airtight container until needed for analysis.

5.3.4 Preparation of Whole Grain Sorghum Flour

Sorghum (*Sorghum bicolor (L) Monench*) was sorted out to remove excess dirt and thoroughly washed as per the method reported by Adeyeye, 2016. The cleaned grains were oven (Schutzart DIN 40050 – IP20) dried at 65^{0}C for 8 hours then milled using a commercial hammer mill into fine flour. The dried flour was packaged in a clean airtight container for further use.

5.3.5 Preparation of Green Gram Flour

Green grams (*Vigna radiata*) flour was produced using the method of Dabels et al. (2016). The grains were manually cleaned to remove all the dirt then washed with clean water. The clean seeds were then spread on tray and placed in an oven (Schutzart DIN 40050 – IP20) dried at 65^0C to dry for 4 hours. The dried green grams were then milled into fine flour using a commercial hammer miller (GM25- TEFC). The flour was then passed through a 1mm sieve and packed in a clean airtight container for further use.

5.3.6 Drying of Mango (*Mangifera indica L.*) Fruits

In preparation of the dried mango powder, the method of Sengev et al. (2015) was adopted with slight modifications. Twenty large-sized ripe mangoes bought from the market were sorted out, washed, peeled and the mesocarp was sliced manually to a thickness of 2.0mm. The thin slices were spread on a tray lined with aluminum foil and oven (Schutzart DIN 40050 – IP20) dried at 60^0C for 24 hours. The dried slices were then ground (350W- 220V-AK 300, Nunix 2 in 1 Blender) into powder using a grinder and sieved through a 0.5mm sieve to obtain the dried mango powder that was stored in an airtight container stored at room temperature.

5.3.7 Experimental Design and Sample Formulation

The experiment was laid out as a 3×4 factorial design with three levels of sorghum flour (whole grain, malted, fermented) and four levels of green gram flour and dried mango powder substitution (100:0.0, 80:10:10, 70:20:10, 60:30:10) for each of the treatments as shown in **Table 5.2**. The ratio of the flour blends was labelled CLF (Cereal: Legume: Fruit) to represent the sorghum: green gram: mango powder ratio in the formulations. Sample CLF1 with 100% whole grain sorghum flour served as the control sample **(Table 5.2)**. Formulation (**Table 5.1)** was done based on the recommended minimum levels of protein, fiber, fat by FAO/WHO which were achieved using NutriSurvey Linear Programming Package 2004 version (Amegovu et al., 2013).

Table 5.1: Formulation of Ingredients Used in the Sorghum-Green Gram Cookies

Ingredients	Amount (grams)
Flour *	200
Sugar	50
Margarine	50
Salt	0.35
1 Whole Egg	30
Skimmed Milk Powder	8
Baking Powder	1.25
Vanilla Essence	1.25

*Sorghum flour, green gram flour and dried mango powder

Table 5.2: Ratio of the Flour Blends

Flour Blends	Whole-Grain Sorghum Flour (%)	Malted Sorghum Flour (%)	Fermented Sorghum Flour (%)	Green Gram Flour (%)	Dried Mango Powder (%)
CLF 1	100	0	0	0	0
CLF 2	80	0	0	10	10
CLF 3	70	0	0	20	10
CLF 4	60	0	0	30	10
CLF 5	0	100	0	0	0
CLF 6	0	80	0	10	10
CLF 7	0	70	0	20	10
CLF 8	0	60	0	30	10
CLF 9	0	0	100	0	0
CLF 10	0	0	80	10	10
CLF 11	0	0	70	20	10
CLF 12	0	0	60	30	10

Key: CLF = Cereal: Legume: Fruit Blend

5.3.8 Formulation of the RTE Cookies

The ingredients used in the sorghum-green gram cookie preparation included the different ratios of the sorghum flour (whole grain, malted and fermented), green gram flour and mango fruit powder. The cookies were prepared according to the American Association of Cereal Chemists (AACC, 10-50D) with slight modifications. For each of the experiment, 200g of the blended flour (CLF – Cereal: Legume: Fruit) was used. Other ingredients included sugar, margarine, skimmed milk powder, baking powder, eggs, vanilla essence and cinnamon powder were used in the cookie recipe. Sugar was creamed with the margarine to a fluffy consistency using a hand mixer (Geepas Hand Mixer – GM6127). Whole egg and vanilla essence were added to the creamed mixture and whisked to a soft texture. Flour and all the dry ingredients were sieved together and mixed gently with the creamed mixture to obtain a stiff paste. The dough obtained was rolled out on a board and cookie cutters used to shape out the cookies. Cut-out pieces of cookies were placed on a tray lined with parchment paper and baked at 180^0 C for 15-20 minutes. Cookies were cooled and stored in 500g plastic containers at ambient temperature (30 ± 2^0C) for further analysis.

5.4 ANALYTICAL METHODS

5.4.1 Sensory evaluation of RTE sorghum-green gram cookies

The ready-to-eat cookies were assessed by 30 semi-trained panelists between the ages of 20-38 years. The panelists consisted of undergraduate students, graduate students and staff from the Department of Food Science, Nutrition and Technology in University of Nairobi, Kenya. The cookie samples were coded randomly with a three-digit number and presented to the panelists for assessment. A 9-point hedonic scale (1-dislike extremely, 9-like extremely) by Lim (2011) was used to evaluate the color, smell, taste, texture, crunchiness and overall acceptability of the cookies developed from the different formulations. The panelists were provided with portable water for rinsing their mouths after tasting each sample to minimize masking of sensory attributes and any error that could have occurred during the tasting.

5.4.2 Shelf-life evaluation of RTE sorghum-green gram cookies

Cookie samples prepared were packaged in two different types packaging materials (laminated resealable pouches and certified thermopak recyclable plastic containers) and stored in an incubation chamber where temperature and humidity were kept constant. The chamber simulated conditions of accelerated storage at 55^0C and 55% RH for a storage period of 5 days (1day – 1 month) (Fu and Labuza, 1997). Each of the packages was subjected to shelf-life evaluation to establish how long the cookies will keep before they become unfit for consumption.

5.4.2.1 Microbial analysis

5.4.2.1.1 Determination of Total viable count

Total viable counts (TVC) of the cookie samples were done as per AACC method 42-11-01 (2000). Diluents were prepared using 0.85% sodium chloride for serial dilutions and 5g of the sample was used. Molten plate count agar was prepared according to the manufacturer's directions. Pour plate method was used where 1ml of each dilution was plated. The plates were incubated upside down at 37^0C for 24 hours after which bacterial counts were enumerated using a colony counter and converted to $\log_{10}$ cfu/g of the sample.

5.4.2.1.2 Determination of Yeast and molds

The yeast and mold counts were done as per method 42-50-01 of AACC (2000). 5g of sample was transferred into 0.85% sodium chloride diluents prepared for serial dilutions. Molten potato dextrose agar (PDA) media was prepared as per the manufacturer's directions. A micro-pipette was used to transfer 1ml of the serial dilutions into sterile petri-dishes. Pour plate technique was used and the plates were incubated upside down at 30^0C for 48 hours. Enumeration was done using a colony counter and microbial counts expressed in log cfu g^{-1} .

5.4.2.1.3 Determination of *Staphylococcus aureus*

ISO method 6888-1; 1999 (ISO 1999) was used detect *Staphylococcus aureus* in the developed cookies. 5g of sample was transferred into 0.85% sodium chloride diluents prepared for serial dilutions. Baird Parker agar was prepared as per the manufacturer's directions. 50 ml of egg yolk emulsion was added to the molten media. Spread plate technique was used and the plates were incubated upside down at 37^0C for 24-48 hours. Observation was made for colonies that appeared shinning grey or back surrounded by a partially opaque zone. Staphylococci present was expressed as cfu/g of the sample.

5.4.2.2 Peroxide Value (PV)

Determination of PV was done using the titrimetric method of AOAC (2010). About 2g of each sample and 30 ml glacial acetic chloroform solution was added in a conical flask and swirled to dissolve. 0.1 ml of saturated potassium iodide was added to the mixture. After one minute, 30 ml of distilled water and 0.5 ml of 1% starch was added and then titrated with 0.01N $Na_2S_20_3$ (sodium thiosulphate) until the blue color disappeared. A blank titration was carried was carried out. The peroxide value was calculated using the following equation:

$$Peroxide\ Value\ (\frac{meq}{kg}) = \frac{1000\ (\mathrm{A} - \mathrm{B})N}{\text{Sample Weight (g)}}$$

Where; A = Volume of $Na_2S_20_3$ used for sample, B = Volume of $Na_2S_20_3$ used for the blank, N= Normality of $Na_2S_20_3$.

5.4.3 Statistical Analysis

All data collected in this study was carried out in duplicate and expressed as mean ± standard deviation. Statistical analysis of the data was by analysis of variance (ANOVA) at 5% significance level using the Genstat Version 15 and multiple comparisons done using Fisher's unprotected LSD. Sensory analysis was also done using the Genstat software version 15 at $P<0.05$ significance level.

5.5 RESULTS

5.5.1 Sensory attributes of the developed sorghum-green gram mango cookies.

Table 5.3 show the sensory evaluation results of the formulated sorghum-green cookies. Significant differences in the sensory attributes of the developed cookies were observed in cookies made from whole grain, malted and fermented flours. Formulation 60:30:10 made from fermented sorghum flour had the highest sensory attributes and overall acceptability as seen in **Figure 5.1** as compared to all the other formulations.

Table 5.3. Sensory attributes of the developed sorghum-green gram-mango cookies

Attributes		Color	Odor	Taste	Texture	Crunchiness
WG	100:0:0	$6.55±1.71^{bc}$	$6.55±1.46^{cd}$	$4.68±1.22^{a}$	$5.66±1.43^{a}$	$6.86±0.91^{b}$
	80:10:10	$6.58±1.49^{bc}$	$6.50±1.70^{d}$	$4.69±1.84^{a}$	$5.86±1.28^{ab}$	$7.01±0.93^{b}$
	70:20:10	$6.63±1.65^{bc}$	$6.48±1.56^{d}$	$4.71±1.22^{a}$	$5.91±1.01^{b}$	$7.03±1.26^{b}$
	60:30:10	$6.68±1.06^{bc}$	$6.46±1.70^{d}$	$4.74±1.21^{a}$	$5.97±1.43^{b}$	$7.05±1.26^{b}$
MF	100:0:0	$6.63±0.95^{bc}$	$6.51±1.48^{cd}$	$5.83±1.24^{bc}$	$5.91±1.27^{ab}$	$6.80±1.37^{b}$
	80:10:10	$6.65±1.35^{bc}$	$6.48±1.20^{cd}$	$5.85±1.20^{bc}$	$6.25±1.09^{bcd}$	$6.85±1.24^{bc}$
	70:20:10	$6.72±1.16^{c}$	$5.75±1.41^{ab}$	$5.85±1.64^{bc}$	$6.35±0.93^{cde}$	$6.88±1.07^{bc}$
	60:30:10	$6.78±1.04^{c}$	$5.55±1.50^{a}$	$5.88±0.86^{bc}$	$6.41±1.61^{cde}$	$6.90±1.32^{b}$
FF	100:0:0	$6.81±1.06^{cd}$	$6.68±0.98^{d}$	$6.70±1.23^{ef}$	$6.46±1.26^{cde}$	$5.75±1.44^{a}$
	80:10:10	$6.85±0.93^{cd}$	$6.63±0.88^{d}$	$6.78±1.10^{ef}$	$6.51±1.03^{de}$	$6.10±1.43^{a}$
	70:20:10	$7.06±1.03^{d}$	$6.51±1.72^{cd}$	$6.81±1.07^{ef}$	$6.60±0.86^{def}$	$6.78±1.22^{b}$
	60:30:10	$7.46±1.03^{ef}$	$6.48±1.49^{cd}$	$6.93±1.31^{f}$	$6.66±1.35^{ef}$	$7.51±1.09^{c}$

Notes: Values represent mean ± standard deviation of (n=3) replications. Different superscripts in a column are significantly different ($p \leq 0.05$). WG – Whole Grain Sorghum Flour, MF – Malted Sorghum Flour, FF – Fermented Sorghum Flour.

The fermented samples scored highest in the attributes where color was scored in the range of 6.81 – 7.46, odor 6.48 – 6.68, Texture 6.70 – 6.93, Taste 6.46 – 6.66 and crunchy score of between 5.75 – 7.51. Malted samples came second however they recorded lowest in odor with a score range of 5.55 – 6.51. Cookie samples from wholegrain flour despite being acceptable recorded the lowest scores in taste attribute ranging from 4.68 – 4.74. In the overall acceptability of the prepared cookies, fermented cookies were most acceptable while those from wholegrain sorghum flour were least acceptable.

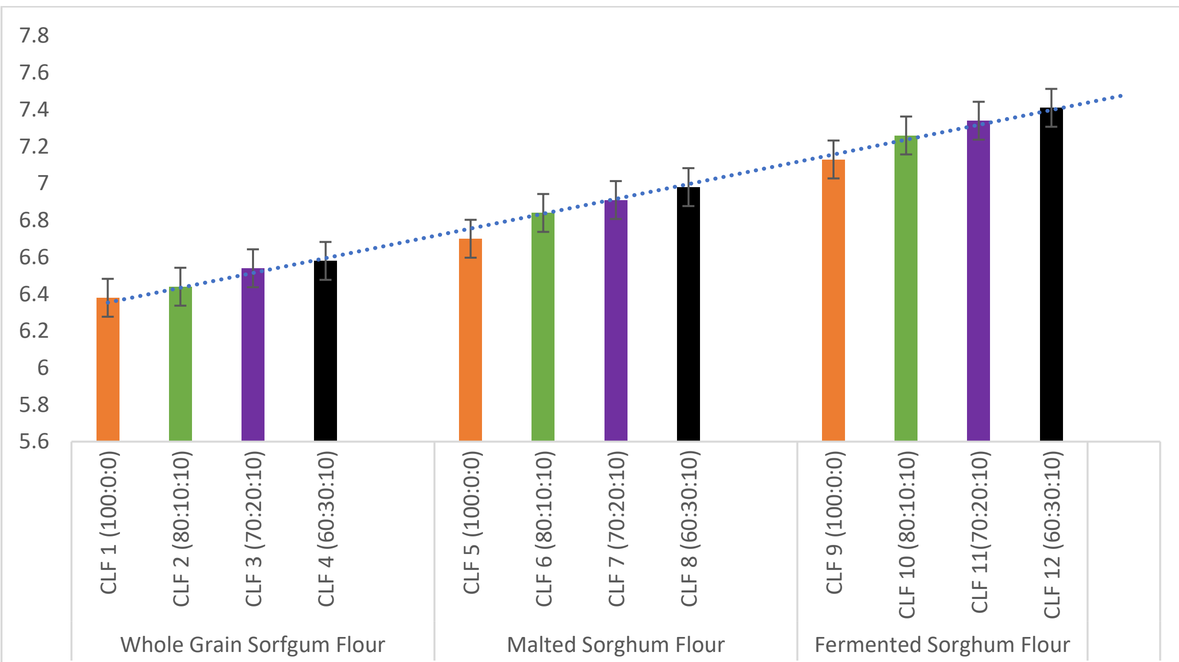


Figure 5.1 Effect of processing on the overall acceptability of the developed cookies

[a]CLF: Cereal-Legume-Fruit blend. [b]WSF- Whole Grain Sorghum Flour, MSF- Malted Sorghum Flour, FSF- Fermented Sorghum Flour, GGF- Green Gram Flour, DMP-Dried Mango Powder..

Samples CLF 1 = 100%WSF, CLF 2 = 80%WSF:10%GGF:10%DMP, CLF 3 = 70%WSF:20%GGF:10%DMP, CLF 4= 60%WSF:30%GGF:10%DMP, CLF5 =100%MSF, CLF6=80%MSF:10%GGF:10%DMP, CLF7=70%MSF:20%GGF:10%DMP, CLF8=60%MSF:30%GGF:10%DMP, CLF9=100%FSF, CLF10=80%FSF:10%GGF:10%DMP, CLF11=70%FSF:20%GGF:10%DMP, CLF12=60%FSF:30%GGF:10%DMP.

5.5.2 Effect of packaging and storage time of the sorghum-green gram cookies

Figures 5.2 and 5.3 represent the changes in total viable count and yeast and mold of the formulated cookies stored in different types of packaging material for a period of 5 days under accelerated conditions of at 55^0C and 55% RH during the storage period. The total viable count of the cookies increased significantly ($p<0.05$) in both packaging materials from day 1 up to day 5 of storage. In laminated resealable pouches, the total viable count of the cookies increased from log 2.13 to log 3.70.

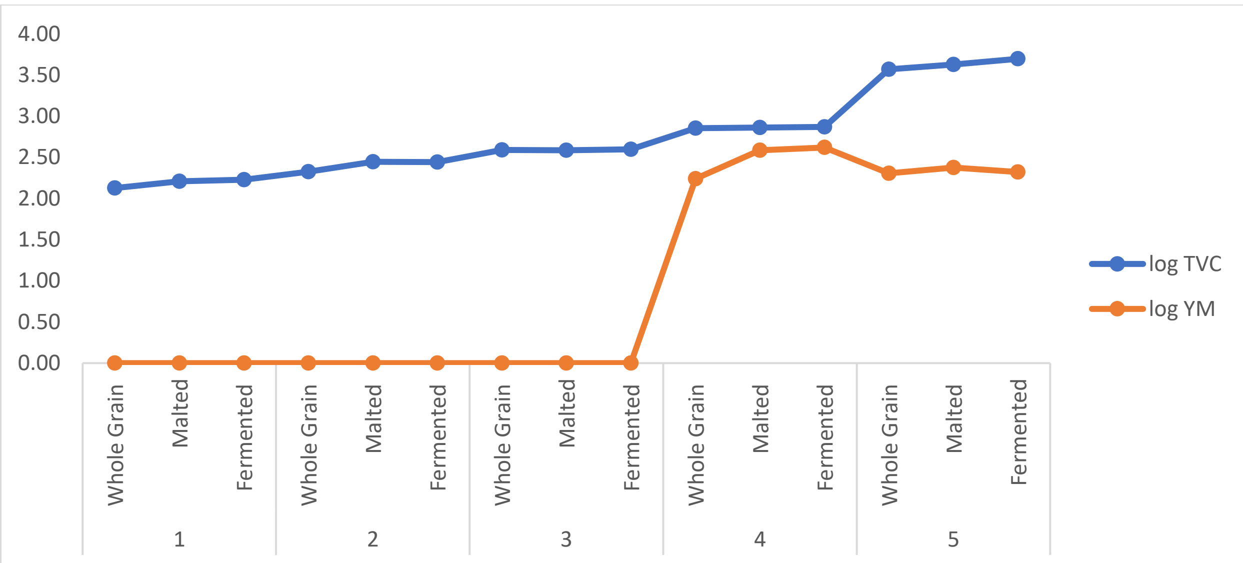


Figure 5.2 Changes in the TVC and YM of cookies packed in rsealable pouches

In the certified thermopak recyclable plastic containers, the total viable count ranged from log 2.29 to 3.86 (Figure 3.2.1). Yeast and mold of samples in laminated resealable pouches ranged from log 2.24 to log 2.62 and log 1.80 to log 2.73 in plastic containers. Yeast and molds could not be detected up to day 3 of accelerated storage in laminated resealable pouches as observed in Figure 3.2.2 below

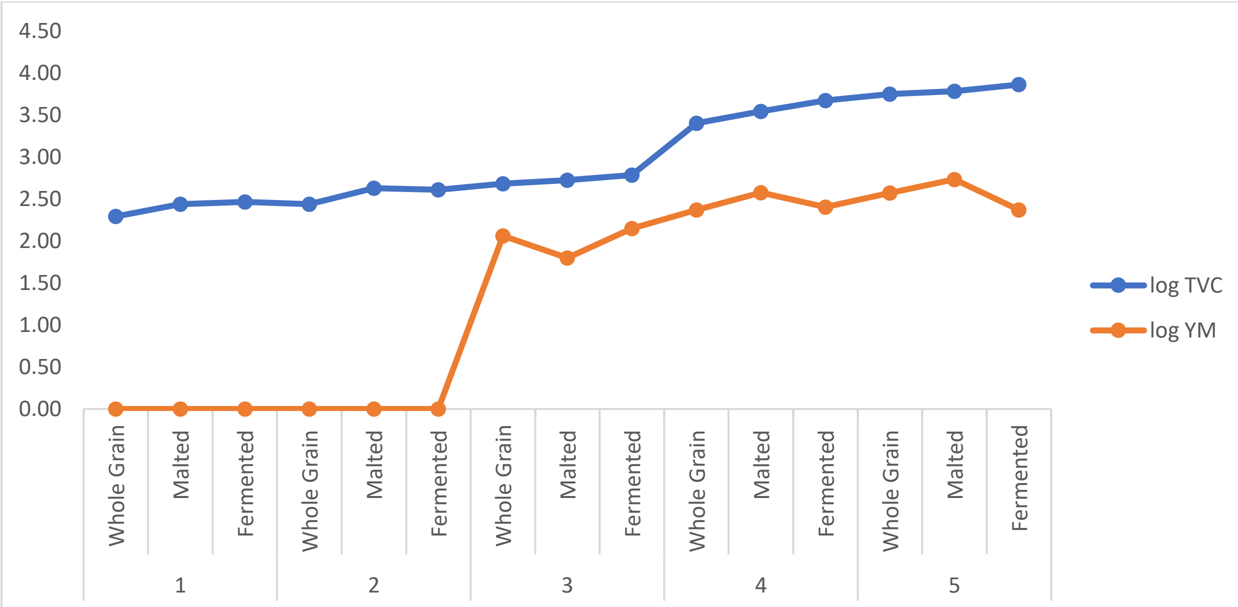


Figure 5.3: Changes in the TVC and YM of cookies packed in recylable plastic containers

5.5.3 *Staphyloccoccus aureus* count in baked cookies

Staphyloccoccus aureus in the formulated cookie samples ranged from log 1.27 – log 1.32 in cookies packed in the laminated resealable pouch and log 1.18 – log 1.24 in cookies packed in plastic containers. Staphylococcus counts were only obtained once on day one of the storage process. From results displayed in **Table 5.4**, there were no significant differences (P>0.05) stapphylococcus counts in the different packaging materials.

Table 5.4: ***Staphylococcus aurues*** **in the baked cookies (log cfu/g)**

SAMPLES	PACKAGING A	PACKAGING B
CLF 1 (100:0:0)	1.32±0.70[a]	NIL
CLF 2 (80:10:10)	NIL	NIL
CLF 3 (70:20:10)	1.27±0.00[a]	1.18±0.70[a]
CLF 4 (60:30:10)	NIL	1.24±2.12[a]
CLF 5 (100:0:0)	NIL	NIL
CLF 6 (80:10:10)	NIL	NIL
CLF 7 (0:20:10)	NIL	NIL
CLF 8 (60:30:10)	NIL	NIL
CLF 9 (100:0:0)	NIL	1.16±0.00[a]
CLF 10 (80:10:10)	NIL	NIL
CLF 11 (70:20:10)	1.30±1.41[a]	1.18±0.70[a]
CLF 12 (60:30:10)	NIL	NIL

Notes: Values represent mean ± standard deviation of (n=3) replications. Different superscripts in a column are significantly different ($p \leq 0.05$). CLF – Cereal -Legume-Fruit flour. Whole Grain Sorgum (CLF 1 – CLF 4), Malted Sorghum (CLF 5 – CLF 8), Fermented Sorghum (CLF 9 – CLF 12).

5.5.4 Peroxide value changes in the cookies during storage period

Figure 5.4 shows the peroxide values of the cookies packed in the two packaging material and stored under accelerated conditions for 5 days. The peroxide value increased significantly in both pacakaging materials during the storage period. Peroxide value was not detected in day of storage as seen in figure 3.3.1. In laminated releasable pouches, peroxide value was the range of 1 meq/kg to 17.06 meq/kg and a range of 4 meq/kg to 20.52 meq/kg in recyclable plastic containers. Peroxide values of cookies packed in laminated resealable pouches were lower than those packed in the certified thermopak recyclable plastic containers.

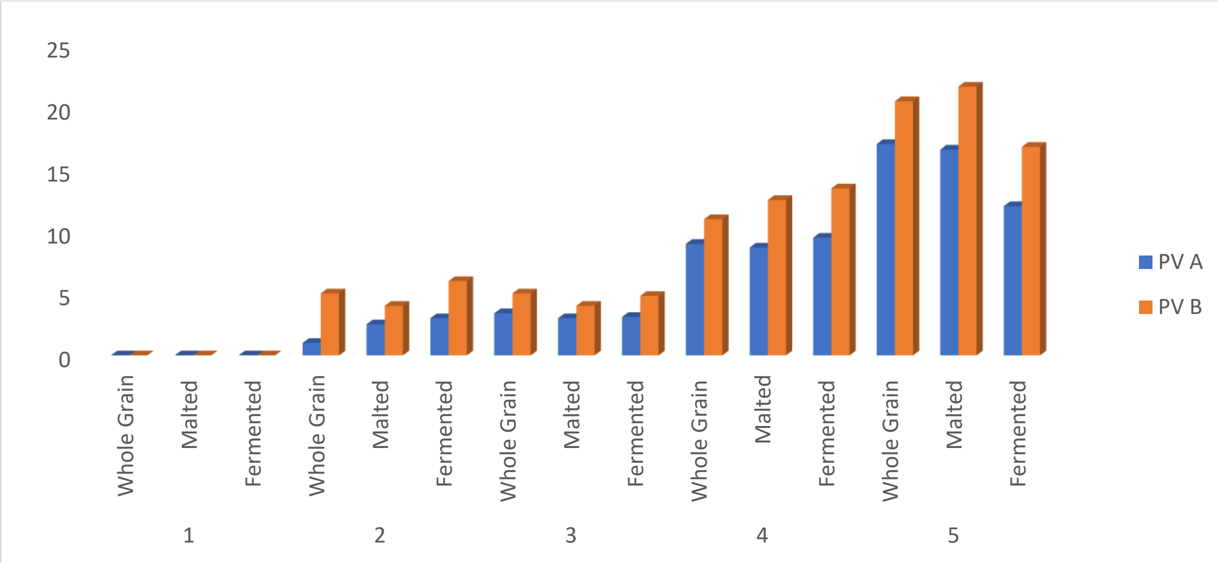


Figure 5.4 Peroxide changes in the sorghum-green gram cookies packed in different packaging material.

A – Laminated resealable pouch, B – Plastic container.

5.6 DISCUSSION

5.6.1 Sensory attributes of the developed sorghum-green gram mango cookies.

The sensory assessment of the cookies produced from sorghum-green gram blends supplemented with dried mango powder are presented in **Table 5.3**. The mean scores of color, taste, texture, crunchiness and odor are significantly different ($p<0.05$) from one another. Attributes such as color, taste, texture and crunchiness of the cookie samples increased significantly with increase in green gram flour substitution. Increase in level of green gram substitutions has been reported to improve sensory attributes such as color, taste, texture and appearance in biscuits fortified with green gram flour (Shukla et al., 2016). Odor of the cookies had an inverse relationship with increase in green gram substitution. The decrease in odor of the cookies with increased green gram substitution could be attributed to the strong significant smell of green gram flour which led to decreased scores by the panelists. According to Dokic et al. (2015), most consumers are not used to the specific color and aroma associated with green gram flour. The taste scores from wholegrain and malted flour were much lower than those of the fermented products. The low taste scores could be attributed to the astringent taste observed in the cookie samples as a result of presence of antinutrients in sorghum flour (Mohapatra et al., 2017).

Malting and fermentation process improved the sensory attributes of the developed cookies. Generally, the overall acceptability scores of the sorghum-green gram cookies developed ranged between 6.38 to 7.41, $p<0.05$. Final products with 60:30:10 sorghum green gram formulations scored highest in overall acceptability with fermented products recording the highest at 7.41, followed by malted with 6.98 and lastly wholegrain with 6.58. Fermentation of starchy products using selected microorganisms has been reported to improve nutritional as well as sensory quality of different food products (Ntsamo et al., 2020). Natural fermentation by the naturally occurring lactic acid bacteria (LAB) inhibits enteropathogenic bacteria leading to improved palatability and acceptability of food products improving the sensory attributes of a particular food (Mohapatra et al., 2017). Ayo and Ogunsakin, (2013) found that sensory properties of maize-cardaba banana complementary food was improved by the process of fermentation.

In this study, malting was found to improve the nutritive, bioactive and consume quality of the formulated products. This is due to the endogenous enzymes that hydrolyze starch breaking it into small molecular weight increasing the total sugar content contributing to improved food flavour in the products (Olamiti et al., 2020). No significant differences ($p>0.05$) were obtained in the color of the cookies from the malted sorghum flour however the color was above the acceptable limit. The intensity of color increase with malting process is mainly due to the increase in phytochemicals (Asuk et al., 2020). The high scores in malted and fermented cookies with 30% green gram substitution could be as a result of the increased protein content by the green gram flour. This leads to increase in Maillard reaction (non-enzymatic) between lysine protein and reducing sugars contributing to caramelized products which are desirable in baked goods. According to Sudha et al. (2007), darker colored products are associated with increased fiber content. Similar findings of improved sensory attributes with malting were reported by Adhikari and Acharya, 2015, Egbujie and Okoye (2019)

5.6.2 Effect of packaging and storage time on the sorghum-green gram cookies

Cookie samples stored in the laminated resealable pouches showed a slower growth of total viable count as compared to cookies stored in the thermopak recyclable plastic containers. This suggests that the cookies could have a longer shelf life when packed in laminated resealable pouches. Nagi et al. (2012) reported that the impervious nature of laminated packaging materials contributes to less absorption of air and water vapor during storage period of biscuits. Cookies stored in laminated resealable pouches showed a slower growth of yeast and mold as compared to those packed in plastic containers suggesting that products can have a longer shelf life if packed in laminated resealable pouches. Despite the significant differences ($p<0.05$), microbial load was present all through the storage period.

Baked products are an excellent source for microbiological growth thus are mostly subjected to fungal contamination limiting their shelf life during the storage period (Alpers et al., 2021). Yeast and mold counts were not present until day 3 and 4 in plastic container and laminated resealable pouches respectively. This could be attributed to the high baking temperatures which may have destroyed the yeast and molds present in the cookie samples. Similar to the results of this study, Ravimannan (2016) reported presence of microbial load as from day 4 which increased significantly during the storage period. The study showed that there were some heat-resistant microorganisms not destroyed by baking temperatures and could subsequently be found d in the dough. Fore baked products, the baking process is an effective kill-step in controlling foodborne pathogens (Channaiah et al., 2021).

Microorganisms present in food products play a crucial role in determining the shelf life of the product. Deterioration in baked products is mainly caused by microbial spoilage. According to Jayaweera et al. (2018), a high aerobic plate count indicates the presence of mixed population of microorganisms. In order to keep food safe for human consumption, limits of microbial counts have been recommended for different types of food products. According to Sibian and Riar, 2020, the total aerobic count for ready-to-eat foods should be less than 10^4 – 10^6 cfu/g which is in agreement with the microbial load limit set by the International Commission for Microbiological Specification. The microbial load of the cookies which ranged from log 2.13 – log 3.70 (TVC), log -2.24 -2.68 (YM) in laminated resealable pouches and log 2.29 – log 3.86 (TVC), log 1.80 – log 2.73 (YM) in thermopak recyclable plastic containers are well within the acceptable limits despite the increment in microbial load. From Figures 3.2.1 and 3.2.2, microbial load increased linearly with storage period. These results are similar to those of Priya and Ramaswamy, 2016, and Tikle and Mishra, 2019 in a study to evaluate the physical, microbial and sensory qualities of cookies produced from composite flour.

Table 5.4 shows the *staphylococcus aureus* counts of the developed cookies done on day one of storage. Most of the samples registered an absence of staphylococcus aureus in them (CLF 2,4, 5,6,7,8 9,10,12) in packaging A and CLF 6 1,2,5,6,7,8,9,10,12 in packaging an indication that there was proper handling during the cookie preparation process. The values are also well below the recommended limit of bacterial load by the International microbiological standard. B. *Staphylococcus aureus* ranged between log 1.27 – log 1.32 in laminated resealable pouches and log 1.18 – log 1.24 in plastic containers. According to Kadariya et al. (2014), *Staphylococcus* *aureus* are commensal and opportunistic pathogens that cause a wide range of infections that could be severe to fatal. S*taphylococcus aureus* can cause food contamination especially during preparation and processing. Determination of *S. aureus* was done to ascertain whether any contamination occurred during the preparation since these organisms can remain viable on hands of the cookies and during the baking process. *Staphylococcus aureus* is a significant cause of food-borne diseases (Kadariya et al., 2014). A study by Ayciek et al. (2005), showed that food that require more handling especially during preparation are more vulnerable to *S. aureus* contamination. Proper handling of food during preparation should be done to prevent food borne disease outbreaks especially as a result of *Staphylococcus aureus*.

5.6.3 Peroxide Value

Peroxide value is the amount of peroxide oxygen that is generated per 1 kilogram of fat or oil. Peroxide value is used a measure of the degree of oxidation occurring in a food sample, also known as rancidity. During autoxidation reaction, peroxides are the intermediate compounds synthesized. Autoxidation being a free radical reaction involving oxygen leads to deterioration of fats and oils contributing to off-flavor and off-odors. In case of rancidity, the peroxide value range is between 30-40 meq/kg (Ahmad et al., 2017). Fat oxidation during the storage period contributes to increased peroxide values. From **figure 5.4**, peroxide values were not detected on day 1 of storage in both packaging materials. Studies have shown that peroxide values are low or absent at the beginning of the shelf life of a food product (Ahmad et al., 2017, Mathi, 2016).

Peroxide values were highest in the last day of storage under accelerated conditions. Cookies packed in laminated resealable pouches had peroxide values ranging from 12.05 meq/kg – 17.06 meq/kg on day 5 of storage. Cookies in plastic containers had a peroxide value range of 16.83 meq/kg – 20.52 meq/kg on day 5 of storage. FAO/WHO recommends a peroxide value of ≤ 10 meq/kg. Cookies packed in laminated resealable pouches had peroxide values of less than 10 meq/kg up to day 4 of storage indicating that the cookies could be stored for a period of 4 months and still be ok for consumption. After four months they are no longer safe for consumption since the peroxide values were way above the recommended limit by FAO/WHO.

Cookies packed in thermopak recyclable plastic containers could only be stored for 3 months since peroxide values were beyond the recommended limit by the 4th day of storage under accelerated conditions. **Figure 5.4** shows significant differences ($p<0.05$) Peroxide value changes but with no significant trend in cookies made from whole grain sorghum flour, malted sorghum flour and fermented sorghum flour. The low water activity of baked products such as cookies, crackers, biscuits and breadsticks contribute to the long shelf -life of baked products. During the storage periods, the quality of baked products deteriorates due to loss of crispiness as a result of moisture absorption and development of lipid oxidation (Manzocco et al., 2020). Several studies have highlighted the effectiveness of using peroxide value as an indicator to study oxidative status of bakery products (Calligaris et al., 2008, Calligaris et al., 2016).

5.7 CONCLUSION

The results of the study showed that sorghum-green gram cookies from fermented sorghum were most preferred as compared to those from whole grain and malted flours. Therefore, simple processing techniques such as fermentation can be used in baking to produce acceptable products. The keeping quality of baked products such as cookies can be greatly improved by use of laminated resealable pouches as packaging materials. From the study the keeping quality of cookies in laminated resealable pouches was up to 4 months of storage which was prediction from the accelerated storage conditions. During the storage period, peroxide values which are an indicator of rancidity in the products were well within the recommended limit up to day 4 of storage. Ready to eat cookies can be stored in laminated resealable poches and be consumed for a period of 4 months before being unfit for consumption.

CHAPTER SIX: GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 GENERAL CONCLUSION

The study showed that sorghum can be used with other legumes such a green gram to develop quality and nutritious products which can be sold commercially. Use of sorghum and green grams in this study could provide opportunities to prevent the under-utilization of cereals and legumes in Kenya. From the study, sorghum flour can be combined with different levels of green gram flour and supplemented with a fruit powder to produce acceptable and high-quality gluten-free cookies. The high nutritional quality of green grams helps to improve sorghum-based products. Malting and fermentation have long been used at the household level with no commercialized products in the market. From the study, malting and fermentation process of sorghum were seen to contribute to the improved quality of the formulated cookies. The study further showed a storability period of 4 months for the formulated gluten-free cookies packed in laminated resealable pouches with no preservative being used which is a good attribute especially because most gluten-free products have a short shelf-life period. Use of non-wheat flours such as sorghum and green grams in addition to simple processing techniques such as malting and fermentation can be used to increase consumption of healthy shelf-stable functional food products while addressing their underutilization in food product development in Kenya.

6.2 GENERAL RECOMMENDATION

Malting and fermentation processes are cost-effective methods which can be adopted by food industries to produce quality and nutritious products in the market. Utilization of sorghum and green grams in value addition will curb the underutilization of such cereals and legumes in the country. It is recommended to increase awareness in the country on the benefits of other cereals other than maize and wheat which are largely being used. Awareness should also be done on the benefits of legume production which can be used for value addition minimizing post-harvest losses and the issue of lack of markets. Increasing awareness in the country of the use of non-wheat flours in product development can help boost commercialization of non-wheat products of high nutritional quality. It is also essential for further work to be done to ensure a longer storage period for the developed ready-to-eat products.

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8.0 APPENDICES

APPENDIX 1: SENSORY EVALUATION QUESTIONNAIRE OF SORGHUM COOKIES PREPARED

Respondent Number…………………………………….. Date…………………………

Gender………………………………………….

You have been presented with 12 differently formulated cookie samples. Kindly observe, taste each of the samples provided and rate the sensory attributes as per the scores given below**.** ***Rinse your mouth with clean water provided between each of the tastings***. You may re-taste the samples if you wish.

Score Scale

9 = Like Extremely
8 = Like Very Much
7 = Like Moderately
6 = Like Slightly
5 = Neither Like nor Dislike
4 = Dislike Slightly
3 = Dislike Moderately
2 = Dislike Very Much
1 = Dislike Extremely

Sample Code	Color	Smell	Taste	Texture	Crunchiness	Overall Acceptability
120						
109						
136						
124						
104						
009						
119						
004						
138						
005						
110						
128						

THANK YOU FOR PARTICIPATING