EVALUATION OF WHEAT-SORGHUM-CHICKPEA COMPOSITE BREAD FOR PHYSICAL, NUTRITIONAL AND SENSORY QUALITY

LUCY N. MARIERA

A Thesis Submitted to the Graduate School in Partial fulfilment of the Requirements for the Award of Master of Science Degree in Nutritional Sciences of Egerton University

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DECLARATION AND RECOMMENDATION

Declaration

I declare that this research thesis is my original work and to the best of my knowledge has not been presented for an award of a degree, diploma or certificate in this university or any other institution

Signature……………………………………Date…………………………………………

Lucy N. Mariera

HM18/3662/13

Recommendation

This research thesis has been submitted with our approval as supervisors.

Signature: ……………………………..……Date…………………..…………………………

Dr. Maureen Cheserek

Department of Human Nutrition

Egerton University

Signature: ……………………………..……Date… ………………..…………………………

Prof. James O. Owuoche

Department of Crop, Horticulture and Soil Sciences

Egerton University

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DEDICATION

This work is dedicated to the Almighty God, my late son Engineer Alex Nyachieki Mariera for his inspiration and selfless support, Carol Mariera, Kelvin Mariera for their loving care and support.

ACKNOWLEDGEMENT

I would like to thank the almighty God for giving me the courage, endurance and power for the completion of this study. The financial support from Kenya agricultural productivity programme (KAPP) and technical support from Egerton University and Kenya Agricultural and livestock Research Organization (KALRO) is highly acknowledged. I sincerely thank Mr. Maurice Mutumba (Egerton Laboratory), Mr. Ndungu (Cereal laboratory KALRO) and their teams for their support throughout the research period. I greatly thank my supervisors Dr. Maureen Cheserek and Prof. James Owuoche for expertise, knowledge and skills.

ABSTRACT

The incorporation of whole chickpea (*Kabuli)* flour into sorghum (*Sorghum bicolor* L. Moench) wheat composite flour could improve nutritional quality of wheat (*Triticum aestivum* L.) bread. The aim of the study was to produce bread using sorghum genotype EUS130 and chickpea, compare physical and baking properties, nutrient levels, *in vitro* protein digestibility, shelf life and sensory acceptability. Randomized Complete Block Design was used to compare different breads. Sorghum-wheat composite bread was prepared in proportions wheat: sorghum; 100:0 (control), 96:4, 92:8, 88:12and 84:16. Results showed that specific loaf volume (SLV) was low in composite loaves, protein content was highest ($p<0.05$) in 8% sorghum bread, ash and fibre were high in composite loaves compared to control. The shelf life were also higher in sorghum composite bread (9 days) than control (6.3 days) while *in vitro* protein digestibility was not significantly ($p>0.05$) different among the loaves. Sorghum composite flour was further enriched with chickpea in proportions wheat: sorghum: chickpea was 100:0:0 (control), 96:0:4, 92:0:8, 88:0:12 and 84:0:16 (0%). Sorghum 4% bread blended in proportion 92:4:4, 88:4:8, 84:4:12 and 80:4:16 (4%); 8% bread proportion 88:8:4, 84:8:8, 80:8:12 and 76:8:16 (8%). The 12% bread proportion 84:12:4, 80:12:8, 76:12:12 and 72:12:16 (12%) while 16% was mixed in proportion of 80:16:4, 76:16:8, 72:16:12 and 68:16:16 (16%). The composite bread had similar dough length, height, area under curve and energy (W) with control, values decreased with increase in wheat substitution. Fibre, oil and protein contents were significantly ($p<0.05$) high in chickpea enriched bread compared to control, values increased with increase in wheat substitution. Carbohydrate content was low ($p<0.05$) in enriched bread, values decreased with increase in substitution level. In addition, the *In vitro* protein digestibility was low ($p<0.05$) in wheat-sorghum-chickpea. Sensory scores; texture, taste, chewiness and general acceptability were highly rated in control and bread from 92% wheat:4% sorghum:4% chickpea proportion. Microbial counts (cfu/g) were lowest ($p<0.05$) in 12% chickpea enriched sorghum bread while shelf life was significantly ($p<0.05$) high in sorghum and chickpea containing bread compared to control. In conclusion, bread from proportion 92% wheat:4% sorghum:4% chickpea was most acceptable. It is recommended that, as a policy in Kenya, this composite flour be used for production of bread and other confectionaries to further improve nutritional and sensory quality of wheat based products.

Key words: physical properties, nutritional quality, sensory acceptability.

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LIST OF ABBREVIATIONS AND ACRONMYS

AACC	Approved Method of American Association of cereal chemists
AOAC	Association of official Agrigultural Chemist
GSL	Sorghum grain lipids
HDL	High density lipoprotein
KACE	Kenya Agricultural Commodity exchange
KALRO	Kenya Agricultural and livestockResearch Organizaton
KNBS	Kenya National Bureau of Statistic
PDA	potato dextrose agar
PEM	Protein energy malnutrition
RDA	Recommended dietary allowance
UNICEF	United Nations International Children's Emergency Fund
VLCFA	Very long chain fatty acids
Zn	Zinc

CHAPTER ONE
INTRODUCTION

1.1 Background information

Food insecurity is a major global health concern, over 841 million (12% of world population) people are suffering from starvation (UNICEF, 2018). The rates of under nutrition among the rural communities have been increasing worldwide (28% in 2004 to 29% in 2006) which hinders the achievement of sustainable development goals (FAO, 2015). About 239 million people in Sub Saharan Africa suffer from hunger with two million children dying annually from under nutrition (Black *et al.,* 2008). In Kenya, 26 % of children are stunted, 4% wasted and 11% underweight (KNBS and ICF Macro, 2015). Undernutrition is higher in the urban slums estimated at 40% (United Nations International Children's Emergency Fund UNICEF, 2016). This has been attributed to poverty and food insecurity partly because over 75% of the total area in the country is arid and semi-arid land (ASAL). The area experiences unpredictable and poorly distributed rainfall aggrevating food hunger and food insecurity (Ogeto *et al.*, 2013). Notably, wheat contributes 50% of global dietary needs (Kerneay, 2010). Bread is an important staple food in both developed and developing countries, consumption has increased due to urbanization and population increase (Abdelghafor *et al.*, 2011). Awumi *et al.* (2017) confirms that in Nairobi wheat consumption is higher than maize since 1990, therefore an important item in the food budget share. Wheat (*Triticum aestivum*) flour has been the major ingredient in leavened bread because of its unique viscoelastic property attributed to gluten (Ohimain, 2014). In Kenya, wheat production is decling, the supply is 360,000 tonnes against a national requirement of 900,000 tonnes (Mahojan, 2014). Importation to meet bread and confectionary demand increases food insecurity due to high demand for other cereals, over dependence on foreign foods and high bread prices (Ohimain, 2014).

Non-wheat growing countries use locally grown crops such as cassava and plantain in different proportions so as to reduce demand for imported wheat, making composite bread an attractive option (Abdelghafor *et al.,* 2011). Composite flour has been defined as wheat flour that has been blendedwith non-wheat flours (Tiimub, 2013; Ohimain, 2014). In Nigeria, inclussion policy of 10% cassava flour in bread making in 2012 was estimated to save the country of ₦ 63.5 billion, the country is considering addind ahigher proportion of cassava flour (Ohimain, 2014).

Kenya needs to venture into dry areas by introducing drought resistant crops such as sorghum (*Sorghum bicolor)* and millet (*Elusine spp)* that can be used to add value to wheat products (Ogeto *et al.,* 2013). Most importantly, awareness creation on the consumption of variety of foods is necessary for dietary diversity as majority of children consume less than four food groups in a day (Bukania, 2014). Researchers have created awareness and demonstrated that dietary diversity helps to ensure adequate nutrient intake for better growth and development (Hooshman and Odipi, 2013; Utilla-Coello *et al.,* 2014; Proieti *et al.,* 2015) Development of chickpea-enriched sorghum- wheat bread enhances the nutritional and sensory qualities of bread (Uttrilla-Coello *et al*., 2014). It provides most of the essential minerals, protein, fat, dietary fibre, vitamins and carbohydrates (Ratnavathi and Patil, 2013). Thus, deficiency of these nutrients in the body is often reduced through fortification of wheat flour products with legumes such as chickpea. This also improves dietary diversity, colour, texture and affordability through reduced production cost (Abdelghajor *et al*., 2011; Ogeto *et al*., 2013).

Protein deficiency is observed in majority of food insecure households especially among children, pregnant and nursing mothers in developing countries. Refined wheat bread is popular for its convenience but consumers are not keen on the low nutritional value (Tiimub, 2013). As wheat production declines, Kenya loses Kes. 2 billion from importation and it is currently targeting sorghum to feed 52% of food insecure population (Mwadalu and Mwangi, 2013). Kenya is producing approximately 159,877 tonnes of sorghum annually in the ASAL area, more farmers are already cultivating better yielding variety and researches are creating awareness on its nutritive value and importance as a cereal (Chepngetich *et al.,* 2013). The cereal is nutritionally rich, has a neutral smell and blends well with wheat and often used as a wheat substitute in flat bread. However, nutrient bioavailability is inhibited by antinutritive factors (Ratnavathi and Patil, 2013). Jukanti *et al.* (2011) observed that when chickpea is consumed alongside other cereals such as sorghum or wheat, any deficiency in amino acids requirement is met. Chickpea is also gaining consumer acceptability for its nutritional value. Kenya is supporting farmers through KALRO to grow dry land crops and it is already producing approximately over 20,000 tonnes annually (Ogeto *et al*., 2013). When used to enrich sorghum-wheat composite bread; it is likely to improve protein content (especially lysine amino acid) that is vital for protein synthesis, minerals (such as zinc and iron) fibre and energy especially for breakfast meal of food insecure households.

1.2 Statement of the problem

Globally, 821 million children are suffering from chronic undernutrition consequently resulting in two million deaths annually (UNICEF, 2018). In Kenya, undernutrition has reached 40% in urban areas and 26% children are stunted (UNICEF, 2016). Wheat is food to 50.4% Kenyans; wheat bread consumption has increased versus declining wheat production. This has necessitated importation of approximately 600,000MT to meet demand. This increases food insecurity due to high demand for other, there is over dependence for foreign foods and bread price increases. Sorghum is nutritious, grown in dry areas, has a neutral flavour, costs less (Kes 50.00/kg) in the local market compared to wheat flour (Kes 65.00). However, the use of sorghum to partly replace wheat in bread production has not been exploited. Sorghum is nutritious but its potential to reduce food insecurity and improve dietary diversity has not been practiced in Kenya. Chickpea provides essential amino acids (such as lysine), minerals, vitamins fibre and carbohydrate. Their deficiency can be reduced by incorporating chickpea into wheat and sorghum products (such as bread) an important value addition not in Kenya where malnutrition is a challenge in many families. This study aimed to develop chickpea enriched sorghum-wheat composite bread, evaluate the nutrient composition, sensory quality, microbial count, shelf life and *in vitro* protein digestibility.

1.3 Main objective

To determine the physical properties, nutrient content, sensory acceptability and microbial counts of wheat-sorghum-chickpea composite loaves of bread.

1.3.1 Specific objectives

i. To compare the physical and baking properties of total wheat, sorghum-wheat composite and chickpea enriched sorghum-wheat composite loaves of bread.
ii. To determine nutrient composition of the total wheat, sorghum-wheat composite and chickpea enriched sorghum-wheat composite loaves of bread and *in vitro* protein digestibility of the chickpea enriched bread.
iii. To determine organoleptic acceptability of the total wheat, sorghum-wheat composite and chickpea enriched sorghum-wheat composite loaves of bread.
iv. To determine microbial counts and shelf life of the total wheat, sorghum composite and chickpea enriched sorghum-wheat composite loaves of bread.

1.4 Hypotheses

i. Physical and baking properties of total wheat, sorghum-wheat composite and chickpea enriched sorghum-wheat composite loaves of bread are not significantly different.

ii. Nutrient compositions and *in vitro* chickpea protein digestibility of the total wheat, sorghum- wheat composite and chickpea enriched sorghum-wheat composite loaves of bread do not have significant differences.

iii. Use of sorghum composite flour and chickpea enriched sorghum-wheat composite flour in bread making does not affect its acceptability.

iv. Microbiological counts and shelf life of total wheat, sorghum-wheat composite and chickpea enriched sorghum-wheat-composite loaves of bread do not significantly differ.

1.5 Justification

Undernutrition and related complications are a cause of death to two million children annually worldwide (Black *et al.*, 2008). Wheat provides 50.4 % of dietary needs in Kenya, wheat bread demand has steadily increased due to urbanization, increased population, consumer preference and convenience as a snack. However, wheat yield is declining and approximately 350,000 tonnes is produced versus a demand of 900,000 tonnes annually. This deficit increases demand of other cereals; malnutrition and food insecurity are aggravated in most ASAL areas. Kenya loses about Kes. 2 billion annually on wheat importation to meet demand (Ogeto *et al.,* 2013). sorghum is a traditional food in Kenya however, most people believe in Western diets which are not affodable (especially wheat based products) in food insecure areas. Furthermore, most of the food aids are sorghum based products such as Ready to Use Therapeutic Food (RUTF). It is time that Kenya encourages consumption of sorghum because like other cereals sorghum is nutritious and may contriute in reduction of the 26% stunted children in Kenya due to prolonged inadequate food intake (Ndemwa *et al.,* 2017). It is drought tolerant, yields highly in ASAL areas, cheaper (Kes 2,700 per 90 kg bag) compared to wheat (Kes 3,200 per 90 kg bag) hence if adapted as food, it will help in reduction of malnutrition and food insecurity. The use of sorghum in bread making is to improve nutrient intake of consumers through nutrient diversification and to stabilize bread price. Income from sale of sorghum to bakers by farmers will make them self sustainable and thus reduce malnutrition.

Wheat bread is protein and mineral deficient (1.568-1.89375 g protein in every 2 slices) (Motbainor *et al*. 2015). Sorghum, like other cereals is protein defiecient, therefore, enrichment of sorghum-wheat composite bread with chickpea improves protein and mineral. Chickpea is a dry land crop, rich in essential amino acids (especially lysine), has high mineral content (folate, iron), Vitamins (such as A and E) and fibre. It was preferred in this study because it is superior to soya in eight (8) essential amino acids has been recommended for infant formulas and as a milk substitute. Sorghum and chickpea can complement each other to improve nutrient content of bread so as to reduce protein energy malnutrition and mineral deficiency, improve health status of consumers' particularly pregnant women, lactating mothers, growing children and school performance. Sale of sorghum and chickpea by farmers will increase self-sustainability and reduce malnutrition and food insecurity in the community. This will contribute to Kenya's achievement of Sustainable Development Goals, provision of quality and quantity food, improved livelihoods of Kenyans and the attainment of Kenya Vision 2030.

CHAPTER TWO
LITERATURE REVIEW

2.1. Increase in bread demand

Bread may be described as a confectionary product that is produced mainly from wheat flour, yeast, water and salt, by a process that involves a series of mixing, kneading, proofing, shaping and baking (Ndife *et al.,* 2011). It was known as food even before 2100 BC and consumption of white flour bread has been associated with prosperity, though often opposed on nutritional grounds (Jones, 2007). Wheat flour is the main bread ingredient due to the functional protein gluten (Bot, 2011). It is the most widely consumed food crops and fundamental to world food security as it is among crops that provide 50% of dietary needs (FAO, 2014).

Globally, billions of people rely on wheat for a large part of their diets (Henderson *et al.*, 2007). In Sub-Saharan Africa (SSA) between the year 2000 and 2009, *per capita* wheat consumption increased by 0.35 kg per annum and it is projected that consumption will be approximately 1.12 million tonnes between 2010 and 2020, and 1.28 million tonnes by 2030. Kenya is among the top wheat importing countries where wheat is considered to be the second most important crop (Tosi *et al.,* 2011) with consumption of 50.4% of which, 75% is used in the urban area (USAID, 2015). Its consumption has increased beyond wheat production due to convenience (Abdelghafor *et al.,* 2011), rising incomes, growing population, urbanization and women participation in the labour force. Bread has been the second most widely consumed non indigenous food leading to importation and loss of foreign exchange (Olaoye *et al.,* 2007). The Kenya Agricultural Commodity exchange (KACE, 2012) however indicates that wheat output dropped by 37% due to unpredictable rainfall while the United State Department of Agriculture,(2015) reported that its demand rose from 350,000 tonnes to 900,000 tonnes (2009) contrary to 28% increase in consumption in the year 2008.

Nyangito *et al.* (2008) explains that the decline in wheat production has been caused by high production costs, biotic stress, pest and lack of credit to farmers, low level of technology adaptation, reduced arable land and unpredictable weather believed to be a consequence of climate change. The crop yield losses have led to food insecurity and undernutrition (Sandiswa *et al.,* 2014). The high cost of bread in many developing countries has led to the use of composite flours from locally grown crops to mitigate against doubling of international food prices (especially wheat and maize) and hoarding of cereals (Sasson, 2012). High food prices have been linked to poverty and food crisis in Africa (FAO, 2012).

There is therefore need to venture into dry land farming by introducing drought, disease and pest resistant crops such as sorghum and millet to bridge the gap (Ogeto *et al.,* 2013). Awareness creation on consumption of variety foods is necessary for dietary diversity (Bukani *et al.,*2014). The increased use of local cereals like sorghum is intended to improve the nutritional quality and diet diversity especially for poor populations in developing countries (Tiimub, 2013).

2.2 Sorghum utilization

Global sorghum production is 61.5 million tonnes from which 21.6 million tonnes come from Sub-Saharan Africa (Proietti *et al.,* 2015), and 50% is a staple diet to 300 million food insecure people in developing countries (Dicko *et al.,* 2006; Henley, 2010). In Kenya, approximately 159,877 tonnes are produced in the ASAL areas and the *per capita* consumption is 2.25kg among poor household group and 5.58kg among high income household group (Munyanga *et al*., 2003). It is valued as the fourth most important crop after maize (*Zea mays*) in Kenya, rice (*Oryza sativa*) and barley (*Hordeym vulgare*) (Timu *et al.,* 2014). Kenyans use it for malting beer, porridge and *Ugali* (Timu *et al.,* 2014). The food security programs targets sorghum and other legumes for reduction of food insecurity to 52% in food insecure population especially in the dry land such as Kitui, Nyanza and Mbeere (Chepngetich *et al.,* 2014).

In Tanzania, sorghum is boiled, popped and can be ground into flour and mixed with wheat flour to make *chapatti, mandazi*, weaning flour for children's porridge or for people with opportunistic infections (associated with HIV/AIDS) and making local brews (Rohrhach and Kiriwaggulu, 2007). The cereal has been used to fortify bread, cookies and other confectionaries so as to improve the nutrient content, dietary fiber and sensory properties. It is also used as an antioxidant supplement, natural food preservative and anti-caloric agents for obese individuals (Awika and Rooney, 2004; Kim and Park, 2012). Ratnavathi and Patil (2013) also produced sorghum composite cake and noodles from non-pigmented sorghum composite flour.

Sorghum bran is used in replacement of 50% proportion of cocoa in chocolate (to give a brown colour) or 100% substitution. Sorghum kernels and other parts are also milled for animal feed and fodder, fencing materials, wood fuel, construction material, industrial starch, fuel and natural dye or food colour (Mikbeb, 2009; Ratnavathi and Patil, 2013). Sorghum composite flour has also been used for production of therapeutic foods (RUTF) such as plumpy nuts (Awadalkareem *et al.,* 2008) and to increase energy, protein and minerals in biscuits (Mridula *et al.*, 2006; Adebowale *et al*., 2011). Developing countries use sorghum in order to reduce costs associated with wheat importation (Mohammed *et al.,* 2011). In Sudan and Senegal, experiments on sorghum composite bread were found to be acceptable (Abdelghafor *et al.,* 2011).

2.2.1 Physical and baking properties of wheat- sorghum composite bread

Baking and physical characteristics are affected when sorghum flour is increased to 30% in bread making. The loaf volume decreases significantly between 5.04 and 5.27 cc/g as compared to the control 5.95cc/g (Abdelghafor *et al*., 2011) as it affects rheological properties of the dough (Carson and Sun, 2011). This is attributed to the low levels of gluten which affects the ability of the dough to rise due to weaker cell structure (Tinureh, 2012). Water absorption capacity ranges between 3.47 and 4.12 but in sorghum composite flour, it is 86.8 to 92.5: an indication that sorghum flour has a higher water binding capacity than wheat flour, and this improves the constitution ability and structural properties of the dough (Ajanuka *et al.,* 2012). This is as a result of the loose structure of starch polymers (Oladipo and Anwokocha, 2011).

An increase in sorghum proportion affects the crumb colour values (Eduardo *et al.,* 2014) thus, when preparing the dough, more sugar, yeast, or pre-fermentation is necessary. More proofing time (50 minutes and baking at 250 °C for 8-10 minutes) is needed for wheat bread as compared to sorghum composite bread (45 minutes proofing time, at 212 ^{o}C baking temperature for 18 minute) (Ratnavathi and Patil, 2013). Sorghum lacks gluten; hence substitutes such as gums, methylcellulose, xanthan gum, carboxy methyl cellulose are used as gluten replacement to enhance the strength of the dough (Boswell and Rooney, 2010; Bot, 2011).

2.2.2 Nutritional quality of sorghum kernels

Sorghum contains 56-73% starch of which 70-80% is amylopectin and 20-30 % is amylase (Rehman *et al*., 2006). The cooking quality of sorghum is influenced by amylase content, soluble protein, swelling power and solubility of the starch (Adebowale *et al*., 2011). The starch digestibility is low (33-38 %) compared to (53-58%) maize (Ratnavathi and patil, 2013) which reduces absorption leading to low glycaemic index (Abdelghafor *et al.,* 2011). In a study where animals were fed on high tannin sorghum (3.5% catechin equivalent), a 10 % weight reduction was associated with low enzymatic action (Al-Mamay *et al.,* 2011).

Protein content in sorghum is approximately 7-15%, which are divided into Kafirins (prolamins) 26%, albumin and globulins together (15%) and glutenines (44%). Prolamin is the major protein constituting of 50-70% (FAO, 1995), it is protease resistant, and responsible for the poor nutritional quality of sorghum (Evans and Taylor, 2003). Sorghum contains very little lysine (essential for growth, bone health and for conversion of fats into energy) but has high levels of proline, glutamic acid and leucine. Digestibility of protein *in vitro* and *in vivo* ranges between 49.5 to 70% as compared to wheat 81%, maize 73% and rice (66%) however, decortication, fermentation and malting improve sorghum digestibility (Adebowale *et al*., 2004). Pearling significantly (P< 0.05) lowers the phytic acid level in 30% sorghum composite flour by 14%-16% (Rehman *et al.,* 2006).

Sorghum has approximately 3% lipid in the germ (scutellum) (Ogunsakin *et al.,* 2015). Fatty acids in sorghum includes, the polyunsaturated fatty acids linoleic (49%), linolenic acid (2.7%), oleic (31%), palmitic acid (14%) and stearic acids (2.1%) (Stefosca-Needham *et al.,* 2015). It also contains very long chain fatty acids called policosanols (VLCFA) which affects lipid profile and cholesterol biosynthesis. A consumption of 50 mg/kg body weight significantly (*P*=0.004) reduces total cholesterol and non-high density lipoprotein (LDL) cholesterol (*p*=0.007) without affecting HDL levels (Wang *et al.,* 2005). A study on Hamsters fed on diets containing 5% GSL for 4 weeks had 19% increase (*P*<0.05) in HDL cholesterol, 36% decrease in plasma non LDL cholesterol A and positive correlation was observed between cholesterol absorption and plasma non-LDL cholesterol concentration *(r*=0.97, P=0.035) (Grundy *et al.,* 2004). Unlike other cereals, sorghum kernels contain fat soluble vitamins A, D (tocopherol 1), E and K (Mella, 2011) and water soluble vitamins such as riboflavin, pyridoxine and thiamin (Dykes and Rooney, 2004). The B vitamins thiamin and nicotinic acid can become more bio-available through fermentation (Pontieri *et al.,* 2014).

Fibre is an endogenous component of the plant material which is resistant to digestion by human enzymes. Sorghum kernel consists of 2.3% - 2.9% fibre and a single serving of sorghum provides 48% of the Recommended Dietary Intake (25g for women and 38g for males). Dietary fibre includes cellulose, hemicelluloses, pectin and lignin while insoluble fibre consists of pectin, arabinoxylan and *B*-glucans. These fibres have the ability to lower blood serum cholesterol (Rehman, 2011) through bulking and binding of cholesterol and prevents its digestion resulting to reduced blood serum cholesterol (Horn, 2014). The processes help in elimination of carcinogenic and harmful substances for protection against heart diseases, atherosclerosis, obesity, diabetes, cancers and maintenance of gastrointestinal health (Rooney and Waniske, 2000). Urban populations have become aware that sorghum is a healthy food for diabetic patients (Mridula *et al.*, 2007) which has led to an increase in the consumption of sorghum composite flour products such as bread especially among Indians (Ratnavathi and Patil, 2013).

Bread from sorghum-wheat composite flour also contains phytochemicals such as flavonols, flavones, flavanones, isoflavones, catechines, 3- deoxyanthocyanin, flavan-3-ols, anthocyanins. They are associated with the blue, purple and red colour in plants such as strawberries, blackberries which reduce the risk of chronic diseases ((Awika and Rooney, 2007; Rooney and Awika, 2012) such as CVD through improved endothelial function, inhibition of platelet aggregation and protection against the risk of atherosclerosis (Dykes and Rooney, 2006). This occurs through lowering of cholesterol level, interacting with carbohydrates to form resistant starch which lowers digestibility consequently resulting in weight loss, reduced serum blood glucose; a benefit in diabetic patients (Taylor *et al*, 2005).

The levels of minerals such as Ca, P, K, Fe, Zn, Mg and Cu are high in sorghum, but tannin makes them unavailable for absorption within the gastric system (Pontieri *et al*., 2014). According to Afify *et al*. (2011), soaking, germination or fermentation improves the absorption of iron 12 times more than the unfermented sorghum (Bot, 2006). In the process of reducing phytic acid, minerals are also lost, hence, enrichment with legumes such as chickpea is necessary to reduce Zn and Fe deficiency; consequently, lowering undernutrition which has serious health consequences (EL-Money *et al*., 2011). Pearling of kernels reduces tannin and increases iron bioavailability, cooking lowers phytic acid in flour by 78% in baked bread (Bot, 2006) while traditional roasting of grains effectively reduces anti-nutritional factors, improves the aroma, colour and extension of storage life of foods (Sanni *et al*., 2008).

2.3 Chickpea utilization

Chickpea is a legume that is grown in the Kenyan highlands during the short rains, it is popular in Koibatek, Bomet, Mbeere, Garaba (*http://grainlegumes.cgiar.org/farmwersin-kenyan-highlands-taking-to-chickpea-cultivation-during-short -rains/).* Kenya produces over 20,000 tonnes of chickpea (MOA, 2012; FAO, 2006). The legume is used as a rich source of protein in human diets (Verma *et al.,* 2015), its protein quality outscores that of soy in seven of the ten essential amino acids in quantity and therefore found useful in supplementation of chickpea based infant formula (Malunga *et al.,* 2014). Rosiak *et al.* (2013), established that chickpea flour is used in supplementation of bread, snacks and chips as it reduces production of acrylamide (a carcinogenic substance produced during cooking). Mateljan, (2016) noted that in the Mediterranean, chickpea is used for making flat bread (sacrament during *lent)* and relied on as a high protein legume. Indians use chickpea in cold salads, stews and eaten with pastas, meat or sausages, ground into flour for making of fried balls *(falafel)* or made into a batter for coating vegetables before deep frying (fritters). The green leaves are used as vegetables while a liquid extraction derived from chickpea can be commercially used as an egg white replacement for making of meringue. It has been used to replace soy in animal feed for better milk and egg production (*https ://dornsife.usc.edu>news>stories*).

2.3.1 Nutritional value of chickpea (*Cicer arietinum*)

Refined wheat bread is popular and often associated with wealth, but its nutritional quality is low, as wheat is deficient in essential amino acids such as lysine amino acid (Jideani and Onwubali, 2009). As noted earlier, sorghum is nutritionally rich but many nutrients are bound by tannin rendering them not bio-available (Ratnavathi, 2013). Rosiak *et al.* (2013) reported that supplementation of sorghum with chickpea flour increases amino acid content; contributes to improved health particularly children, lactating mothers, pregnant women from food insecure households or vegetarians (Jukanti *et al*., 2012; Ratnavathi, 2013). Protein is essential in production of enzymes, antibodies and hormone; essential for bone health through increase in calcium absorption (Maneju *et al*., 2011). Rosiak *et al.* (2013) established that chickpea is rich in amino acids (lysine, methionine and tyrosine). Protein (Lysine amino acid) is a building block for synthesis of body protein and peptides which participate in all biochemical reactions and physiological activities of all cells and tissues and an important source of energy (Wu, 2013).

Furthermore legume is rich in protein, minerals (potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), Fe, Zn, copper (Cu) and manganese (Mn). It also contains vitamins A, C, E, K, B6, thiamin, niacin, folate, riboflavin and pantothenic acid as well as dietary fibre (Pradhan *et al.* 2014). The biological value, net protein and protein efficiency ratio of chickpea is higher compared to soybean *(Glycine max*) and other legumes (Mhlanga *et al.* 2014). The *Beta* carotene is also easily converted to vitamin A and thus if consumed could possibly help in reduction of cornea damage (xerophthalmia). Jukanti *et al*., (2012) reported that consumption of dietary fibre in chickpea is associated with faecal bulking and increased frequency of elimination, binding of bile salts which lower cholesterol digestion consequently reducing the risk of CVD. It reduces total plasma cholesterol level (215 to 182 mg/dl) in obese persons and from 49-65.4% in rats fed on chickpea diet compared to 46-62% in the control. Cakes fortified with legumes are shown to have higher protein 6.66%

compared to the control 6.01%. Soaking, germination and boiling for 15 minutes at 95-97 ^{0}C followed by dehulling improves protein digestibility (Salve and Mehrajfatema, 2011).

Chickpea protein digestibility is higher (65.3-79%) than soybean (62.7-71.6%), various processing and cooking methods that affect anti-nutritive factors also influence protein digestibility of legumes. *In vivo* and *in vitro* methods are used to determine protein digestibility, but *in vivo* is preferred because it is less time consuming, less expensive and produces equally reliable results (Sudesh *et al.*, 1998; Vanucchi *et al.,* 2005). Chickpea is soaked to reduce antinutritive factors, protein carbohydrates and lipids are therefore broken down easily into simpler forms, thereby increasing protein bioavailability (Afify *et al.*, 2012). In *in vitro* digestibility, pepsin and pancreatin enzymes are used, and the extent of hydrolysis is determined by the increase of free amino groups.

2.4 Acceptability of sorghum- wheat composite bread

The appearance of sorghum composite bread has been associated with the sorghum ratio. Increased sorghum flour by 5 – 10% produces a darker crumb than the control (Ndife *et al*., 2011; Serrem *et al.*, 2011) but at 20%, bitter taste is observed. As the ratio of non-wheat flour increases, acceptability decreases (Ijah *et al*., 2014) but most studies point out that there is no significant difference between the bread baked from 5–10% sorghum- wheat composite flour compared to bread baked from total wheat flour as far as acceptability is concerned (Abdelghafor *et al.*, 2011). Sudan Kissra bread enriched with Bambara nuts had declining acceptability with increase in the level of nuts (Yagoub and Abdalla, 2007).

2.5 Microbial count of composite bread

Afolabi e*t al.* (2015) defines total viable counts (TVC) as a count of the total number of living bacteria in a sample (aerobic colony count at 30° C) that is used as a measure of microbiological quality in reference to levels of bacteriological contamination. It reflects the condition in which food was produced, stored, handled (personal and food hygiene and packaging) and this can be implicated in foodborne illnesses or used to predict the shelf life of the product. Food spoilage is eminent when the levels of TVC reaches 10-100 million per gram of the product. Acceptable level on nutrient agar ranges from 9.0×10^2 to 1.5×10^3 or TVC < 10, 100(colony forming units) cfu/g. Bread can be contaminated by bacteria; which alters its quality; a potential source of infection to consumers. Pepe *et al.* (2003) established that white wheat bread develops rope spoilage within 5 days from bacillus subtillis (61%) and smaller percentages from *Bacillus licheniformis, Bacillus cereus and Bacillus clause,* but in fermented bread, this occurs within 7 days.

Table 1: Comparison of the nutrient composition in 100 g dry chickpea, sorghum and wheat**.**

Food group		Chickpea g/100g	Sorghum g/100g	Wheat g/100g
Protein	-	13.5 - 28	8.6 – 18.9	8.9-16
	Lysine	1.291	0. 226	0.404
	Methionine	0.853	0.169	0.634
	Phenylalanine	1.034	0.546	0.808
	Isoleusine	0.832	0.433	0.541
	Leucine	1.374	1.1491	1.038
	Threonine	0.716	0.349	0.433
	Tryptophan	0.28	0.124	0.195
	Histidine	2.4	0.346	0.33
	Valine	1.009	0.561	0.953
	Arginine	1.4	0.355	0.702
	Cystine	1.1	0.127	0.274
	Alalanine	2.7	1.033	0.555
	Glutamate	7.7	2.439	4.946
	Glycine	0.7	0.346	0.621
	Proline	1.8	0.852	1.68
	Serine	3.4	0.462	0.63
	Tyrosine	0.6	0.321	0.127
	Aspartic acid	0.881	0.743	0.694
Minerals	Phosphorus	382mg	338mg	322mg
	Potassium	440mg	464mg	340mg
	Sodium	5mg	2.61mg	2mg
	Zinc	4.21mg	2.6mg	2.78mg
	Iron	7.23mg	5.7mg	3.6mg
	Copper	0.04mg	0.3mg	0.41 mg
	Selenium	-	0.2mg.	70.7µg
	Calcium	290mg	28mg.	25mg
	Magnesium	4.8mg	1.8mg	4.05mg
Vitamins	Vitamin A	41 IU	-	9 IU
	Vitamin B6	0.5mg	0.47mg	0.336mg
	Vitamin E	0.8 µg	-	1.01mg
	Vitamin K	120mg	-	1.9. µg
	Thiamin	1.1mg	0.26mg	0.54mg
	Folate	299 µg	-	43 µg
	Niacin	2.9mg	2.91mg	5.71mg
Carbohydrate	-	60.7g	76.6g	68g
Dietary fibre	-	17.4g	6.6g	12g
Fat	-	6g	3.3g	2g

Source: Queiroz *et al*. (2015); Mulanga *et al*. (2014); Kaijage *et al*. (2014); USDA. (2014). Samiha *et al.* (2011); International Centre for Agricultural Research in dry areas (1989) ICARDA.

Rosenkrist and Hansen, (1995) observed that bacterial spoilage in bread includes unpleasant odour, enzymatic degradation of the crumb which becomes soft and sticky from extracellular slimy polysaccharides. Ropiness and spore germination occurs at temperatures between 25-30°C under humid environment. Spores of *B. subtillis* can also be found in raw materials, bakery environment, yeast, preservatives, additives or gluten and some resist baking temperatures of between 97-101°C. Ijah *et al.* (2014) recorded bacterial spoilage after 6 days (3.0 x10^6) in sweet potato composite bread (ratio 90:10), but none in the control.

Coliforms are non-sporing facultative anaerobic rod-shaped bacteria that ferment lactose to produce acid and gas within 48 hours at 35°C (Nzung'a *et al*., 2013).They are not pathogenic but their presence within the environment is an indication of favourable condition for presence of pathogens. *E.Coli* is a coliform originating from faecal contamination (Giwa *et al.,* 2012). Total fungi count (potato dextrose agar -PDA) between 3.1 x 10^2 to 1.0 x 10^3 cfu/ is acceptable, both mould and fungi cause various degree of deterioration and decomposition of food. They invade and grow on any type of food at any time. Their growth is manifested by spots of different sizes and colour, slime, white cotton mycelium or highly coloured mould and abnormal odours and flavours. Fungi count (8.0 x10^1) were observed after 7 days in Irish potatoes and after 8 days in sweet potato composite bread Ijah *et al.,* (2014).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Area of study

The study was conducted in the Animal Science Laboratory for nutrient content and *in vitro* protein digestibility while Microbial counts, shelf life and sensory evaluation were carried out in Food Science Laboratory at Egerton university (0° 23' south, 35° 35' East). Physical and baking properties were done in the Cereal Chemistry Laboratory at Kenya Agricultural and Livestock Research Organization (KALRO), Njoro (0° 20' south, 35° 56' East).

3.2 Experiment 1: Development of sorghum- wheat composite bread and evaluation of nutritional, physical and sensory quality

3.2.1 Experimental design

Randomized Complete Bock Design was adopted in this study. Experimental treatments were wheat flour and sorghum flour mixed in the ratio of sorghum: wheat 0:100 (control), 4:96, 8:92, 12:88 and 16:84. The experimental loaves of bread were baked in triplicate for precision, grouped into three (3) blocks so that treatments may be compared under homogenous condition. Blocks were formed with loaves of bread from the same level of treatment, each treatment level occurring exactly once in a block and randomization done separately in each block.

3.2.2 Preparation of sorghum –wheat composite flour

Composite flour was prepared according to Abdelghafor *et al*. (2011) method. Dry brown sorghum grain sample from variety EUS130 that was developed at Egerton University (a line that was identified as suitable for bread baking) was used. The samples were winnowed and cleaned in order to remove husks and any foreign materials. They were dried to approximately 12% moisture content, before milling to fine whole flour using a perten laboratory bench mill to produce fine whole meal flour

3.2.3 Procedure for baking sorghum-wheat composite bread

The sorghum composite bread was made by weighing wheat flour into a plastic clean container using a digital weighing scale. Sorghum flour was weighed and incorporated with the commercial wheat flour in the ratio (sorghum: wheat) 0:100 (control), 4:96, 8:92, 12:88 and 16:84. The composite sorghum-wheat flour was mixed thoroughly using a hand blender to a homogenous blend. In each composite flour blend, approximately, 3% shortening/fat, 5% sugar, 3% milk powder, 1.5% instant yeast, 1% salt, 0.01% dobrin, 0.01% calcium propionate and the water warm water (approximately 37 ^{o}C) used in dough making was determined using Brabendar Farinograph.The dough was made by gradually adding water to each sample of the homogenous ingredients while kneading until the dough formed a smooth paste. For each treatment, the dough was portioned into 100g pieces, shaped into rectangular shapes and then put into pre-greased baking pans and allowed to proof. Once it rose to the required size, the dough pans were placed in pre-heated oven maintained at 250 ^{o}C for 10 minutes or until the crust caramelized. The baked loaves of bread were then cooled at room temperature (approximately 20 ^{o}C for I hour) before being packed into polyethylene packing bags. Within 24 hours, sensory evaluation was done by a panel of 50 people by filling a 9-point hedonic questionnaire with minimum rating of 6 per sample. To make the other samples, the same procedure and materials were used.

3.2.4 Determination of physical and baking properties of sorghum-wheat composite bread

Physical and baking properties, dough height, dough length, P/L ratio and Energy (J) dough were determined using the Chopin-Alveograph machine. Loaf weight and loaf volume were determined by the method AOAC (2000). Loaf volume was determined by rapeseed displacement after bread was removed from oven and weighed. A 400g dummy loaf used in machine calibration was placed in the machine, the bread was placed on top of the dummy loaf and the quantity of seeds displaced considered being the bread volume. Specific loaf volume % was determined by dividing load volume (cc) by weight (g) (volume/weight) (SLV= cc/g).

3.2.5 Moisture content determination

Moisture was determined using the oven drying method AOAC (1990). Clean marked medium dishes were placed in the oven set at 105 ^{o}C for 30 minutes after to dry, then cooled in air tight desiccators and weighed. Approximately 2g of the bread sample were placed in the crucible and placed in a thermostatically regulated hot air oven at 105 ^{o}C for 8 hours. The crucible and sample were removed, cooled in a desiccator and then re-weighed to determine the weight of the dry matter. The moisture content was calculated using the following formula:

Moisture = Initial mass of sample – mass after drying) / Initial mass of sample used × 100

This will be expressed as a percentage of the initial weight of the bread. The loss in weight of the sample will be taken as the moisture content of bread.

3.2.6 Ash determination

Ash was determined according to the method AOAC (1990). Sample pieces of loaves of bread of approximately 2g were weighed into crucibles then put into a pre-heated furnace at 550 ^{o}C for 2 hours. The crucible and sample were removed, cooled in the desiccator and then re-weighed, and the per cent ash content calculated.

% Ash = (Mass of ash /Mass of dry sample used) ×100.

3.2.7 Crude fibre

Crude fibre was determined by adopting the method from AOAC (1990). Approximately 2g of each sample from loaves of bread were weighed into a 500 ml beaker and 200 ml of hot water was added, 1.25ml of conc. H_2SO_4 was added into the beaker containing the sample. The beaker containing the samples was heated on a hot plate with periodic stirring in order to digest the samples for 30 minutes. The content was filtered through a cheese cloth using suction pump and washed with hot water 2-3 times maintained at 80 ^{o}C until the acid was neutralized. The residue was washed back into the beaker with 200 ml of hot water then 0.03M NaOH was added. The sample in the beaker was again boiled for 30 minutes then filtered through a linen cloth and washed with boiling water. The residue was transferred into a pre-weighed crucible (W_1), washed with 25ml of 95 % Ethanol (C_2H_5OH) and dried at 130 °C in an oven for 2 hours. The crucible was cooled in a desiccator and weighed (W_2) then ignited in a pre-heated muffle furnace at 550 °C for 2 hours. The crucible was removed, cooled and weighed (W3). The content of the crude fibre for each sample was computed as follows:

% crude fibre = (loss in weight on ignition (W_3 –W_1 / W_2-W_1) × 100

3.2.8 Crude protein

Crude protein using the *Kjeldahl* method (AOAC, 1990). Weight (0.5 g) of finely ground samples was determined and put into *Kjedahl* digestion flask. Then 25 ml of 98% sulphuric acid (H_2 SO_4) was added to each sample. Further into the alequote mixture of Cu_2SO. 5 H_2O and K_2SO_4 were added and the mixture digested in the *Kjeldahl* flask until the alequote turned blue in solution. The digest was transferred into 100 ml volumetric flask and the volume raised to the mark of 100 ml with distilled water. Approximately 10mls of the sample was added into the decomposition chamber of the distillation apparatus, then 15 ml of

40 % (10M) NaOH solution was added and the ammonia released was trapped into 20 ml of 2 % boric acid solution containing mixed indicator. The colour change from pink to green indicated ammonia gas (NH_3). Distillation continued for 5 minutes and the boric acid-ammonia solution obtained was titrated against 0.1 M HCl. The protein content percentage was calculated as follows:

Nitrogen = (T-B × N14.007×100× 5.7 F (conversion factor for sorghum))/weight of sample where:

T-Titration volume of sample (ml), B-Titration volume for blank (ml), N-Normality of acid to 4 decimal places, F-conversion factor for nitrogen.

3.2.9 Crude fat determination (*soxhlet* method)

A method described by AOAC (1990) was adopted in lipid determination. The procedure involved weighing 2g of a loaf of bread from sample then put into a thimble which was plugged with cotton wool and then inserted into a soxhlet extractor. It was connected to a pre-weighed flat-bottomed flask in which 150 mls of petroleum ether (boiling point 40-60 ^{o}C) will be added. The flasks were heated on an Iso-mantle and extraction allowed to continue for 16 hours with 150 mls of petroleum ether. After removal, the solvent was evaporated on a steam bath in the hood, the flask and its content dried in the hot air oven at 130 ^{o}C for 30 minutes, cooled in the desiccators and weighed. The weight of the fat extracted was computed as follows:

% crude fat = (Mass of the flask + oil - Mass of the flask / Dry mass of sample used) × 100

3.2.10 Carbohydrate determination

Determination of carbohydrate will be done as described by AOAC (1990) was used. Carbohydrate content was derived by subtracting the proportion of crude protein, crude fat, crude fibre and ash content.

3.3 *In Vitro* Soluble Protein Digestibility

This was determined by adding 200 mg sample to a 100 ml Erlenmeyer flask containing 35 ml 0.1 mol/l sodium citrate tribasic (pH 2.0) with pepsin (1.5 g pepsin/l, sigma P-7012). The mixture was incubated for 3 hours in a shaking water bath at 37 °C and then centrifuged at 10,000 rpm for 15 minutes. The residue was washed in 10ml 0.1 mol/l phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm for 15 minutes, then re suspended in 35 ml 0.1 mol/l phosphate buffer (pH 8.0) with pancreatin solution (1.5 g pancreatin/l, sigma

P-1750). The mixture was then be incubated in a shaking water bath at 37 °C for 1 hour. This was followed by centrifugation at 10,000 rpm for 15 minutes, washing the residue in 10 ml phosphate buffer (pH 7.0) and centrifugation at 10,000 rpm for 15 minutes. The residue was collected on nitrogen- free filter paper and washed with 10 ml phosphate buffer (pH 7.0). The dried residue was analysed for nitrogen using *Kjeldhal* method as described in method 3.3.4. The residual protein was subtracted from total protein and the difference expressed as a percentage of the total protein and reported as IVSP digestibility.

Soluble nitrogen was determined by weighing 1 g sample into 50 ml centrifuge tube and dispersed in 20 ml distilled water. The dispersion was mechanically shaken for 1 hour and centrifuged at 10,000 rpm for 15 minutes before collecting the supernatant. The residue was re-suspended and centrifuged twice in 10 ml distilled water. The combined supernatants was analysed for soluble nitrogen by the *Kjeldhal* method. Calculation of *in vitro* insoluble protein (IVISP) digestibility was computed using the formula: (Insoluble protein- Residual protein) / (Insoluble protein × 100)

Where insoluble protein = total protein – soluble protein, residual protein =protein remaining after pepsin hydrolysis.

3.4 Sensory Evaluation

In sensory evaluation, affective method was used to determine the degree of liking and which loaf was best liked from the different sorghum proportions as described by Stone, (2018). Sensory evaluation was done by 50 panellists (25 ladies and 25 gentlemen), above 18 years but below 55 were selected from Egerton University through interview to ascertain whether one was in a state of good health, was interested in the evaluation, had acuity and ability to listen and follow instructions. Those who participated were presumed to be bread consumers, willing to participate and able to read and understand instructions. They were selected based on first come basis, as long as each met the stated qualification. Panellists were trained about the product attributes (texture: hardness of crust or softness, appearance: colour of the crust, size of the cells (air spaces) and general appeal, flavour (taste), smell (aroma) and overall acceptability. They were instructed to evaluate one sample, fill the information on the 9-point hedonic scale questionnaire before moving to the next sample. Sensory evaluation was done in a well-lit room, clean bottle drinking water was provided for rinsing of the palate before evaluating the next sample and samples were presented in identical containers, which were coded with a three digit ranking to prevent bias. The sample order was randomized to avoid bias from the order of presentation. Panellists were eventually required to rank the bread samples for acceptability and order of preference. It was considered to be acceptable when rated minimum 6 by semi-trained panellists. Approximately 30 loaves of bread samples were prepared for evaluation at Egerton University and Kenya Agricultural and Livestock Research Organization (KALRO) Njoro, Kenya. Samples were provided at the venue and subjects with any food allergy were not allowed to participate in the evaluation. For confidentiality, age, gender and dates were indicated but no names were entered on the questionnaire.

3.5 Determination of microbial counts and shelf life of the products

The method AOAC (2000) was used. All samples from the loaves of bread were aerobically evaluated for Total viable counts, fungi and mould counts at Egerton University laboratory. Approximately, 25g from each loaf of bread was transferred aseptically and homogenized in 225 mls of sterilized buffered peptone water using a blender for 2 minutes (10^{-1}). To make serial dilutions, approximately 1 ml of the homogenate was transferred to 9mls of the distilled water (peptone) dispensed in test tubes and labelled 10^{-2}. The 10^{-2} content was mixed and 1 ml drawn and transferred to another 9mls of peptone and labelled 10^{-3}. About 1 ml of the dilution samples was transferred into plates and the media added. MacConkey media was used for *E. coli,* and Coliforms while Potato Dextross Media was used for yeast and mould. After mixing the media, Plates for *E-coli* and Coliforms were incubated at 37 ^{o}C for 36 hours, while plates for the yeast and mould were kept at room temperature (approx. 20 ^{o}C plates) for 60 hours. Shelf life of white wheat bread ranges between 3-5 days, the composite loaves of bread will be packed in polyethylene bags after cooling, placed on the shelf at room temperature and physical observation done to determine how long the bread will take before spoilage by fungi, mould or strange odours. Samples from each loaf of bread were in four replicates and count of visible colonies observed was expressed as $\log_{10}$ CFU / g sample.

3.6 Data analyses

Data for physical and baking properties, microbial count, shelf life and nutritional content were subjected to analysis of variance (ANOVA) in Statistical Analysis System (SAS) using the following statistical model:

$$Y_{ij} = \mu + t_i + B_j + \varepsilon_{ij}$$

Where: Y_{ij} = the observation of the i^{th} treatment in the j^{th} replicate; μ = the overall mean, t_i = effect due to the i^{th} treatment; B_j = effect of the j^{th} replicate; ε_{ij} = random error term. The treatment sum of squares were partitioned using the orthogonal contrasts procedure in SAS version 8. Means of treatments were separated using Least significant difference (LSD) test at $p \leq 0.05$. Sensory properties of loaves from different treatments were subjected to descriptive statistics analysis where means were compared using the standard error.

3.7 Experiment 2: Development of chickpea enriched sorghum-wheat composite bread and evaluation of physical, nutritional, sensory properties and shelf life.

3.7.1 Experimental design

Experimental design was as described in section 3.2.1. Experimental treatments were wheat, sorghum and chickpea flour mixed in the proportion of wheat: sorghum: chickpea; 100:0:0 (control), 96:0:4, 92:0:8, 88:0:12 and 84:0:16. Sorghum 4% composite bread enriched with 4, 8, 12 and 16%, chickpea (4) was 92:4:4, 88:4:8, 84:4:12 and 80:4:16. Sorghum 8% bread was enriched with 4, 8, 12 and 16% was blended in the proportion 88:8:4, 84:8:8, 80:8:12 and 76:8:16. The 12% sorghum composite bread was enriched with 4, 8, 12 and 16% chickpea proportion 84:12:4, 80:12:8, 76:12:12 and 72:12:16. The contained 16% sorghum was made using wheat: sorghum: chickpea proportion of 80:16:4, 76:16:8, 72:16:12 and 68:16:16.

3.7.2 Preparation chickpea enriched sorghum-wheat composite flour

Samples of chickpea (*Cicer arietinum-Kabul variety*) grains obtained from Egerton University were cleaned to remove any foreign materials, washed in 70% alcohol (ethanol) to disinfect and soaked in 1:10 (w/a or vinegar) for 12 hours to reduce antinutritive factors such as trypsin inhibitors. They were rinsed in cold water to remove the alcohol flavour then cooked in boiling water at approximately 97 °C for 15 minutes. They were immediately rinsed in hot water to remove foam before drying in the oven for 12 hours at 60°C. Milling was done using a hammer mill (Hutler model) and thereafter the flour was passed through a 200 micrometer sieve at KALRO Njoro. Other ingredients such as commercial wheat flour (*Pembe* Bakers' flour), shortening/fat, salt, sugar, yeast and calcium were purchased from ordinary shops. Ingredients were purchased in bulk to ensure quality consistency throughout the experiment. Equipment such as oven, digital weighing scale, farinograph, hammer mill were obtained from KALRO Njoro and Egerton University Laboratories. All chemicals procured were of analytical grade.

3.7.3 Procedure for baking chickpea enriched sorghum- wheat composite bread

Chickpea flour was blended into the sorghum-wheat composite flour in proportions described in section 3.2. The baking was done following the procedure described in section 3.2.3.

3.7.4 Physical and baking properties

Dough height, length, area under curve, energy (W), P/L ratio specific loaf volume and loaf weight were determined using the method 3.2.4 as described in chapter 3.

3.7.5 Sensory evaluation

Sensory evaluation was determined by semi-trained panelists using a 5- point hedonic scale to evaluate sensory attributes (general appearance, aroma, taste, smell, crumb and crust colour, texture, chewiness and general acceptability) as described by See *et al*., (2007). The same procedure used in method 3.8 of chapter 3 was used.

3.7.6 Microbiological quality and shelf life

Bread samples were aerobically evaluated for total viable bacterial counts (*E-coli,* Coliforms) and fungi (yeast and mould counts) to determine microbial load using AOAC (2000) method as described in method 3.9 in chapter 3.

3.7.7 Nutritional content

Nutrient content: moisture, crude protein, crude fibre, crude fat, ash and carbohydrate content were determined using the method AOAC (1990) as described in 3.2.5, 3.2.8, 3.2.7, 3.2.6 and 3.2.9 of chapter 3.

3.7.8 In vitro protein digestibility

In vitro protein digestibility was determined using the method described by Akesson and Stahman (1964) and as used in method 3.7 chapter 3.

3.8 Data Analyses

Data were analysed as described in section 3.10 of chapter 3.

CHAPTER FOUR

RESULTS AND DISCUSSION

Developing of sorghum-wheat composite bread and evaluation of nutritional, physical and sensory quality

4.1 Abstract

The incorporation of whole sorghum (*Sorghum bicolor* (L.) Moench) flour to develop bread improved nutritional and sensory quality of refined wheat (*Triticum aestivum* L.) bread. The objective was to develop sorghum composite bread, evaluate nutrient content, physical and baking properties, sensory evaluation and microbial counts. This study used a new sorghum genotype, *EUS130*, to develop sorghum-wheat composite breads and compare physical and baking properties, nutrient levels, *in vitro* protein digestibility, shelf life and sensory acceptability with wheat bread. A Randomized Complete block Design was adopted for data analysis. Sorghum flour was used to partially substitute wheat flour in proportions; 0:100 (control), 4:96, 8:92, 12:88 and 16:84 (0, 4, 8, 12 and 16% sorghum respectively). Loaf height (P-value) and P/L ratio values were higher ($p<0.05$) in 8% sorghum than in control while energy (W) was lowest in 8% sorghum. Protein content (g/100g) was higher in 8% sorghum bread than control and increased with increase in sorghum level however, at 12% sorghum, the protein content declined. *In vitro* protein digestibility in sorghum and control breads were not significantly different. Crude fat was high in 8% sorghum bread compared to control while carbohydrate content was lower in composite breads compared to control than. Microbial counts in 4%, 8% and 16% sorghum bread were not significantly different ($p<0.05$) from control while sorghum composite breads had longer shelf life. The texture, crumb colour, mouth feel, cell size, flavour, smell and general acceptability were not significantly different between control, 4%, 8% and 12% sorghum bread. In conclusion, 8% sorghum flour can be partially substituted with wheat flour to develop bread with improved nutritional and sensory quality. The study recommends partial replacement of wheat flour with wholemeal 8% sorghum flour as a policy add nutrient value to wheat based products, improve nutrient intake and reduce food insecurity.

Keywords: *In vitro* digestibility, Sensory Quality, Shelf life, Nutritional Quality

4.2 Introduction

Refined wheat bread is one of the most commonly consumed item for breakfast and other meals but not affordable in developing countries that rely on wheat importation

(Wambua *et al*. 2016). Wheat flour has been the main bread ingredient due to the functional protein gluten. In Kenya, bread consumption has increased due to urbanization and changes in lifestyle. Wheat yield continues to decline with consequent increases in bread price due to high cost of importation making it unavailable to many households (Sasson, 2012). Studies that explore the possibility of partly substituting wheat with locally grown crops have been done using sorghum (*Sorghum bicolour*) and millet (Abdelghafor *et al.,* 2011), sweet potatoes (Ijah *et al.,* 2014), rice (Rai *et al.*, 2012), maize and sorghum (Nkhubutlane *et al.,* 2014). However, the newly produced sorghum genotype EUS130 and proportions below 5 have not been studied in the baking industry.

Wheat is a nutritious cereal, but the use of roller mill to produce refined wheat removes the bran and germ making the flour nutritionally inferior. More than 50% vitamins B, E and nearly all fibre is lost in the bran which has led to nutritional disorders (Heshe *et al.,* 2015). Whole grain has been associated with reduced risk of mortality from cardiovascular disease and cancers (Aune *et al.*, 2016). Sorghum is a drought tolerant crop, it has a neutral smell and blends well with wheat (Adebowale *et al.,*2012; Ogeto *et al.*, 2013). It contains 7-15% protein (*kafirins* and prolamins), fatty acids mainly polyunsaturated fatty acids such as linoleic linolenic acid and oleic acid (Whelan and Fritsche, 2013), fat soluble vitamins A, D, E and K and water soluble vitamins such as riboflavin, pyridoxine and thiamine 2.3% - 2.9% dietary fibre and phytochemicals such as flavonols, flavones (Rooney and Waniska, 2004). It also contains minerals such as Ca, P, K, Fe, Zn, Mg and Cu but tannin makes them unavailable for absorption (Afify *et al.*, 2012).

It has been established that when 5-10% wheat is substituted with sorghum flour, acceptability rate is similar to the control but a darker crumb and bitter taste are observed. It was observed that as sorghum ratio increases, acceptability declines (Abdelghafor *et al.*, 2011). This applied to studies on sorghum composite biscuit and potato composite bread (Ijah *et al.*,2014).The use of large particle size, damaged starch and high fibre content of sorghum flour also adversely affects physical and baking properties of bread (Trappey *et al.,* 2015). Studies have indicated that partial substitution of wheat flour at various levels can produce acceptable bread. Composite flour helps to improve nutritional quality, utilize local crops, reduce cost of production as well as produce variety of products (Sibanda *et al.*, 2015). Abdelghafor *et al.* (2013) observed that sorghum composite bread with large proportions of sorghum had effects on dough extensibility, bread volume and was bitter in taste. Therefore, the objective of this study was to develop sorghum-wheat composite bread with properties close to wheat bread through the use of small proportions of finely milled whole meal sorghum flour from the new sorghum genotype (EUS130).The genotype has not been used in any study known to the researcher as it had not been released into the market by the time of the study; To achieve this objective, sorghum composite breads and wheat bread produced were compared for sensory acceptability; physical and baking properties, nutrient contents, *in vitro* protein digestibility and shelf life.

4.3 Results

4.3.1 Physical and baking properties of dough and breads

Statistical analysis of various parameters was subjected to change due to influence of different levels of sorghum flour. Table 4.1 showed significant (p<0.005) difference between treatment means and between control *vs* 4, 8, 12, 16% sorghum bread and 8% *vs* 16% sorghum bread had significant (p<0.005) difference in dough height (mm), length (mm), P/L ratio, Energy (J), SLV and loaf weight. In the 8% *vs* 12% sorghum composite bread, dough height (mm), length (mm) and Energy (J) means were p<0.005 different.

Physical dough characteristics of of bread constituting 8% sorghum-wheat exhibited the highest (9.8 ± 0.03mm) height (p<0.05) compared to loaves of bread baked from wheat flour (9.6 mm) while bread containing 16% sorghum had the lowest (Table 4.2). Wheat bread showed the longest dough length (L-value) of 10.8 mm compared to 7.37mm observed on dough that contained 8% sorghum. The 12% and 16% sorghum composite bread had the lowest length suggesting that addition of sorghum reduces dough length. High specific loaf volume of 5.7cc/g was observed on loaves of bread baked from wheat flour. Addition of sorghum reduced specific loaf volume. The highest P/L ratio was in the 8% bread 1.5± 0.024 while the control bread 0.90 had the lowest ratio. Deformation energy (J) value was highest (p<0.05) in 12% sorghum composite bread 367.8J compared to the wheat bread 298.7 (J) while 8% sorghum bread had the lowest 245.7J.

Table 4.1: Means Squares for Physical and Baking Properties of Sorghum Composite Dough and Bread using Orthogonal Contrast

Source	df	Dough Height	Dough length	P/L ratio	Energy (W)	Specific loaf volume	Weight (g)
Replicate	2	0.00	0.00	0.01	2.86	0.00	3.10
Treatment	4	34.23***	30.69***	1.02***	18570.44***	3.97***	60.28***
Control(wheat bread) vs 4, 8, 12 and 16% sorghum breads	1	32.19***	108.64***	0.81***	51354.23***	14.48***	143.22***
8% vs 12% sorghum breads	1	0.14***	1.50***	0.03*	1519.09***	0.03	30.83*
12% vs 16% sorghum breads	1	0.14***	4.86***	1.82***	2880.73***	0.46***	9.13
8% vs 16% sorghum breads	1	0.54***	11.76***	2.32***	8583.63***	0.74***	73.50***
Error	8	0.01	0.23	0.01	3.48	0.01	4.83
CV (%)		1.45	3.13	5.39	1.03	2.19	1.48
R^2		0.99	0.99	0.98	0.99	0.99	0.83

Mean ± standard deviations;

Values with *, **, *** are significantly different at p<0.05, p<0.01and p<0.001 respectively using analysis of variance (ANOVA) and least significant difference (LSD) for post doc analysis.

0 %= 100% bakers' flour (control): 4%=96% bakers flour, 4% sorghum flour, 8%=92% bakers' flour, 8% sorghum flour, 12%=88% bakers, 12% sorghum flour, 16%= 84% bakers, 16% sorghum flour.

Table 4.2: Physical and baking properties of wheat and sorghum-wheat composite dough and breads

Sorghum %	Height (mm) (P-value)	Length (mm) (L-value)	P/L Ratio	Energy (J)	Specific loaf volume(cc) (SLV)	Loaf weight (g)
0: (100% wheat flour (control)	9.6±0.02[b]	10.8±0.02[a]	0.90±0.03[d]	298.7±0.03[b]	5.7±0.11[a]	140.2±0.87[a]
4 (96% wheat :4% sorghum)	9.4 ±0.04[c]	8.8±0.04[b]	1.2±0.01[d]	289.9±0.05[c]	3.8±0.05[b]	142.6±0.40[a]
8 (92% wheat: 8% sorghum)	9.8±0.03[a]	7.37±0.20[c]	1.5±0.02[a]	245.7±0.01[e]	3.7±0.15[b]	144.5±3.21[a]
12 (88% wheat, 12% sorghum)	9.7±0.02[b]	6.5±0.02[d]	1.3±0.03[b]	367.8±0.06[a]	3.5±0.04[b]	142.2±0.16[a]
16 (84% wheat: 16% sorghum)	7.5±0.02[d]	6.5±0.04[d]	1.25±0.03[c]	277.3±0.03[d]	3.5±0.17[b]	143.1±0.63[a]

Mean ± standard deviations;

Values in a column with different superscript letters are significantly different, $p<0.05$ using analysis of variance (ANOVA) and least significant difference (LSD) for post hoc analysis.

4.3.2 Sensory evaluation

Sensory evaluation texture scores ranged between 6.38 and 7.38, 4%, 8% and 12% sorghum composite bread had similar while 16% sorghum composite bread had ($p<0.05$) the lowest (Table 4.3). Mouth feel scores were highest (7.40) in 8% sorghum bread and lowest in 12% sorghum composite bread. Crumb colour, cell size, flavour, smell and general acceptability were similar with control bread.

Table 4.3 Means for Sensory properties of wheat and sorghum-wheat composite breads

Bread	Sensory Attributes							
(Wheat :sorghum)	Texture	Mouth feel	General appearance	Crumb colour	Cell size	Flavour (taste)	Smell (aroma)	General acceptability
100:0 control	7.38± 0.02^{a}	7.70±0.06^{a}	7.96±0.04^{a}	7.20±0.02^{a}	7.04±0.01^{a}	7.30±0.01^{a}	6.12±0.03^{a}	6.96±0.01^{a}
96:4	7.28±0.05^{a}	7.28±0.01^{b}	7.38±0.03^{ab}	7.54^{a}±0.03^{b}	7.02±0.01^{a}	7.10±0.01^{a}	6.84±0.02^{a}	7.16±0.06^{a}
92:8	6.94±0.01^{b}	7.12±0.02^{b}	7.08±0.01^{bc}	7.04±0.02^{a}	7.02 ±0.02^{a}	7.34±0.04^{a}	6.78±0.01^{a}	7.12±0.05^{a}
88:12	7.34±0.03^{ab}	7.40±0.02^{ab}	6.67±0.05^{bc}	7.02±0.06^{a}	7.16 ±0.07^{a}	7.46±0.03^{a}	6.94±0.08^{a}	7.08± 0.01^{a}
84:16	6.38±0.05^{c}	7.08±0.01^{b}	6.32±0.04^{c}	6.14±0.03^{c}	7.08±0.02^{a}	7.14± 0.01^{a}	7.02±0.02^{a}	6.86± 0.04^{a}

Data are Means ± standard deviations

Values in a column with different superscript letters are significantly different, $p<0.05$ using analysis of variance (ANOVA) and least significant difference (LSD) for post hoc analysis.

0 %= 100% bakers' flour (control): 4%=96% bakers flour, 4% sorghum flour, 8%=92% bakers' flour, 8% sorghum flour, 12%=88% bakers, 12% sorghum flour, 16%= 84% bakers, 16% sorghum flour.

Means ± standard deviations of scores from 9-point hedonic scale, 9- extremely like; 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.

4.3.3 Microbial count and shelf life of bread

The microbial counts were different between 12 vs 16% bread while shelf life was significantly (*P*<0.01) different between treatments, control vs 4, 8, 12, 16% and 12 vs 16% Table 4.4 . Shelf life for sorghum composite ranged between 6.7 to 9 days with control bread having the shortest (*p*<0.05) shelf life(Table 4.5).

Table 4.4 Means Squares for microbial counts and shelf life breads in orthogonal contrast

Source of variation	df	Cfu/g	Shelf life (days)
Replicate	2	0.12	0.27
Treatment	4	1.18	13.67***
Control (100% wheat bread) vs 4% (96% wheat: 4% wheat), 8, 12 and 16% sorghum	1	1.06	41.67***
8% (92% wheat:8% sorghum) vs 12% (88% wheat:12% sorghum)	1	0.60	4.17*
12% (88% wheat:12% sorghum) vs 16%(sorghum 84% wheat:16% sorghum	1	3.62**	10.67***
8% (92% wheat:8% sorghum) vs 16% (84% wheat:16% sorghum)	1	1.28	10.67**
Error	8	0.38	0.7
CV (%)		15.17	10.73
R^2		0.55	0.70

Values with *, **, *** superscript are significantly different at p<0.05, p<0.005 and p<0.0005 using analysis of variance (ANOVA) for analysis.
0 %= 100% bakers' flour (control): 4%=96% bakers flour, 4% sorghum flour, 8%=92% bakers' flour, 8% sorghum flour, 12%=88% bakers, 12% sorghum flour, 16%= 84% bakers, 16% sorghum flour.

Table 4.5 Means for total viable counts and shelf life

(Wheat :sorghum)	Cfu/g	Shelf life (days)
100:0 control	4.22±0.01[a]	6.3±0.19[c]
96:4	3.43±0.34[ab]	8.03±0.33[ab]
92:8	3.47±0.42[ab]	8.33±0.06[ab]
88:12	1.80±0.50ab	8.33±0.19[ab]
84:16	3.95±0.50[ab]	9.00±0.33[a]

Means ± standard deviations. Values in a column with different superscript letters are significantly different, p<0.05 using analysis of variance (ANOVA) and least significant difference (LSD) for post doc analysis.

Key: 0% = 100% bakers' flour (control): 4%=96% bakers flour, 4% sorghum flour, 8%=92% bakers' flour, 8% sorghum flour, 12%=88% bakers, 12% sorghum flour, 16%= 84% bakers, 16% sorghum flour.

4.3.4 Nutrient content in loaves of the bread

Table 4.6 in orthogonal contrast showed significant (*p<0.005)* difference between treatments, control vs 4, 8, 12,16% and 8 vs 16% sorghum composite bread. In 8 vs 12% sorghum bread fibre content was (*p<0.05)* while ash content in 12% versus 16% was different. In Table 4.7, bread containing 92% wheat: 8% sorghum had higher (*p*<0.05) protein (13.22g/100g) content than wheat bread (10.21g/100g). Moisture level was lowest in 16% sorghum bread. Crude fat and fibre were high in sorghum composite bread while carbohydrate content was lower in composite bread compared to control.

4.3.5 In vitro protein digestibility

In vitro protein digestibility was significantly different (*p*<0.005) between treatments and control vs 4, 8, 12,16% and 8 vs 16% sorghum composite bread similar(Table 4.6). Differences were also significant (*p*<0.05) in 8% vs 16% and 8% vs 16% sorghum composite loaves of bread. There was no difference (p>0.05) in *in vitro* protein digestibility (Table 4.7).

Table 4.6 Mean squares for nutritional content of wheat bread, sorghum-wheat composite loaves of bread and flours in orthogonal contrast

Source of variation	df	Protein	Moisture	Fat	Fibre	Ash	Carbohydrate	*In vitro* digestibility
Replicate	2	7.92	3.67	0.00	0.44	0.01	2.79	0.01
Treatment	4	21.34**	2.80	2.67***	6.67***	0.39***	83.58***	2.15***
Control vs 4, 8, 12 and 16% sorghum	1	74.73***	5.69	7.22***	7.37***	0.27***	219.69***	5.68***
8 vs 12% sorghum	1	2.90	1.73	0.13**	4.23***	0.03*	18.53*	0.01
12% vs 16% Sorghum	1	0.44	0.37	0.12**	1.14	0.63***	13.62*	1.30*
8 vs16% Sorghum	1	5.59	3.68	0.51***	9.73***	0.96***	63.90***	1.5*
Error	8	2.90	1.93	0.01	0.28	0.00	1.60	0.10
CV (%)		11.57	3.55	1.09	17.69	3.5	1.71	0.36
R^2		0.75	0.37	0.99	0.90	0.97	0.95	0.90

Values in a column with different superscript *, **, *** are significantly different at $p<0.05$, $p<0.01$and $p<0.001$ respectively using analysis of variance (ANOVA) and least significant difference (LSD) for post doc analysis.

KEY: 0 %= 100% bakers' flour (control): 4%=96% bakers flour, 4% sorghum flour, 8%=92% bakers' flour, 8% sorghum flour, 12%=88% bakers, 12% sorghum flour, 16%= 84% bakers, 16% sorghum flour.

Table 4.7: Nutritional content of wheat bread, sorghum-wheat composite loaves of bread and flours

Ratios (wheat :sorghum)	Protein (g/100g)	Moisture (g/100g)	Fat (g/100g)	Fibre (g/100g)	Ash (g/100g)	Carbohydrate (g/100g)	*In vitro* protein digestibility (%)
Sorghum 0% (100:0)	10.21±0.49[b]	40.16±1.71[a]	5.77±0.030[c]	3.08±0.70[b]	1.52±0.27[a]	80.17±1.42[a]	85.45±0.20[a]
Sorghum 4% (96:4)	12.83±1.35[ab]	40.70±0.80[a]	5.83±0.12[bc]	4.54±0.35[a]	1.53±0.09[a]	75.57±0.75[b]	84.42±0.24[ab]
Sorghum 8% (92:8)	13.22±1.25[a]	39.64±1.27[a]	6.57±0.28[a]	4.44±0.89[a]	1.55±0.21[a]	74.21±0.96[b]	84.38±0.42[ab]
Sorghum 12% (88:12)	12.85±1.03[ab]	39.36±1.67[a]	6.12±0.04[b]	4.78±0.88[a]	1.56±0.27[a]	75.70±1.72[b]	84.29±0.41[ab]
Sorghum 16% (84:16)	12.06±0.49[ab]	36.87±1.69[b]	6.30±0.28[ab]	4.27±0.70[a]	1.86±0.21 [a]	75.51±0.66[b]	84.13±0.37[ab]
Wheat flour	12.81±0.49	12.82±3.45	5.73±0.68	3.2±0.72	1.52±0.46	75.74±0.35	
Sorghum flour	12.23±0.63	12.99±7.45	3.55±0.23	6.49±7.31	1.55±0.36	75.19±9.27	

Means ± standard deviations

Values in a column with different superscript letters are significantly different, $p<0.05$ using analysis of variance (ANOVA) and least significant difference (LSD) for post hoc analysis.

0 %= 100% bakers' flour (control): 4%=96% bakers flour, 4% sorghum flour, 8%=92% bakers' flour, 8% sorghum flour, 12%=88% bakers, 12% sorghum flour, 16%= 84% bakers, 16% sorghum flour.

4.3.6 Association of nutrients and In vitro protein digestibility in sorghum-wheat composite loaves of bread

Moisture was inversely correlated (*r*=-0.573*) with protein content and also positively associated (*r*=0.564*) with carbohydrate content (Table 8). A significant negative correlation (*r*=-0.805**) was also found between protein and carbohydrates. Shelf life positively correlated with ash (*r* =0.781**) and fibre (*r*=0.946**), and inversely with lipid content (*r*=-0.919**). There was a positive correlation between shelf life, ash and fibre (Table 4.8).

Table 4.8 Association of nutrients in sorghum-wheat composite loaves of bread

Variables	Moisture	Ash	Fibre	Lipid	Protein	Carbohydrate	Shelf life	*In vitro* protein digestibility
Moisture	-	1.22	-0.78	0.44	-0.57*	0.56*	-0.14*	-0.35
Ash		-	-0.33	-0.17	-0.07	0.20	0.78*	0.42
Fibre			-	0.27	0.08	-0.69	0.95*	-0.30
Lipid				-	0.48	-0.69	-0.92*	-0.56*
Protein					-	-0.81***	-0.53	-0.29
Carbohydrate						-	0.06	0.15
Shelf life							-	-0.35

Values with *, **, *** are significantly different at p<0.05, p<0.005and p<0.000 respectively.

4.4 Discussion

4.4.1 Physical and baking properties of wheat and sorghum-wheat composite dough and loaves of breads

The height (P-value: resistance to elasticity), length (L-value resistance of the dough to expansion and extensibility) and configuration ratio (P/L) were obtained from the alveograph. Gluten protein in wheat is responsible for dough structure formation; high P-value is an indicator of strong dough with the ability to trap gases resulting to less dense loaf and good quality bread (Muhammed *et al.,* 2013). In this study, 8% sorghum composite dough had the highest P-value compared to 4% sorghum and control (Table 4.2). This may be attributed to the high protein content in 8% sorghum composite bread compared to 4% sorghum and control. High protein content in bread has been associated with dough strength, stability and high P-value. In this study dobrin improver was used to contribute to improvement of dough stability and quality of bread however significant differences between means were observed in the physical and baking properties (Table 4. 2). High P-value was an indication of high dough resistance to deformation and it is related to stability that the dough showed during proofing stage. Such dough exhibits resistance to elasticity and good gas holding capacity resulting to desirable bread volume (Bakare *et al.,* 2016). The L-value is an indicator of protein gluten quality and predicts handling of the dough (Hordes *et al.,* 2008). Gliadin is responsible for making the dough highly extensible while high fibre disrupts the viscoelastic system. In high L-value, dough has low ability to trap gases resulting in higher density bread with low volume and less desirability (Gomez *et al.,* 2003). In the study sorghum composite dough had the lowest extensibility compared to wheat bread which may be attributed to dilution of gluten protein by non -wheat flour. Bread produced was more soft, less springy, and crumbly during slicing compared to wheat bread (Table 4.2). Studies (Bakare *et al.,* 2016) have shown that at 10% composite dough, L-value and P-value were higher in wheat flour dough compared to composite dough. However, P/L ratio was highest in composite dough compared to wheat dough. Bakers prefer moderately strong dough for optimum bread production and this encourages use of dough improver by bread bakers.

Configuration ratio (P/L) is an index of gluten behaviour; indicating the balance between dough strength and stability. In this study, P/L value was higher in 8% sorghum compared to control (Table 4.1) possibly due to high protein level. This may be an indication the dough in sorghum composite bread and control was strong; suitable for bread production. Specific loaf volume (SLV) is a reliable measure of loaf size and guides bakers to produce standard sizes of bread. However, dietary fibre (DF) reduces gluten network and compromises bread volume (Bakare et al. 2016), It was observed that SLV was lower in sorghum composite breads compared to wheat bread ascribed to low gas retention from gluten dilution and high fibre in whole meal sorghum flour (Table 4.2). This observation has been reported in fruit bread (Bakare *et al.,* 2016) and cassava composite bread (Sibanda *et al.*, 2015).

Energy (J) is the work required to stretch the dough until it ruptures. Depending on wheat variety, strong dough requires more energy. Good quality wheat ranges from 220 to 300J; above this the wheat is categorised under improving wheat (Hordes et al. 2008). Thus, energy values in control, 4, 8 and 16% breads were under good quality category. However, 12% sorghum composite had above 300J (Table 4.2) thus was under improving wheat category an indication that other factors affect dough rheology. Inconsistency in sorghum dough stability, thickness and texture of the dough are likely to have affected energy requirement in 12% sorghum composite bread. Differences in energy requirements among the different bread proportions may be attributed to dough texture and dough handling processes. Studies have shown that energy (J) errors may occur due to friction between the plates and sample, high strain applied during air blowing, thickness and texture of the dough and inconsistency in composite dough stability (Chalaramides *et al.,* 2002). It is for this reason Dobrin dough improver is used in baking industry to help improve on gas retention and gluten restructuring. In the study, dobrin was used which may have improved the dough strength, enhanced dough tolerance and bread quality.

4.4.2 Sensory evaluation

Sensory properties of bread are important however, highly substituted wheat flour affect sensory attributes of bread. Texture is the structure formed by strands of gluten including the loaf crust. In this study, texture was measured for crumb and crust hardness. The 4%, 8% and 12% sorghum composite breads had similar texture scores with wheat bread while the 16% bread had the lowest. Low bread volume and close texture results to bread denseness (Sibanda *et al.*, 2015). The low score in 16% sorghum bread may be attributed to the low bread volume, crumbly texture and inability to spring back when pressure was applied. Mouth-feel attribute is affected by high dietary fibre, ash, texture and protein level (Onoja *et al*., 2014). The mouth feel scores were not different between composite breads and wheat bread which may be attributed to the non-stickiness property of sorghum baked products and fineness of flour. The sorghum grain used in this study was milled twice in order to reduce flour particle size for improved gas retention and bread quality;

Crumb colour influences purchase or rejection of a product, a uniform golden brown colour is preferable (Hossian *et al.,* 2014). This study observed that crumb colour was significantly lower in 16% sorghum bread compared to other samples, crumb colour of samples up to 12% sorghum was desirable and likeability decreased at 16% sorghum. The low score in the 16% sorghum composite bread may be associated with darker colour of bread from the high proportion of red sorghum which panellists may not have been familiar with. Abdelghafor *et al.* (2011) observed reducing crumb colour scores in high sorghum level, consumers preferred a lighter colour which was associated with raw materials used for wheat bread. Cell size is air space in the loaf crumb often determined by the gluten protein quality and quantity. In the study, cell size was not different among the samples (Table 4.3). This is possibly due to fine milling of sorghum flour which might have likely helped maintain gas retention resulting to evenly distributed cell size during fermentation process. Also, the dobrin dough improver that was used may have played a role of increasing gas retention and dough strength thus contributing to insignificant difference in cell size among samples. Similar scores in loaf flavour (taste) and smell (aroma) were observed in the study attributable to the mild flavour of sorghum flour and low wheat substitution level. On the contrary, Abdelghafor *et al.* (2011) observed a bitter taste at 10% sorghum which was associated with phenolic compounds from the grain coat. Importantly, general acceptability is influenced by taste, aroma and tenderness. All bread samples were rated above 6 (like slightly) with insignificant difference on general acceptability (Table 5.3), In the 4% and 8% sorghum composite bread, general acceptability met the KEBS (2009) threshold score of 7.10 to 7.11.

4.4.3 Microbiological quality

This study showed that sorghum composite breads had lower cfu/g level compared to wheat bread (Table 4.4) which positively related to the lower moisture level in sorghum composite breads and high ash content in sorghum. The high loss of moisture during baking and high fibre content may have contributed to low moisture content thus extending the shelf life of sorghum composite bread. Table 4.5 showed that different levels of wheat replacement with sorghum flour affected shelf life of loaves of bread. Fezilah *et al.* (2015) obtained low cfu/g in baked fruit bread and attributed to low moisture. In the study, fungi were not present in all the bread samples implying that the breads were safe for human consumption. This may also be attributed to the use of calcium propionate in this study. Tarar *et al.,* (2010) reported a decrease in yeast activity in breads containing calcium propionate preservative. This study observed the lowest 1.80 cfu/g in 12% sorghum and associated it to handling of bread samples, the microbial level was within acceptable Kenyan standards.

4.4.4 Nutrient content of the breads

This study showed that the protein content in sorghum-wheat composite breads was higher than in wheat bread (Table 4.7). Higher protein content was also observed in sorghum-millet composite cookies compared to control (Rai *et al.,* 2014). It was noted that the protein level was highest in 8% sorghum, however, as the sorghum level increased, the protein level decreased possibly due to high carbohydrate levels. A negative correlation was found between protein and carbohydrate levels (Table 8), this may be attributed to the variation in chemical composition of wheat gluten that is associated with reduced protein. Previous studies (Sibanda *et al.*, 2015; Abdualrahman *et al.,* 2016) reported that low protein in sorghum kernel contribute to low protein in sorghum composite bread. A similar protein trend was observed in cassava composite bread (Wambua *et al.*, 2016). It is important to note that the recommended level of protein intake is 0.75g/kg /day for adult female and 0.84 g/kg /day for adult male; healthy persons above 19 years (Campbell *et al.,* 2007). Thus, consumption of sorghum composite bread would provide 10.18 to 13.93% recommended dietary intake protein for adult persons per day in the 0 to 16% bread.

Carbohydrate was lowest in 8% sorghum as mentioned earlier, carbohydrate and protein levels were inversely correlated. Similar trend was obtained by Adebowale *et al*. (2012). This finding concurs with a recent study that observed low carbohydrate in sorghum composite biscuits (Serrem *et al.,* 2015). In addition, the low carbohydrate level in sorghum composite breads than in wheat bread might be due to lower carbohydrate level in sorghum flour compared to wheat flour. Sorghum tannins have been found to interact with carbohydrates which lowers digestibility by inhibiting the action of amylase enzyme; an advantage to diabetic persons.

Furthermore, the moisture content reduced with increased sorghum level which concurs with previous studies (Bibiana *et al.,* 2014; Adebowale *et al.,* 2012). Low moisture in sorghum composite bread is attributed to high moisture loss during and after baking, the high hydrophobic characteristic of sorghum protein and binding water capacity of fibre. Low moisture content in confectionaries is an advantage in reduction of microbial proliferation thus prolonging storage period of products (Sanni *et al.*, 2006). In the study, low moisture in sorghum composite bread may have contributed to low counts of microbes and long shelf life of bread. Crude fat content was higher in sorghum composite bread compared to wheat bread (Table 4.6). Reduced moisture content lowers sorghum fat extraction while large flour particle size of sorghum flour were found to hinder heat transfer between solids and solvents during extraction (Wang *et al.,* 2005). The composite sorghum breads also contained higher ash than wheat bread, an indication of higher levels of minerals in sorghum composite bread. Notably, ash values increased with increase in sorghum level. Sorghum contains high amounts of Ca, P, K, Fe, Zn, Mg and Cu (Afify *et al.,* 2012). The presence of high ash content in sorghum composite bread confirms that sorghum composite bread can be used to deliver essential micronutrients to populations for reduction of micronutrient deficiency.

4.4.5 *In vitro* protein digestibility

Sorghum digestibility is poor when wet cooked due to cross-linking of disulphide bonds. However, dry cooking methods such as popping have been shown to reduce phytic acid content and thus improve protein and starch digestibility in sorghum (Nathakattur *et al.,* 2013). In this study, *in vitro* digestibility of protein was lower in sorghum composite breads compared to wheat bread. High fibre content in sorghum composite bread may have bound the protein consequently reducing the content as well as inhibiting *in vitro* digestibility, exogenous and endogenous factors in sorghum may have adversely affected *in vitro* protein digestibility. Haud, (2010) observed that dietary fibre binds nutrients resulting to indigestion in small intestines. Afify *et al.,* (2012) observed that interaction of sorghum protein with non-protein (such as lipids and phytates) and sorghum protein *karifins* resistance to peptidase digestion reduces sorghum protein digestibility. Notably, *in vitro* digestibility was not significantly different among the composite samples which may be attributed to the small difference in sorghum proportion added.

4.4.6 Association of nutrients

Table 8 showed that protein content was highest in 8% sorghum bread, value decreased with increase in sorghum proportion while carbohydrate decreased. The inverse correlation between carbohydrate and protein was associated with high carbohydrate in wheat flour Mariera *et al.,* (2017) reported high carbohydrate content in wheat flour compared to sorghum flour. The study observed a negative correlation between carbohydrate and fibre. This was ascribed to the fact that dietary fibre is the indigestible carbohydrate. Lunn and Butrics, (2007) reported that complex carbohydrates are polymers of glucose (straight chainamylose) and branched chain (amylopectin) which are hydrolysed into glucose, fructose and galactose energy. Dietary fibre is the indigestible carbohydrate by the human digestive tract; associated with reduction of risks against lifestyle diseases such as cardiovascular diseases. The study showed that ash positively correlated with shelf life (Table 4.8), this was ascribed to ash or salt property that inhibits microbial growth. Tarar *et al.* (2010) found out that addition of salt to bread influences microbial growth; reduces yeast activity, decreases microbial growth and increases shelf life. *In vitro* protein digestibility negatively correlated with fat and fibre, the study attributed this to low *in vitro* protein digestibility, Degen *et al.,* (2006) reported that fibre interacts with protein thus inhibiting digestibility.

4.4.7 Conclusions

The study demonstrated that substitution of wheat flour with sorghum flour affects specific loaf volume due to gluten dilution. Sorghum improved protein, fat and fibre content of bread, 8% sorghum-wheat composite bread had the highest protein level. Sorghum composite bread was acceptable and met KEBS (2009) standards on sensory evaluation requirement. Shelf life increased with increase in proportion of sorghum an advantage to rural communities who have inadequate storage facilities. Therefore, partial substitution of wheat with 8% whole sorghum flour can produce acceptable and nutritious bread with comparable long shelf life.

4.4.8 Recommendation

(i) More studies should be done on handling of composite dough as current technology focuses mainly on wheat properties.

(ii) The Kenya Government should incorporate sorghum in wheat flour for bread and other confectionaries for improved nutrient diversity of bread and reduced food insecurity

(iii) More studies on sorghum composite flour for value addition of wheat flour to reduce malnutrition through consumption of local foods are needed.

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CHAPTER FIVE

Experiment 2: Development of Chickpea Enriched Sorghum Wheat Composite Bread and Evaluation of Physical, Nutritional, Sensory Properties, Acceptability and Shelf Life

5.1 Abstract

The incorporation of whole chickpea (*Cicer arietinum-Kabul variety)* flour into sorghum (*Sorghum bicolor* L. Moench) wheat composite flour is necessary to improve nutritional quality of wheat (*Triticum aestivum* L.) bread. This study used a new sorghum genotype *EUS130* to develop chickpea enriched sorghum-wheat composite bread. The objective of the study was to further enrich sorghum-wheat composite bread with different levels of chickpea to bread, compare physical and baking properties, nutrient levels, *in vitro* protein digestibility, shelf life and sensory acceptability with wheat bread. A Randomized Complete Block Design was employed in this study. Bread was developed using proportions of wheat: sorghum: chickpea 100:0:0 (control). Sorghum (4%) bread proportions 92:4:4, 88:4:8, 84:4:12 and 80:4:16 respectively. Sorghum (8%) bread proportion 88:8:4, 84:8:8, 80:8:12 and 76:8:16 (8%). The 12% bread proportion 84:12:4, 80:12:8, 76:12:12 and 72:12:16 (12%) while 16% sorghum was in proportion; 80:16:4, 76:16:8, 72:16:12 and 68:16:16 (16%). Results showed control bread had the significantly ($p<0.05$) high Length, Height, P/L, loaf volume, SLV in control and loaf from 92:4:4 proportions were similar. Water added and weight of loaf was high in chickpea enriched bread. Fibre, oil, protein were significantly ($p<0.05$) high in chickpea enriched bread while carbohydrate content was high in control. *In vitro* protein digestibility was significantly ($p<0.05$) high in 16% sorghum enriched with chickpea. Sensory sores; loaf shape, texture, colour, aroma, taste, chewiness, cell size and general acceptability for control and enriched bread proportion 92:4:4 were similar. Among the enriched loaves, 4% sorghum (92:4:4) bread had the highest acceptability score. Colony forming units (cfu/g) were within acceptable limit while shelf life was highest in chickpea enriched loaves. In conclusion bread from 4% sorghum (proportion 92:4:4) can be used to partially substitute wheat flour to further improved nutritional and sensory quality.

5.2 Introduction

Refined wheat (*Triticum aestivum* L.) bread is one of the most consumed food products worldwide but not affordable in developing countries who rely on wheat importation

(Gadalla *et al.,* 2017). Refined wheat flour contains calories and other nutrients but the protein is of low nutritional value (Ndife *et al.,* 2011). Moreover, during milling, wheat germ and bran are lost consequently leaving refined wheat flour deficient in dietary fibre, minerals and vitamins which are a leading cause of high prevalence of constipation and digestive disorders (Heshe *et al.,* 2015). However, the wheat protein gluten is a main ingredient due to the viscoelastic property that is essential in obtaining quality bread (Abdelghafor *et al*., 2011). Wheat provides 50% of dietary needs worldwide, its production is low compared to domestic requirement which necessitates the use of composite flour from locally grown crops such as sorghum, millet or root vegetables in non-wheat growing countries to meet bread demand (Adebowale *et al*., 2012).

The use of sorghum (*Sorghum bicolor* L. Moench) to partly substite wheat so as to reduce cost of importation is an attractive option (Abdelghafor *et al.,* 2011). Sorghum is a drought resistant crop, has a neutral smell, blends well with wheat and used as a wheat substitute in flat bread. However, it is poor in essential amino acids such as lysine (Gadalla *et al.,* 2017). Partial substitution of wheat with sorghum can add nutritional value to wheat bread (Ogeto *et al.,* 2013). However, nutrient bioavailability is inhibited by antinutritive factors (Ratnavathihi and Patil, 2013). Consumer awareness for healthy diet has increased globally, prompting producers to introduce legume supplemented cereal based products such as bread with whole cereals flour to make them nutritionally superior but also acceptable (Bolarinwa *et al.,* 2015). Benefits of consuming whole grain products include reduction of risks against some cancers, obesity, coronary heart diseases. This has been due to high dietary fibre, minerals, vitamins antioxidants, fats, phenolic compounds and starch found in the bran and germ (Slavin *et al.,* 2001).

Malnutrition in pregnant and breastfeeding mothers can lead to child mortality, retarded growth and low productity (FAO, 2013). Chickpea (*Cicer arietinum*) is a legume grown in semi-arid areas, it contains high value of complex carbohydrate, high protein with high digestibility, B vitamin and mineral (Kumral, 2015). It is rich in lysine amino acid but contains low amounts of sulphur containing amino acids such as methionine. Since cereals are nutritionally inferior, when used with legumes in a product, they complement each other (Gadalla *et al.,* 2017). The use of chickpea in composite bread has great potential in improving nutritional value particularly in food insecure families. Legumes such as chickpea have low glycaemic index whose health benefits include risk reduction of diabetes, obesity and coronary heart diseases. Chickpea has been drawing attention of consumers due to its high nutritional value, although antinutritive compounds inhibit its utilization however, heat treatment increases protein digestibility (Bolarinwa *et al*., 2015). Studies Xu *et al*. (2016) and Man *et al* . (2015) observed that chickpea improves nutrient content of a product such as protein, lipids, fibre and ash and improves protein digestibility. It is a suitable milk imitation and meat substitute (Zhang *et al*., 2007). However, it reduces viscoelastic properties of the dough; reduces dough strength, elasticity and bread making potential (Hefnewy *et al*., 2012; Suleiman *et al.,* 2013). This study aimed to develop chickpea enriched sorghum wheat composite bread so as to improve protein content, compare nutrient content, physical and baking properties and shelf life of the breads with control.

5.3 Results

5.3.1 Physical and baking properties of dough and breads

The physical and baking properties showed that dough length had significant ($p<0.001$) difference among treatments, control bread and 4% enriched sorghum-wheat composite bread had similar dough length (Table 5.1). Hq3eight values were different ($p<0.001$) when comparison was made between control vs 4%, 8%, 12% and 16% (sorghum), and 8% vs 16% (sorghum) enriched dough. Area under the curve was significantly ($p<0.05$) different between treatments, significantly ($p<0.01$) different in control compared with different breads. Configuration ratio (P/L) was different ($p<0.001$) between treatments, 8% vs 12% and 8% vs 16% (sorghum) enriched bread. Energy values were significantly ($p<0.05$) different between treatments, significantly ($p<0.01$) different between control and all chickpea enriched sorghum composite loaves of bread. Volume values were different ($p<0.05$) between treatments and 8% vs 16% (sorghum) enriched loaves of bread. Loaf weight was, significantly ($p<0.01$) different between control and all sorghum loaves of bread enriched with chickpea enriched, control and all sorghum composite loaves of bread enriched, 8% vs 12% and 8 vs 16% (sorghum) enriched loaves of bread, it was significantly ($p<0.05$) different between 8% vs 12% chickpea enriched sorghum breads. Specific loaf volume was significantly ($p<0.01$) different between treatments and 8% *vs* 16% (sorghum) enriched bread. Comparison between control *vs* all loaves of bread and 12% vs 16% (sorghum) enriched loaves of bread; significant ($p<0.05$) difference was observed. Quantity of water added during dough processing was significant ($p<0.001$) different between control *vs* 4, 8, 12 and 16% (sorghum) enriched, 8 *vs* 16% and 8 *vs* 12% (sorghum) enriched dough and 8 *vs* 16% (sorghum) enriched dough. Table 5.2a and 5.2b shows that bread from 4% sorghum; proportion 92:4:4 had significantly $P<0.05$high length, area under curve, energy (J), specific l loaf volume while P/L ratio and water added were low. Physical and baking properties decreased with increase in wheat substitution level

5.3.2 Sensory acceptability

The study demonstrated that generally loaf shape scores ranged from 2.07 to 3.92, wheat bread had the highest score of 3.92 compared to 2.07 of 16% sorghum-chickpea enriched bread respectively (Table 5.3a and 5.3b). Wheat-chickpea enriched bread had a texture of 3.82±0.21 compared to 1.37±0.18 of 16% wheat -sorghum -chickpea bread, wheat bread had the highest while loaves with 16% sorghum bread enriched with chickpea had the lowest. Similarly, control bread had higher scores in sensory attributes (crust colour, crumb colour, crumb softness, aroma, taste, chewiness, cell size and general acceptability) compared to enriched sorghum composite bread. The crust and crumb colour achieved acceptability score in 4, 8, 12 and 16% enriched sorghum bread while aroma was acceptable in control, 4, and 8(Table 5.3a and 5.3b). In addition, the control and 4% sorghum enriched bread had the highest acceptability score while 16% had the lowest.

Table 5.1: Mean squares from analysis of variance and orthogonal contrasts of treatments properties of (wheat-sorghum-chickpea bread) for physical and baking properties

Source of variation	df	Length	Height	Area	P/L	Energy(W)	Volume	Weight	SLV	Water
Replicate	2	0.01	0.01	1.34	0.00	57.35	560.65	3.59	0.03	0.25
Treatment	4	17.52***	23.07***	523.39*	0.54***	22385.55*	33460.34*	1163.66***	3.44**	69.43***
Control (wheat:chickpea) *vs* 4%,8%,12% and 16% sorghum	1	51.63***	47.00***	1698.81**	0.08	72658.56**	12189.33	1699.63***	3.05*	179.98***
8% *vs* 12% sorghum	1	0.09	17.01**	135.14	1.25***	5779.96	9801.01	215.40*	1.01	40.04***
12% *vs* 16% sorghum	1	2.73	4.51	45.35	0.01	1939.32	45501.04	942.51***	3.45*	54.30***
8% *vs* 16% sorghum	1	1.82	39.04***	23.92	1.52***	1023.25	97537.50*	2059.05***	8.16**	1.08
Error	58	3.10	1.80	173.63	0.07	7172.76	12921.92	27.41	0.68	2.57
CV		32.85	21.25	44.79	20.92	43.99	19.77	3.39	21.98	2.53
R^2		0.29	0.47	0.18	0.36	0.18	0.15	0.75	0.26	0.65

Values with *, **, *** are significantly different at p<0.05, p<0.01and p<0.001 respectively.
0% (control) wheat: chickpea 100: 0, (control) - 96:4, 92:8, 88:12 and 84:16%.
4% sorghum- proportion wheat: sorghum: chickpea -96:4:4, 88:4:8, 84:4:12, and 80:4:16.
8% sorghum- proportion wheat: sorghum: chickpea- 92:8:4, 88:8:4, 84:8:8, 80:8:12, 76:8:16.
12% sorghum- proportion wheat: sorghum: chickpea -84:12:4, 80:12:8, 76:12:12 and 72:12:16
16% sorghum proportion wheat: sorghum: chickpea; 80:16:4, 76:16:8, 72:16:12, 68:16:16

Table 5.2a Physical and baking properties of chickpea enriched sorghum-wheat composite bread

Treatment	Bread (W:S:C)	Length (mm)	Height (mm)	Area under curve (mm^2)	P/L ratio	Energy (J)
OFO (control)	10:0:0	10.77±0.03^a	9.62±0.02^a	45.67±0.31^a	0.90±0.33^j	298.66±2.18^a
4% sorghum						
FFF	92:4:4	7.82±0.01^b	7.32±0.01^c	45.13±0.06^a	0.97±0.07^i	295.12±0.42^a
FFE	88:4:8	7.05±0.03^g	7.04±0.03^d	35.18±0.02^d	1.00 ±0.06^{hi}	230.08±0.10^d
FET	84:4:12	5.02±0.07^i	6.94±0.02^d	30.50±0.01^f	1.38±0.05^c	199.47±0.04^f
FFS	80:4:16	3.42±0.09^m	4.44±0.03^i	11.42±0.01^j	1.30±0.03^d	74.69±0.16^j
8% sorghum						
EXF	88:8:4	5.91±0.01^e	7.06±0.06^d	32.41±0.01^{ef}	1.20±0.06^{ef}	211.94±0.04^{ef}
EWE	84:8:8	5.42±0.01^f	8.23±0.02^b	42.52±0.02^b	1.52±0.08^b	278.10±0.081^b
YET	80:8:12	4.50±0.0^j	6.83±0.03^d	20.72±0.04^h	1.51±0.08^b	135.51±0.01^h
EZS	76:8:16	2.82±0.05^o	6.53±0.02^e	14.13±0.03^i	2.31±0.01^a	92.41±0.14^i
12% sorghum						
TCF	84:12:4	6.09±0.05^d	6.09±0.08^f	33.94±0.02^{de}	1.00±0.01^{hi}	221.97±0.15^{de}
CCE	80:12:8	5.41±0.01^f	5.83±0.02^g	24.63±.02^g	1.08±0.01^g	161.08±0.12^g
CCT	76:12:12	4.23±0.02^k	5.09±0.05^h	6.53±0.01^i	1.20±0.10^{ef}	108.11±0.49^i
CCS	72:12:16	3.41±0.01^o	4.89±0.02^i	1.70±0.03^i	1.43±0.01^c	102.66±0.17^i
16% sorghum						
SSF	80:16:4	4.81±0.01^h	5.00±0.05^h	39.40±0.01^c	1.04±0.01^{gh}	257.68±0.08^c
SSE	76:16:8	4.55±0.03^i	4.93±0.03^h	32.97±0.30^{de}	1.08±0.01^g	215.6±0.16^{de}
SST	72:16:12	3.83±0.02^l	4.45±0.26^m	15.32±0.02^i	1.16±0.07^f	100.19±0.10^i
SCS	68:16:16	3.23±0.02^m	4.05±0.06^j	14.11±0.01^i	1.25±0.02^{de}	92.25±0.04^i

Means ± standard error; Means with different superscript in the same column are significantly difference p<0 05

0% sorghum-wheat bread 100:0: 0, (control) , 4% sorghum- proportion wheat: sorghum : chickpea -96:4:4, 88:4:8, 84:4:12, and 80:4:16; 8% sorghum- proportion wheat: sorghum: chickpea- 92:8:4, 88:8:4, 84:8:8, 80:8:12 and 76:8:16; 12% sorghum- proportion wheat: sorghum : chickpea -84:12:4, 80:12:8, 76:12:12 and 72:12:16; 16% sorghum-proportion wheat: sorghum: chickpea; 80:16:4, 76:16:8, 72:16:12, 68:16:

Table 5.2b: Means for Physical and baking properties of chickpea enriched sorghum-wheat composite bread

Treatment	Bread (W:S:C)	Volume (cc)	Weight (g)	SLV	Water (ml)
OFO (control)	10:0:0	804.00 ± 0.58^{a}	140.17 ± 1.50^{i}	5.74 ± 0.07^{a}	59.57 ± 0.12^{i}
4% sorghum					
FFF	92:4:4	746.69 ± 3.01^{b}	$147.20\pm0.49^{c-f}$	5.42 ± 0.03^{ab}	$62\pm0.11^{g-h}$
FFE	88:4:8	745.20 ± 2.61^{b}	$146.32\pm0.32^{f-h}$	5.09 ± 0.05^{b}	$63\pm0.02^{e-g}$
FET	84:4:12	675.65 ± 0.65^{c}	$149.23\pm2.53^{e-g}$	4.53 ± 0.07^{cd}	$63\pm0.01^{e-g}$
FFS	80:4:16	$571.33\pm2.91^{e-g}$	$153.90\pm0.98^{c-e}$	3.72 ± 0.05^{ef}	$63.33\pm0.33^{e-g}$
8% sorghum					
EXF	88:8:4	694 ± 0.35^{bc}	$150.10\pm2.87^{e-f}$	4.63 ± 0.0^{c}	$64.33\pm0.33^{d-f}$
EWE	84:8:8	594 ± 2.31^{de}	$149.73\pm0.67^{e-f}$	3.97 ± 0.03^{e}	$65.67\pm0.57^{a-c}$
YET	80:8:12	586.34 ± 0.02^{ef}	$151.10\pm0.29^{c-f}$	3.88 ± 0.04^{e}	67.33 ± 0.33^{a}
EZS	76:8:16	$573\pm1.20^{e-g}$	$155.05\pm2.32^{b-d}$	3.68 ± 0.01^{ef}	$63.60\pm2.33^{c-e}$
12% sorghum					
TCF	84:12:4	649.67 ± 2.38^{cd}	157.67 ± 1.16^{bc}	4.12 ± 0.23^{de}	65 ± 0.14^{hi}
CCE	80:12:8	590 ± 3.86^{ef}	$155.00\pm2.23^{b-d}$	3.81 ± 0.27^{ef}	$65\pm1.67^{g-i}$
CCT	76:12:12	527.38 ± 1.11^{fg}	157.27 ± 1.06^{bc}	3.35 ± 0.08^{fg}	$65\pm0.0457^{a-e}$
CCS	72:12:16	516.65 ± 2.01^{g}	159.90 ± 1.43^{b}	3.23 ± 0.03^{g}	$65\pm0.05^{a-e}$
16% sorghum					
SSF	80:16:4	587.75 ± 3.65^{ef}	154.95 ± 1.63^{cd}	3.79 ± 0.07^{ef}	$65\pm0.11^{a-e}$
SSE	76:16:8	526.90 ± 2.16^{fg}	164.27 ± 1.37^{a}	3.20 ± 0.31^{g}	63.35±0.33a-4
SST	72:16:12	408.60 ± 3.67^{h}	166.5 ± 1.80^{a}	2.45 ± 0.01^{h}	$66.93\pm0.33^{a-c}$
SCS	68:16:16	412.45 ± 4.64^{h}	167.50 ± 2.19^{a}	2.46 ± 0.07^{h}	67.03 ± 0.03^{ab}

Means ± standard error. Means with different superscript in the same column are significantly difference p<0 05 using least significant difference (LSD)

0% sorghum-wheat bread 100:0: 0, (control), 4% sorghum- proportion wheat: sorghum: chickpea -96:4:4, 88:4:8, 84:4:12, and 80:4:16; 8% sorghum- proportion wheat: sorghum: chickpea- 92:8:4, 88:8:4, 84:8:8, 80:8:12 and 76:8:16; 12% sorghum- proportion wheat: sorghum: chickpea -84:12:4, 80:12:8, 76:12:12 and 72:12:16; 16% sorghum- proportion wheat: sorghum: chickpea; 80:16:4, 76:16:8, 72:16:12, 68:16:1.

Table 5.3a: Means for sensory quality of chickpea enriched sorghum-wheat composite bread

	Wheat: sorghum: chickpea proportion								
Sensory Attributes	(Control) 100:0:0	FFF (92:4:4)	FFE 88:4:8	FFT 84:4:12	FFS 80:4:16	EXF 88:8:4	EWE 84:8:8	YET 80:8:12	EZS 76:8:18
Shape	3.92±0.12^{a}	3.65±0.13^{ab}	3.51±0.14^{ab}	3.31±0.16^{a-c}	3.49±0.13^{ab}	3.69±0.12^{ab}	3.21±0.14^{a-d}	3.03±0.16^{b-e}	2.59±0.27^{c-f}
Texture	3.82±0.14^{a}	3.64±0.12^{ab}	3.18±0.13^{c}	3.18±0.15^{c}	3.23±0.13^{c}	3.36±0.12^{bc}	3.05±0.13^{cd}	3.05±0.14^{cd}	2.44±0.16^{ef}
Crust Colour	3.61±0.13^{ab}	3.69±0.13^{a}	3.54±0.14^{bd}	3.36±0.13^{cd}	3.45±0.12 ^{c}	3.36±0.14^{cd}	3.23±0.13 ^{d}	3.28±0.16^{d}	3.49±0.17 $^{b-d}$
Crumb colour	3.59±0.14^{a}	3.54±0.14^{ab}	3.38±0.13^{ab}	3.26±0.13^{ab}	3.62±0.12^{a}	3.41±0.13^{ab}	3.31±0.12^{ab}	2.67±0.18^{c}	3.49±0.16^{ab}
Aroma	3.55±0.15^{ab}	3.64±0.1^{a}	3.54±0.12^{ab}	3.23±0.13b^{c}	3.15±0.11^{b-d}	3.44±0.15^{ab}	3.28±0.13^{a}-^{c}	2.38±0.16^{e}	2.82±0.19^{de}
Taste	3.82±0.12^{a}	3.69±0.14^{ab}	3.38±0.15^{bc}	3.00±0.15^{dc}	3±0.14 ^{dc}	3.26±0.13 ^{cd}	3.13±0.14 ^{cd}	2.64±0.17^{fg}	2.72±0.17^{ef}
Chewiness	3.80±0.15^{a}	3.56±1.10^{ab}	3.13±0.14^{cd}	2.67±0.17^{ef}	3.08±0.11^{cd}	3.3±0.14^{bc}	2.95±0.12^{de}	2.56±0.17^{f}	2.31±0.21^{fg}
Cell size	3.72±0.12^{a}	3.56±0.10^{ab}	3.21±0.15^{b-d}	2.74±0.18^{ef}	3.15±0.10^{d-e}	3.49±0.12^{a-c}	3.08±0.15^{ed}	2.28±0.14^{f}	2.51±0.22^{f}
Acceptability	4.18±0.13^{a}	3.97±0.13^{a}	3.00±0.15^{c}	2.51±0.14^{de}	2.82±0.13^{a-d}	3.49±0.13^{b}	3.08±0.11^{c}	2.28±0.13^{e}	2.31±0.19^{e}

Means ± standard error; W:S:C; wheat:sorghum:chickpea.

Means with different superscript in the same column are significantly different p<0 05 using least square difference (LSD)

0% sorghum-wheat bread 100:0: 0, (control); 4% sorghum- proportion wheat: sorghum : chickpea -96:4:4, 88:4:8, 84:4:12, and 80:4:16; 8% sorghum- proportion wheat: sorghum: chickpea- 92:8:4, 88:8:4, 84:8:8, 80:8:12 and 76:8:16; 12% sorghum proportion wheat: sorghum : chickpea -84:12:4, 80:12:8, 76:12:12 and 72:12:16; 16% sorghum- proportion wheat: sorghum: chickpea; 80:16:4, 76:16:8, 72:16:12, 68:16:16.

Table 5.3b: Means for sensory acceptability of chickpea-enriched sorghum-wheat composite bread

	Wheat: sorghum: chickpea Proportions							
Sensory Attributes	CCF (84:12:4)	CCE (80:12:8)	CCT (76:12:12)	CCS (72:12:16)	SSF (80:16:4)	SSE (76:16:8)	SST (72:16:12)	SCS (68:16:16)
Shape	3.05 ± 0.15^{-d}	$2.69\pm0.16^{c-f}$	2.46 ± 0.12	2.13 ± 0.14^{f}	2.18 ± 0.11^{ef}	2.10 ± 0.33^{f}	2 ± 0.11^{f}	2.00 ± 0.12^{f}
Texture	2.08 ± 0.11^{ed}	2.31 ± 0.17^{ed}	2.08 ± 0.11^{f}	1.64 ± 0.16^{g}	1.69 ± 0.12^{g}	1.33 ± 0.14^{gh}	1.23 ± 0.13^{h}	1.23 ± 0.14^{h}
Crust Colour	2.51 ± 0.14^{-e}	$2.85\pm0.11^{b-e}$	$2.31\pm0.17^{b-e}$	2.33 ± 0.11^{de}	$2.36\pm0.14^{c-d}$	$2.56\pm0.16^{b-e}$	2.28 ± 0.12^{ed}	1.77 ± 0.18^{e}
Crumb colour	2.87 ± 0.14^{ab}	3.15±0. 15±	$2.51\pm0.02^{b-e}$	2.33 ± 0.15^{de}	$2.36\pm0.11^{c-e}$	$2.61\pm0.14^{b-e}$	2.28 ± 0.11^{de}	1.77 ± 0.14^{e}
Aroma	2.15 ± 0.16^{de}	2.77 ± 0.12	2.16 ± 0.13^{f}	2.05 ± 0.15^{f}	2.28 ± 0.12^{f}	1.9 ± 0.11^{fg}	1.62 ± 0.15^{gh}	1.41 ± 0.11^{h}
Taste	2.00 ± 0.13^{ef}	2.62 ± 0.11^{f}	2.0 ± 0.15^{h}	2.05 ± 0.17^{gh}	2.15 ± 0.21^{gh}	1.56 ± 0.15^{i}	1.28±0.11i	1.36 ± 0.13^{i}
Chewiness	1.67 ± 0.11^{f}	2.08 ± 0.17^{gh}	1.67 ± 0.13^{i}	1.74 ± 0.18^{hi}	1.67 ± 0.11^{i}	1.54 ± 0.13^{ij}	1.39 ± 0.16^{ij}	1.28 ± 0.12^{j}
Cell Size	1.62 ± 0.01^{f}	1.79 ± 0.11	1.62 ± 0.12^{gh}	1.67 ± 0.16^{gh}	1.72 ± 0.19^{g}	$1.51\pm0.02^{g-i}$	1.283 ± 0.11^{hi}	1.23 ± 0.13^{i}
Acceptability	1.33 ± 0.19^{a}	1.77 ± 0.16^{f}	1.33 ± 0.11^{g}	1.59 ± 0.17^{fg}	1.46 ± 0.11^{fg}	1.36 ± 0.11^{g}	1.23 ± 0.13^{g}	1.23 ± 0.18^{g}

Means ± standard error

Means with different superscript in the same column are significantly different *p*<0 05 using least significant difference (LSD)

0% sorghum-wheat bread 100:0: 0, (control); 4% sorghum- proportion wheat: sorghum: chickpea -96:4:4, 88:4:8, 84:4:12, and 80:4:16; 8% sorghum- proportion wheat: sorghum: chickpea- 92:8:4, 88:8:4, 84:8:8, 80:8:12 and 76:8:16; 12% sorghum- proportion wheat: sorghum: chickpea -84:12:4, 80:12:8, 76:12:12 and 72:12:16; 16%sorghum- proportion wheat: sorghum: chickpea; 80:16:4, 76:16:8, 72:16:12,68:16:16

5.3.3 Microbial counts

Table 5.4 showed microbial count between treatments and 12 *vs* 16% was significant ($p<0.05$) difference Significant ($p<0.001$) difference was observed between treatments, control bread and sorghum enriched breads, 8 *vs* 12% and 8 *vs* 16% chickpea enriched sorghum composite breads. Shelf life (days) was significantly ($p<0.001$) high in sorghum enriched bread compared to control. The number of days loaves of bread remained fresh increased with increase in level of substitution. Table 5.5 shows that microbial count ranged from 2.16 to 4.22cfu/g, 12% chickpea enriched sorghum-wheat bread had the lowest while control had the highest, shelf life ranged between 6.3days to 14.3 days. Wheat bread had the shortest number of days compared to 16% chickpea enriched sorghum –wheat bread, it was evident that shelf life increased with increase in wheat flour substitution.

Table 5.4 Mean squares from analysis of variance and orthogonal contrasts of treatments (wheat-sorghum-chickpea bread) microbial counts and shelf of bread

Source of variation	df	cfu/g	Shelf life
Rep	2	0.16	0.05
Treatment	4	1.17*	113.19***
Control *vs* 4, 8, 12 and 16% sorghum	1	0.87	272.47***
8% Sorghum *vs* 12% sorghum	1	0.98	35.19***
12% Sorghum *vs* 16% sorghum	1	1.76*	0.17
8% Sorghum *vs* 16% sorghum	1	0.11	40.19***
Error	58	0.38	1.16
CV		15.90	10.63
R^2		0.19	0.88

Values with same superscript *, **, *** are significantly different at $p<0.05$, $p<0.01$, $p<0.001$ using orthogonal contrast analysis.

Control-wheat bread proportion wheat: sorghum: chickpea 100:0: 0; 4%-sorghum- proportion 96:4:4, 88:4:8, 84:4:12, and 80:4:16; 8%-sorghum- proportion 92:8:4, 88:8:4, 84:8:8, 80:8:12 and 76:8:16; 12%-sorghum- proportion wheat: sorghum: chickpea -84:12:4, 80:12:8, 76:12:12 and 72:12:16; 16%-sorghum- proportion wheat: sorghum: chickpea; 80:16:4, 76:16:8, 72:16:12, 68:16:16.

Table 5.5 Mean squares of Microbial quality (cfu/g) and shelf life (days).

Treatment	Flour proportions (Wheat:Sorghum:Chickpea)	Cfc/g	Shelf (days)
OFO (control)	10:0:0	$4.22\pm0.35^{a-c}$	6.33 ± 0.37^{i}
4% sorghum			
FFF	92:4:4	$4.22\pm0.09^{a-c}$	7.33 ± 0.67^{hi}
FFE	88:4:8	$4.21\pm0.05^{a-c}$	7.67 ± 0.32^{h}
FET	84:4:12	$4.15\pm0.03^{a-c}$	8.33 ± 0.33^{gh}
FFS	80:4:16	$3.95\pm0.31^{b-d}$	10.33 ± 0.38^{ef}
8% sorghum			
EXF	88:8:4	$3.30\pm0.03^{e-f}$	9.33 ± 0.35^{fg}
EWE	84:8:8	$3.70\pm0.03^{c-e}$	10.33 ± 0.33^{ef}
YET	80:8:12	3.88 ± 0.06^{cd}	11.33 ± 0.67^{de}
EZS	76:8:16	$4.23\pm0.01^{a-c}$	11.33 ± 0.67^{de}
12% sorghum			
TCF	84:12:4	2.16 ± 0.04^{g}	12.00 ± 0.58^{cd}
CCE	80:12:8	3.11 ± 0.03^{f}	12.67 ± 0.33^{cd}
CCT	76:12:12	$3.71\pm0.11^{c-e}$	12.67 ± 0.33^{cd}
CCS	72:12:16	3.49 ± 0.35^{ab}	14.67 ± 0.38^{a}
16% sorghum			
SSF	80:16:4	$3.44\pm0.^{0\ e-f}$	12.33 ± 0.36^{cd}
SSE	76:16:8	$3.42\pm0.06^{e-f}$	12.67 ± 0.32^{cd}
SST	72:16:12	$4.15\pm0.39^{a-c}$	13.33 ± 0.33^{bc}
SCS	68:16:16	4.63 ± 0.11^{a}	14.33 ± 0.31^{ab}

Means ± standard error

Means with different superscript in the same column are significantly different p<0 05 using least square difference (LSD).

W:S:C; wheat:sorghum:chickpea.

0% sorghum-wheat bread 100:0: 0, (control); 4% sorghum- proportion wheat: sorghum: chickpea -96:4:4, 88:4:8, 84:4:12, and 80:4:16; 8% sorghum- proportion wheat: sorghum: chickpea- 92:8:4, 88:8:4, 84:8:8, 80:8:12 and 76:8:16; 12% sorghum proportion wheat: sorghum : chickpea -84:12:4, 80:12:8, 76:12:12 and 72:12:16; 16% sorghum- proportion wheat: sorghum: chickpea; 80:16:4, 76:16:8, 72:16:12, 68:16:16.

5.3.4 Nutrient content

Table 5.5 shows significant (p<0.001) difference in moisture, ash, fibre, protein and carbohydrate due to treatment, significant difference was observed in control vs 4, 8, 12 and 16% in ash, fibre and protein. Moisture content was p<0.001) high in 12 vs 16% and 8 vs 16%. Nutrient content showed significantly (p<0.001) due to treatment, and in control vs 4, 8, 12 and 16% enriched bread, moisture content in 12%vs 16% and 8% vs 16% showed significant (p<0.001) difference (Table 5.6). Moisture ranged from 40.80 to 12.82, the highest was in chickpea enriched 4% sorghum while the lowest was in 18% sorghum. Ash content ranged between 1.48 g to 3.10 g, control bread had the lowest while chickpea enriched 16% sorghum bread had the highest. Fibre content ranged between 1.64 to 6.22g, control had the lowest and enriched 16% sorghum bread had the highest. Oil and protein ranged between 5.02 to 7.09 and 10.23 and 15.28 respectively, control had the lowest while enriched 16% sorghum composite had the highest, nutrient content increased with increase in wheat substitution. Carbohydrate content ranged between 70.21 in and 81.62 in control while In vitro protein digestibility ranged from 85% to 90%, the chickpea enriched 16% sorghum composite had the highest and control bread had lowest level. Table 5.5 showed significant difference in in vitro protein digestibility due to treatment, 8% vs 12% and 8% vs 16% enriched bread.

5.3.5 *In vitro* protein digestibility

In vitro protein digestibility was significantly (p<0.001) different due to treatment, 8% vs 16% and 12 vs 16% loaves of bread (Table 5.5) while means ranged between 85% and 90% (Table 5.6), chickpea enriched 16% sorghum composite bread had the highest digestibility.

Table 5.6: Mean squares from analysis of variance and orthogonal contrasts of treatments properties (wheat-sorghum-chickpea bread) for moisture, ash, fibre, oil, protein carbohydrate and in vitro digestibility

Source	df	Moisture	Ash	Fibre	Oil	Protein	Carbohydrate	*In vitro* protein digestibility
Replicate	2	0.79	0.01	0.01	0.023	0.01	0.29	0.14
Treatment	4	551.10***	1.90***	7.07***	1.32*	9.73***	65.75***	20.58***
Control (wheat: chickpea) *vs* 4,8, 12 and 16% sorghum	1	5317.23	4.69***	23.68***	4.73**	35.718***	230.58***	8.62*
8 Sorghum% Sorghum *vs* 12% sorghum	1	32.83	0.01	0.05	0.02	0.04	0.04	15.25***
12% Sorghum *vs* 16% sorghum	1	1276.33***	0.40	2.09*	0.38	0.79	12.88	11.94**
8% Sorghum *vs* 16% sorghum	1	1712.46***	0.32	1.49	0.22	1.20	11.37	54.09***
Error	58	34.67	0.11	0.54	0.35	1.07	5.44	1.11
CV		16.69	15.41	16.32	9.82	7.47	3.17	1.20
R^2		0.53	0.56	0.49	0.21	0.40	0.46	0.57

Values with *, **, *** supers script are significantly different at $p<0.05$, $p<0.01$, $p<0.001$ using orthogonal contrast analysis
KEY: 0% sorghum- wheat bread enriched with chickpea in proportion wheat: sorghum chickpea 100:0: 0, (control).
4% sorghum- sorghum wheat composite bread enriched with chickpea in proportion wheat: sorghum: chickpea- 96:4:4, 88:4:8, 84:4:12, and 80:4:16; 8% sorghum- sorghum wheat composite bread enriched with chickpea in proportion wheat: sorghum: chickpea- 92:8:4, 88:8:4, 84:8:8, 80:8:12 and 76:8:16; 12% sorghum- sorghum wheat composite bread enriched with chickpea in proportion wheat: sorghum: chickpea -84:12:4, 80:12:8, 76:12:12 and 72:12:16; 16% sorghum- sorghum wheat composite bread enriched with chickpea in the proportion wheat: sorghum: chickpea; 80:16:4, 76:16:8, 72:16:12, 68:16:16.

Table 5.7 Means for nutrient content of chickpea enriched sorghum-wheat composite bread.

Treatment	Wheat:sorghum: chickpea proportion	Moisture (g)	Ash (g/100g)	Fibre (g/100g)	Oil (g/100g)	Protein (g/100g)	Carbohydrate (g/100g)	*In vitro* protein digestibility (%)
OFO (control)	(10:0:0)	40.16±1.96^{a}	1.48±0.02j	1.64±0.03^{m}	5.02±0.04^{h}	10.23±0.02^{l}	81.62±0.09^{a}	85.79±0.76^{d}
4% sorghum								
FFF	92:4:4	33.37±1.33^{bc}	1.64±0.03^{i}	3.91±0.07^{l}	5.68±0.06^{f}	13.51±0.03^{k}	75.26±0.04^{b}	89.78±0.11^{c}
FFE	88:4:8	40.01±0.61^{a}	1.77±0.01 ^{hi}	4.01±0.04^{k}	6.20±0.01^{e}	13.72±0.01^{j}	74.22±0.05^{c}	88.88±0.04^{c}
FET	84:4:12	40.03±0.36^{a}	1.85±0.05^{gh}	4.43±0.06^{g-i}	6.55±0.03^{cd}	14.03±0.01^{g}	73.14±0.04^{e}	86.89±0.29^{c}
FFS	80:4:16	39.62±1.34^{a}	2.20±0.06^{e}	5.41±0.04^{d}	6.82±0.04^{bc}	14.47±0.06^{d}	71.10±0.09^{k}	86.91±0.18^{c}
8% sorghum								
EXF	88:8:4	40.80±0.23^{a}	1.89±0.01^{g}	4.18±0.05^{j}	6.94±0.03^{ab}	13.74±0.04^{j}	73.23±0.03^{e}	86.91±0.28^{c}
EWE	84:8:8	40.69±0.30^{a}	2.15±0.02^{e}	4.57±0.01^{hi}	5.61±0.06^{f}	13.95±0.07^{h}	73.73±0.05^{d}	86.68±0.23^{cd}
YET	80:8:12	40.56±0.05^{a}	2.49±0.02^{c}	4.83±0.0^{fg}	5.70±0.05^{f}	14.37±0.04^{e}	92.61±0.07^{f}	86.77±0.16^{c}
EZS	76:8:16	39.14±0.91^{ab}	2.73±0.03^{b}	5.67±0.01^{bc}	6.32±0.22^{de}	14.75±0.02^{c}	70.52±0.28^{l}	86.88±0.01^{c}
12% sorghum								
TCF	84:12:4	39.16±0.89^{ab}	1.85±0.03^{gh}	4.23±0.01^{i}	5.65±0.08^{f}	14.25±0.02^{f}	74.03±0.04^{cd}	88.32±0.29^{b}
CCE	80:12:8	38.47±0.23^{ab}	2.17±0.02^{e}	4.46±0.02^{gh}	5.77±0.01^{f}	13.86±0.02^{i}	73.86±0.02^{d}	88.43±0.28^{b}
CCT	76:12:12	37.74±1.16^{ab}	2.38±0.02^{d}	4.55±0.01^{f}	6.23±0.02^{e}	14.34±0.02^{e}	72.50±0.05^{ij}	88.44±0.19^{b}
CCS	72:12:16	36.54±2.84^{a-d}	2.76±0.01^{b}	5.65±0.02^{c}	6.66±0.02^{c}	14.71±0.04^{c}	70.21±0.07^{i}	88.49±0.38^{b}
16% sorghum								
SSF	80:16:4	36.40±1.03^{a-d}	2.03±0.08^{f}	4.33±0.02^{i}	5.32±0.24^{g}	14.05±0.02^{g}	74.27±0.27^{c}	89.66±0.04^{a}
SSE	76:16:8	12.82±1.99^{d}	2.36±0.07^{d}	4.91±0.01^{e}	6.12±0.02^{e}	14.38±0.01^{e}	72.22±0.09^{j}	89.71±0.44^{a}
SST	72:16:12	12.99±1.32^{d}	2.71±0.06^{b}	5.78±0.01^{b}	6.80±0.05^{bc}	14.91±0.01^{b}	69.80±0.11^{m}	89.81±0.38^{a}
SCS	68:16:16	31.35±0.95^{c}	3.10±0.03^{a}	6.22±0.09^{a}	7.09±0.03^{a}	15.28±0.05^{a}	68.32±0.04^{n}	90.14±0.36^{a}

Means± standard error for three determinations. Different letters in the same raw are significant difference $p<0.05$, by least significant difference (LSD) W:S:C; wheat:sorghum:chickpea. Bread proportion: 0% sorghum (wheat:sorghum:chickpea): OFO: 100:0:0 (CONTROL), 4% sorghum (wheat:sorghum:chickpea): FFF;88:4:4, FFE; 84:4:8, FFT; 80:4:12, FFS; 76:4:16. 8% sorghum (wheat:sorghum:chickpea): EXF; 88:8:4, EWE; 84:8:8, YET; 80:8:12, EZS;76:8:16 ; 12% sorghum (wheat:sorghum:chickpea): TCF; 84:12:4, CCE; 80:12:8, CCT; 76:12:12, CCS; 72:12:16; 16% sorghum (wheat:sorghum:chickpea: SSF; 80:16:4, SSE; 76:16:8, SST; 72:16:12, SCS; 68:16:16

5.4 Discussion

5.4.1 Physical and baking properties

The dough length (L-value: resistance of the dough to extensibility), dough height (P-value: resistance to elasticity) and P/L ratio (configuration ratio) were obtained from the alveograph, In this study, length value in chickpea enriched wheat dough (control) values decreased with increase in wheat substitution level. The reducing length value may be associated with low viscoelastic network due to interruption by non-gluten protein and fibre from whole meal sorghum and chickpea flours. Dilution of gluten network may have produced dough that was too weak to trap adequate carbon dioxide produced during fermentation resulting to bread with low height and volume. Mrooj (2016) attributed low dough strength and stability to reduced viscoelasticity due to gluten dilution by high fibre and non-wheat flour. The length reducing trend with increase in wheat substitution was in agreement with Sibanda *et al.* (2015) who obtained values that reduced from 132mm to 36mm in sorghum composite bread, Gadalla *et al.* (2017) also reported 175mm, 120mm, 115mm in sorghum and chickpea composite flour. High fibre from whole sorghum and chickpea flour are responsible for disruption of the viscoelastic system of bread dough (Gadalla *et al.,* 2017). The L-value is an indicator of protein gluten quality that predicts handling of the dough (Hordes *et al.*, 2008) when low, the dough has low ability to trap gases making the resulting bread to have low volume, more dense and undesirable (Gomez *et al.*,2010). Wheat gluten protein is responsible for dough structure formation (Gallagher *et al.,* 2003). Gliadin is responsible for making the dough highly extensible.

The high p-value is an indicator of dough strength/ resistance to deformation with ability to trap gases resulting to less dense; good quality bread (Kulamarva *et al.*, 2009). In this study, wheat dough enriched with chickpea (0%) showed significantly ($p<0.05$) high P-value compared to dough made from chickpea enriched 12 and 16% sorghum bread. P-value reduced with increase in wheat replacement level. Low P-value value in breads with high wheat substitution may be attributed to intense incompatibility between chickpea protein and wheat gluten protein. Gluten dilution by high dietary fibre from sorghum and chickpea is likely to have affected dough elasticity reducing its ability to rise to optimum height. This results are in agreement with Gadalla *et al.* (2017) who obtained a reducing P-value with increase in wheat substitution with chickpea or sorghum and Sibanda *et al.* (2015) who obtained a reducing height trend (60.7mm to 49.5mm) in sorghum composite dough. High P-Value in the study may have contributed to high dough elasticity, good gas holding capacity resulting to desirable bread volume. Doxastakis *et al.* (2002) explained that although legumes contain glutenin, addition of non-wheat flour into bread recipe weakens gluten, thus concluded that both protein fractions (gliadin and glutenin) must be present for optimal gluten network development in a specific ratio. The configuration ratio (P/L) indicates the balance between dough strength and stability dough (Torbica *et al.,* 2007). In this study, P/L value was high ($p<0.05$) in control compared to other breads (Table 2). Values decreased with increase in wheat substitution, this is in agreement with Hefnawy *et al.,* (2012) who obtained a reducing P/L ration 3.3, 3.0 and 2.7 as wheat substitution increased, Gadalla *et al.,* (2017) obtained high values in 5-10% sorghum but low values in 15-20% sorghum. Morali *et al*. (2016) obtained reducing P/L values and associated it to high bran from low wheat extraction rate. The study contradicts results reported by Sibanda *et al.* (2015) who obtained an increasing P/L ratio (0.3, 0.5, 0.9 1.5) as the level of wheat substitution increased. This study attributed P/L results to the reducing trend of length and height values with increase in wheat substitution.

Energy (J) is the amount of work required to stretch the dough until it raptures. Depending on wheat variety, strong dough requires more energy. Good quality wheat requires energy that ranges from 220 to 300J (Hordes *et al*., 2008). In the study, energy value for 0% sorghum bread was within the recommended level while other breads fell below good quality category (Table 2). Less energy utilization may be associated with high content of non-wheat protein and fibre from whole meal flour that may have weakened the dough resistance to expansibility and extensibility. Strong gluten flour produces dough with high p-value that can stretch to a thin membrane before breaking; requiring more energy. Strong dough is preferred for bread production (Causgrove, 2004). Since not all wheat flours are strong, dough improvers are used in bread to help improve gas retention and gluten restructuring. This study used dobrin dough improver to improve the dough strength, enhanced dough tolerance and bread quality of composite breads to this study, Bhatt and Gupta (2015) used calcium propionate and dough improver to improve rheological properties of chickpea supplemented bread.

Loaf volume was highest in 8% and lowest in 16% sorghum enriched breads while weight was significantly low in control and highest in 16% sorghum enriched with chickpea. Weight increased with increase in the level of wheat substitution. Wambua *et al.* (2016) and Bakare *et al.* (2016) obtained a similar trend of bread weight and volume with increase in the level of cassava and fruit respectively. In this study, bread volume may have reduced due to disruption of gluten elasticity by fibre from sorghum and chickpea which may have caused the release of gases produced during fermentation. The gelatinization property of chickpea may prevented rising of the dough, uneven cell size and hard texture of chickpea bread. Dietary fibre (DF) reduces gluten network and compromises bread volume (Kurek and Wyrwisz, 2015). Specific loaf volume (SLV) is a reliable measure of loaf size and guides bakers to produce standard sizes of bread (Bakare *et al.,* 2016). It was found out that the SLV was low in chickpea enriched sorghum (16%) wheat composite bread. High loaf weight may be associated with low gas retention from gluten dilution. This observation has been reported in other studies; Maktouf, *et al*. (2016) in pearl Millet bread; Sibanda *et al,* (2015) in cassava composite bread. However, Hefnawy *et al.* (2012) obtained increasing SLV values with increase in chickpea level. The high gelatinization property of chickpea flour may have reduced capacity of the dough to rise as observed in 8, 12 and 16% breads (Table 5.3a and 5.3b). Bread produced with high weight, low score in crumb softness, poor texture, and slices of bread broke during slicing compared wheat bread sorghum bread. Bakers prefer moderately strong dough for optimum bread production (Yeng *et al*. 2015) and this encourages use of dough improvers by bread bakers. Studies Bhatt and Gupta (2015); Carson *et al.* (2000) used gluten and carboxyl methyl cellulose in potato bread, respectively to improve dough rheology of whole wheat flour, as used in this study.

5.4.2 Sensory evaluation

Sensory properties of a food item are important for acceptability and sensory methods and principals used on all foods may also apply to bakery products. Composite products have numerous health benefits however; highly substituted wheat flour affects sensory attributes of bread (Carson, e*t al.,* 2000). In the study, a minimum score of three (3), was considered acceptable. It was observed that wheat bread had significantly ($p<0.05$) high scores while chickpea enriched 16% sorghum-wheat composite bread was ranked lowest. Sensory scores decreased with increase in level of wheat substitution. Loaf shape scores were significantly ($p<0.05$) high for control and lowest for 16% (Table 5.3a and 5.3b). This may be ascribed to high gluten content that enabled high gas holding capacity; leading to acceptable bread volume in the low wheat substitution level with chickpea. From alveograph results, high P-value is an indicator of dough strength and ability to trap fermentation gases resulting in high loaf volume and general acceptability (Kulamarva *et al.,* 2009). High content of the non-wheat flours in enriched 16% sorghum bread may have adversely affected gas holding capacity; loaf volume, shape, texture and undesirability. Texture is the structure formed by strands of gluten including the loaf crust.

In this study, texture was measured for crumb and crust hardness. Scores were significantly ($p<0.05$) high in control and 4%.compared to enriched 16% sorghum bread. Scores decreased with increase in level of wheat substitution. Close cell structure and low volume results to bread denseness and undesirable texture (Sibanda *et al.*, 2015). The high texture score observed in control and enriched 4% sorghum bread may be due to high gluten content in the breads while low texture score in 8, 12 and 16% sorghum enriched breads may be attributed to inability of the loaf to spring back when pressure was applied due to loaf denseness, hard crumb and crust texture with rough surface. The absence of adequate gluten forming proteins in chickpea and sorghum contributes may have been the result of close cell crumb, surface roughness, loaf denseness and coarseness of the breads with high wheat substitution level. Manekazi *et al.* (2013) observed a similar trend in texture scores.

Chewiness was evaluated for gumminess and denseness of the crumb. Score ratings observed in control and 4% (sorghum) enriched bread were significantly ($p<0.05$) high compared to 8, 12 and 16% breads which were low. Gumminess and denseness of loaves may be attributed to gelatinization properties of chickpea flour while and high fibre content. High fibre may have increased coarseness of the crust surface, causing release of gases consequently reducing loaf volume as well as increasing loaf denseness. High ash and low protein level affect loaf texture (Onoja *et al*., 2014). Studies Agu *et al*. (2010);Mwanekesi *et al*. (2015) observed reduced texture scores from 7.15 in control to 6.90 in cassava soybean composite bread and 7.95-7.35 in pumpkin composite bread respectively. The sorghum grain used in this study was milled twice in order to reduce flour particle size for improved gas retention, bread quality and higher mouth feel attribute however, in high wheat substitution level, breads had low chewiness acceptability score.

Crumb colour influences purchase or rejection of a product, a uniform golden brown colour is preferable (Hossian *et al.,* 2014). This study observed that 0, 4, 8 and 12% (sorghum) enriched breads had significantly ($p<0.05$) high crumb and crust colour, desirable and likeability decreased with increase in level of wheat flour substitution. The reducing scores with increase in wheat substitution is in agreement with colour Adeyeye, (2016) obtained 8.25 score in control and 5.70 in sorghum bread. Abdelghafor *et al.* (2011) explained that reducing crumb colour scores was because consumers prefer a lighter colour which is associated with raw materials used for wheat bread. In this study, the low score may be associated with darker colour to high proportion of red sorghum and chickpea which panellists may not have been familiar with. Crumb softness was significantly high in control and 4% breads. In high sorghum and chickpea proportions (8, 12 and 16% sorghum) enriched breads, the crust was hard and slicing was difficult. This may be associated with high fibre, chickpea binding property which may have reduced the dough rising power consequently affecting loaf volume and softness.

Cell size is air space in the loaf crumb often determined by the gluten protein quality and quantity. In the study, cell size scores were significantly ($p<0.05$) high in 0 and 4% (sorghum) enriched breads, values decreased with increase in sorghum level. This is possibly due low content of non-wheat flours. Mariera *et al.* (2017) observed insignificant cell size in sorghum composite bread and associated it to fine milling of sorghum flour however, enrichment of sorghum bread with chickpea adversely affected cell size. The study used dobrin dough improver to increase gas retention and dough strength but significant ($p<0.05$) difference was observed. Similar high scores in wheat bread and 8% for loaf flavour and aroma were observed in the study attributable ascribed to low wheat substitution level. The reducing score with increase in wheat substitution level are in agreement with Agu, 2010); Adeyeye, (2016) in pumpkin seed composite bread and sorghum wheat cookies. Abdelghafor *et al.* (2011) observed a bitter taste at 10% sorghum which was associated with phenolic compounds from the grain coat. This study showed that sensory quality is affected by flour formulation. Control bread and 4% (sorghum) enriched with chickpea were rated significantly ($p<0.05$) high compared to 8, 12 and 16% on general acceptability. Decreased dough strength and extensibility are likely to have influenced bread quality, (Moradi *et al.*, 2006) associated low bran concentration with better rheological properties and bread quality. Importantly, general acceptability was likely influenced by taste, aroma and crumb softness.

5.4.3 Microbial counts and shelf life

Total bacterial counts (cfu/g) was highest in 4% and lowest in 12% sorghum bread enriched with chickpea this may be attributed to handling processes. Shelf life was significantly ($p<0.05$) low in wheat bread compared to sorghum and chickpea (4, 8, 12 and 16%) bread. Shelf life increased with increase in sorghum level. This is likely due to antioxidant activity of red sorghum flour used in sorghum composite bread. Flour type (composition) has also an effect on microbial count and shelf life of a product (Kumral, 2015). Pigmented sorghum bran contains concentrated tannins whose high antioxidant activity (at 25 ^{0}C) makes sorghum bran a potential cheap source of phenolic compounds for use as an antioxidant in industries (Srivastava and Stanlaus, 2016). In potato composite bread, Ijah *et al*. (2014) reported high microbial count in Irish potato composite bread 6 x10^{5} 8cfu/g) compared to sweet potato composite bread (4.8 x 10^{5} cfu/g) potato but nil in control.

The study used calcium propionate preservative as used in the bread making industry in order to inhibit bread spoilage by bacteria such as *Bacillus subtillis* species which causes ropy bread. Spoilage bacteria are destroyed by heat during baking but their pores are resistant to heat, raw materials and unhygienic condition are other factors that cause economic loses in baking industries (Thompson *et al.,* 1998). Bacteria such as staphylococcus are distributed in the environment and occur on human skin, foods are thus likely to be contaminated (Ijah *et al.,* 2012).

5.4.4 Nutrient content of chickpea enriched sorghum wheat composite bread

Nutrient content is important in evaluating the nutritional value of a product. Different compositions of composite flours affect nutrient quality of a product. Table 5.6 shows the moisture, ash fibre, fat, protein, carbohydrate and *in vitro* protein digestibility. Moisture content was significantly ($p<0.05$) low in (16%) sorghum bread enriched with chickpea enriched bread sorghum composite bread, while control, 4, 8 and 12% breads had in significant different. This may be associated with loss of moisture in sorghum during bread making procedures and the low moisture content of chickpea flour. Gadalla *et al.,* (2017) reported 9.3% moisture in chickpea wheat composite flour compared to 11.2% in sorghum wheat composite. Ijah *et al.* (2014) also obtained varying moisture level in Irish and sweet potato composite breads and associated it with baking process. Ahmed *et al.,* (2016) obtained low moisture (3.52±0.01%) content in10% chickpea enriched decorticated sorghum composite biscuits compared to control (5.36±0.02%). High moisture in a product provides a conducive environment for microbial proliferation (Ijah *et al.,* 2014) while low moisture has been ascribed to longer shelf life of a product (Sanni *et al.,* 2008). In the current study, low moisture in composite breads was likely to have contributed to the longer shelf life (days) of chickpea enriched sorghum wheat composite bread with increase in wheat substitution level.

Chickpea enriched sorghum wheat composite bread 8, 12 and 16% breads had significantly ($p<0.05$) high ash content compared to control, 4% and 8% enriched sorghum composite bread. It was evident that control bread had the lowest ash content. High ash in chickpea enriched sorghum composite bread may be ascribed to the high mineral content in chickpea sorghum flour. Gadalla *et al*. (2017) also observed increasing ash level; 0.67 to 2.7g/100g ash; Hefnawy *et al.* (2012) obtained 0.85 to 2.5g/100g ash level. The increasing ash trend is in agreement with Sabanis *et al.* (2006) who associated high mineral content to chickpea flour. High ash is beneficial for human health as chickpea is a rich source of iron, zinc, calcium and magnesium; food insecure households often experiencing micronutrient deficiency and are therefore likely to benefit from incorporation of chickpea and sorghum into bread recipe.

Fibre content was significantly (p<0.05) high in bread containing chickpea enriched 16% sorghum composite bread compared to wheat bread. Fibre level increased with increase in chickpea and sorghum level. The use of whole grain sorghum and chickpea to produce flour may have increased fibre content in the breads. Mrooj. (2016) attributed low dough strength and stability to reduced viscoelasticity due to gluten dilution by high fibre and non-wheat flour. High fibre in chickpea enriched sorghum composite breads is in agreement with Roccia *et al.* (2009); Hefnawy *et al.* (2012); Gadallah *et al. (*2017). High fibre reduces the risk of coronary heart disease through control of cholesterol accumulation (Roccia *et al.* (2009). Other health benefits include improved glucose tolerance, reduction of risks of cancers and obesity (Man *et al*., 2015). However, during bread making process, high fibre reduces viscoelastic properties causing the release of gases produced during fermentation. It also increases water absorption capacity, fat holding capacity, reduces swelling capacity of the dough which affects loaf quality (Man *et al.,* 2015). In the study high fibre was likely to have been the cause of low P-value which was reflected in the are low bread volume increased loaf weight (Table 5.2), loaf reduced loaf texture and general acceptability (Table 11).

Protein content was significantly (p<0.05) high in chickpea enriched sorghum wheat composite bread compared to control. It was evident that the values increased with increase in chickpea and sorghum level. The study associated high protein value to high protein in chickpea flour. These results are in agreement with Gadalla *et al.* (2017) who obtained 12.87, 13.68, 13.81, 14.37 and 14.87 g/100g in chickpea enriched flour. Salve and Mehrajfatema, (2011) also reported 6.66% increase protein in cakes fortified with legumes from 6.01% in wheat bread. Other studies (Hefnawy *et al*., 2012; Sinada *et al.,* 2013; Man *et al.,* 2015) obtained a similar trend in protein value in chickpea enriched bread while Adeyeye (2016) obtained high protein in sorghum composite cookies with increase in sorghum level. Chickpea is rich in lysine amino acid while cereals are rich in sulphur containing amino acids such as methionine and leucine. Bread produced from a combination of both legumes and cereals would provide a balanced intake of essential amino acids (Khattak *et al*., 2007). Lysine amino acid is essential for synthesis of body proteins and peptides that play a role in biochemical reactions (including structural support) of all living cells and tissues, its deficiency causes degradation of body protein especially muscle protein (Rosiak *et al.,* 2013) as witnessed in persons particularly with kwashiorkor. High protein is thus vital for prevention of deficiency effects such as diminished protein synthesis, low immune function in kidney patients, inadequate production of antibodies, mental health, retarded growth, fatigue, anaemia and reproductive disorders Berge, *et al.* (2007). The study observed that carbohydrate values were significantly (p<0.05) low in chickpea enriched sorghum composite bread compared to control, values decreased with increase in chickpea level. This was attributed to low carbohydrate in the chickpea flour and the high level of other nutrients in the bread.

5.4.5 In vitro protein digestibility

In vitro protein digestibility results show that wheat bread had high *in vitro* protein digestibility compared to chickpea enriched sorghum wheat composite breads (4, 8, 12 and 16%) Notably, protein *in vitro* digestibility in sorghum and chickpea containing breads was not significantly different among the composite breads which may be attributed to the small difference in sorghum and chickpea proportion added. Low protein digestibility in sorghum containing bread may have been due to binding of protein by antinutritive factors such as trypsin inhibitors, tannins and phytic acid to form indigestible complexes. Studies (Latimer and Haud, 2010) observed that dietary fibre binds nutrients resulting to indigestion in small intestines. Ahmed *et al*. (2016) observed low *In vitro* protein digestibility of Sorghum composite biscuits (43.51%) compared to 46.59 in maize composite biscuits. Afify *et al.,* (2012) explained that tannins and protein (especially with proline) contained in whole sorghum form complexes which inhibit peptidase enzymes activity. Research on methods to improve digestibility by reducing antinutritive factors have been done. Studies; El-Adawy, (2002); Alajaji and El-Adawy, (2006) observed that boiling significantly (p<0.05) reduced trypsin inhibitors, tannins and phytic acid by 82%, 48% and 28% respectively through denaturing of protein and destruction of antinutritive factors. Mohammed *et al*. (2016) obtained increased level of protein digestibility in decorticated sorghum flour and chickpea flour with increase in wheat substitution. This was attributed to reduction of antinutritive factors (enzyme inhibitors tannins, phytates).

5.5 Conclusion

The study showed that 4% chickpea and 4% sorghum can be used to partially substitute wheat substitution to produce bread with acceptable loaf volume however, at high level, physical and baking properties decrease. Substitution of wheat flour with sorghum and chickpea flour improved protein, ash, fat and fibre contents as well as *in vitro* protein digestability. Chickpea and sorghum can be used to add value to bread and other wheat based products. Shelf life of bread enriched with chickpea and sorghum increased with increase in wheat substitution level; an advantage to rural areas where storage facilities are inadequate. Importantly, 4% sorghum bread enriched with chickpea had the highest general acceptability among the sorghum enriched breads.

5.6 Recommendation

It is important for the government to have a policy that requires the inclusion of 92% wheat 4% sorghum: 4% chickpea in wheat flour or food formulation for improved protein content in wheat based products. There is also a need for more research to understand the rheological properties of chickpea enriched sorghum-wheat composite bread so as to produce acceptable bread recipes for improved nutrient content of bread and other confectionaries. Nutrient dense bread and confectionary recipes will contribute towards reduction of under nutrition especially in developing countries which have potential of producing adequate sorghum and chickpea.

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APPENDICES

Appendix I: Informed consent

The researcher Lucy Naisianoi Mariera, is a Master of Science Student in the department of Human Nutrition at Egerton University.

Purpose of the study

The purpose of the study is to develop sorghum composite bread and enriched sorghum-wheat composite bread. This is intended to reduce wheat importation which increases bread price and makes bread unaffordable to food insecure population. This is likely to stabilize bread price, increase protein content for improved nutritional health of consumers. Farmers in dry land areas will benefit from increased market demand for sorghum and chickpea and thus improve their livelihood, food security as well as helping Kenya in attainment of Vision 2030

Interventions

The hospitals provide F75, F100 and Fortified flour to improve the nutritional status of children with PEM.

Risks: The chickpea has low digestibility.

Benefits: The bread is targeted to improve protein content

Compensation: As a bread consumer, please provide information willingly in order to help in the product development without claim for compensation.

Freedom to withdraw: In case you are unable to continue with the evaluation, you are free to withdraw at any stage as you will not be penalized.

Privacy and confidentiality: All information provided will be handled with utmost privacy and confidentiality

Contact person if need arises

Lucy N. Mariera Mobile number. 0722 271 276

Declaration: I ______________________________ do declare that I have read, understood the content in this form and willing to proceed with the process.

Appendix II: Sensory evaluation

Panelist code--------------------- Date-----------------------

Please look and taste each of the samples of bread in the order as shown below. Indicate how much you like or dislike the following attributes of each sample by marking (×) in the appropriate phrase under the sample code number; aroma, colour, texture, flavour and general acceptability.

Sample code	Loaf shape	Crust colour	Aroma	Texture	Crumb colour	Taste	Gumminess/chewiness	Overall acceptability

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

Comments

Appendix III: (a) Specific bread volume tool

Sample code	Weight of loaves	Volume of rapeseeds	Specific bread volume (cc/g)

Appendix IV: Microbial counts

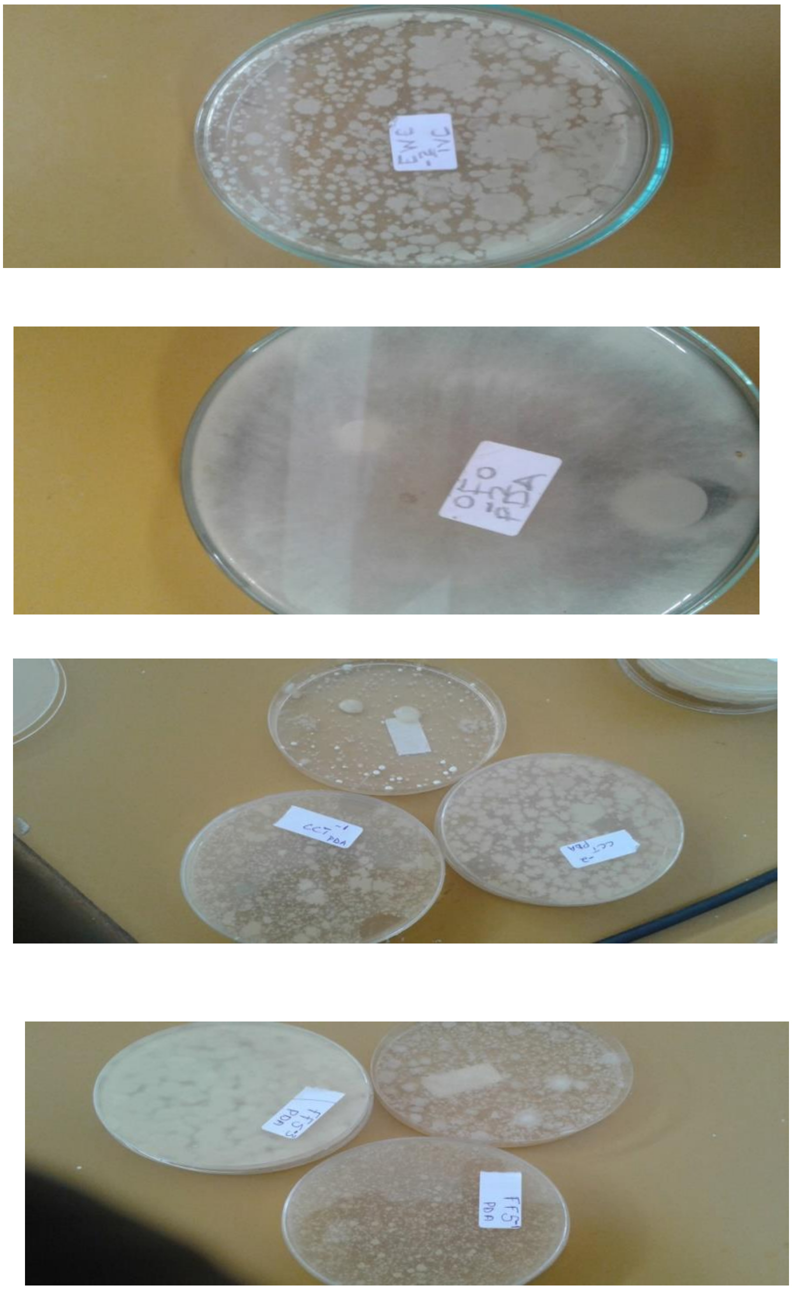

Figure 1 Microbial counts

Appendix V: Baked sorghum composite bread

Appendix VI: Baked composite bread

Figure 2 Sorghum composite loaves of bread 1-16% chickpea:16% sorghum composite bread, 2-wheat bread, 3- assorted composite loaves of bread

Appendix VII: Physical and properties

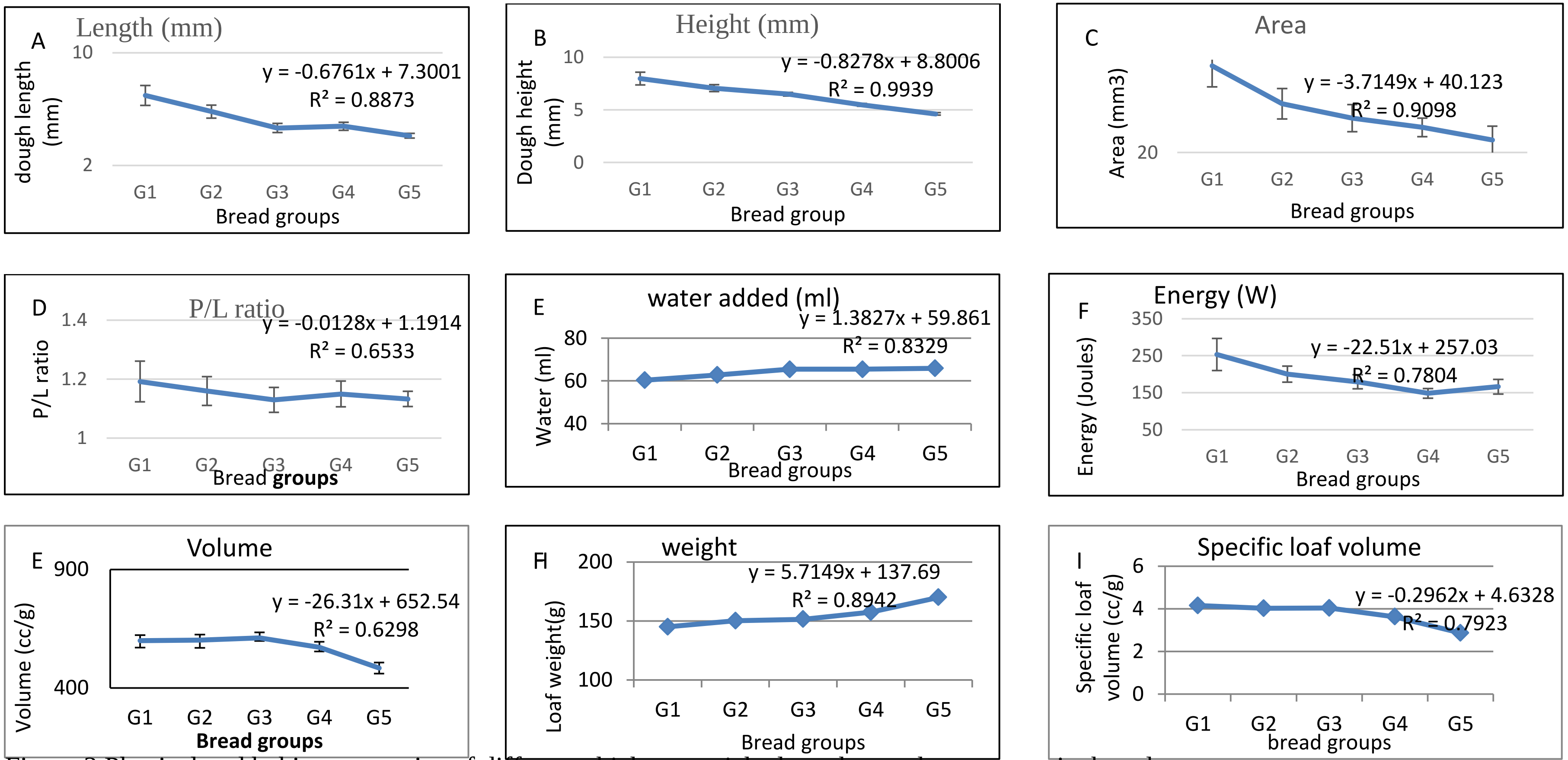

Figure 3 Physical and baking properties of different chickpea enriched sorghum-wheat composite bread

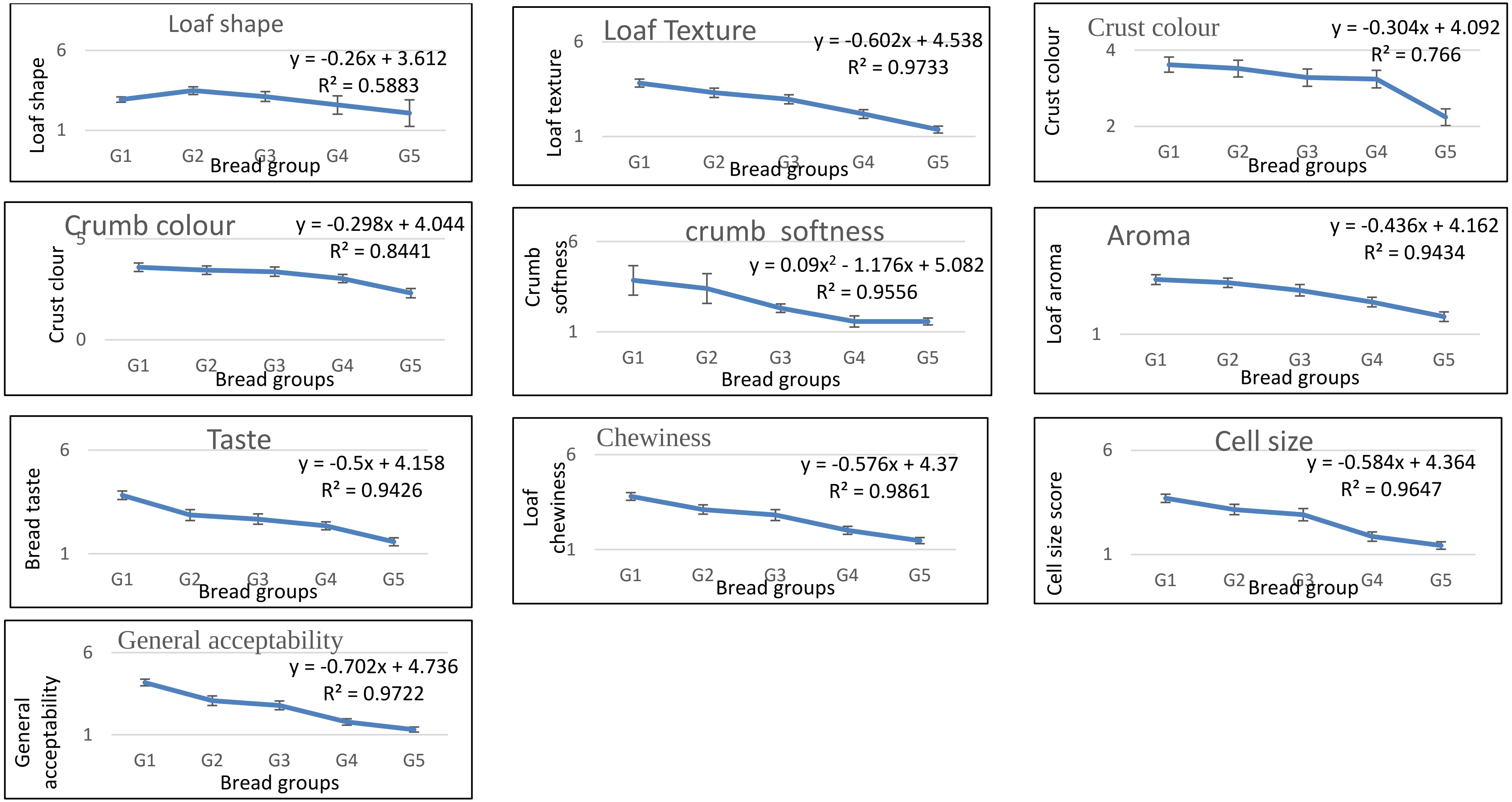

Figure 4: Relationships of relative constitutions of wheat-sorghum-chickpea with different loaf quality and general acceptability.

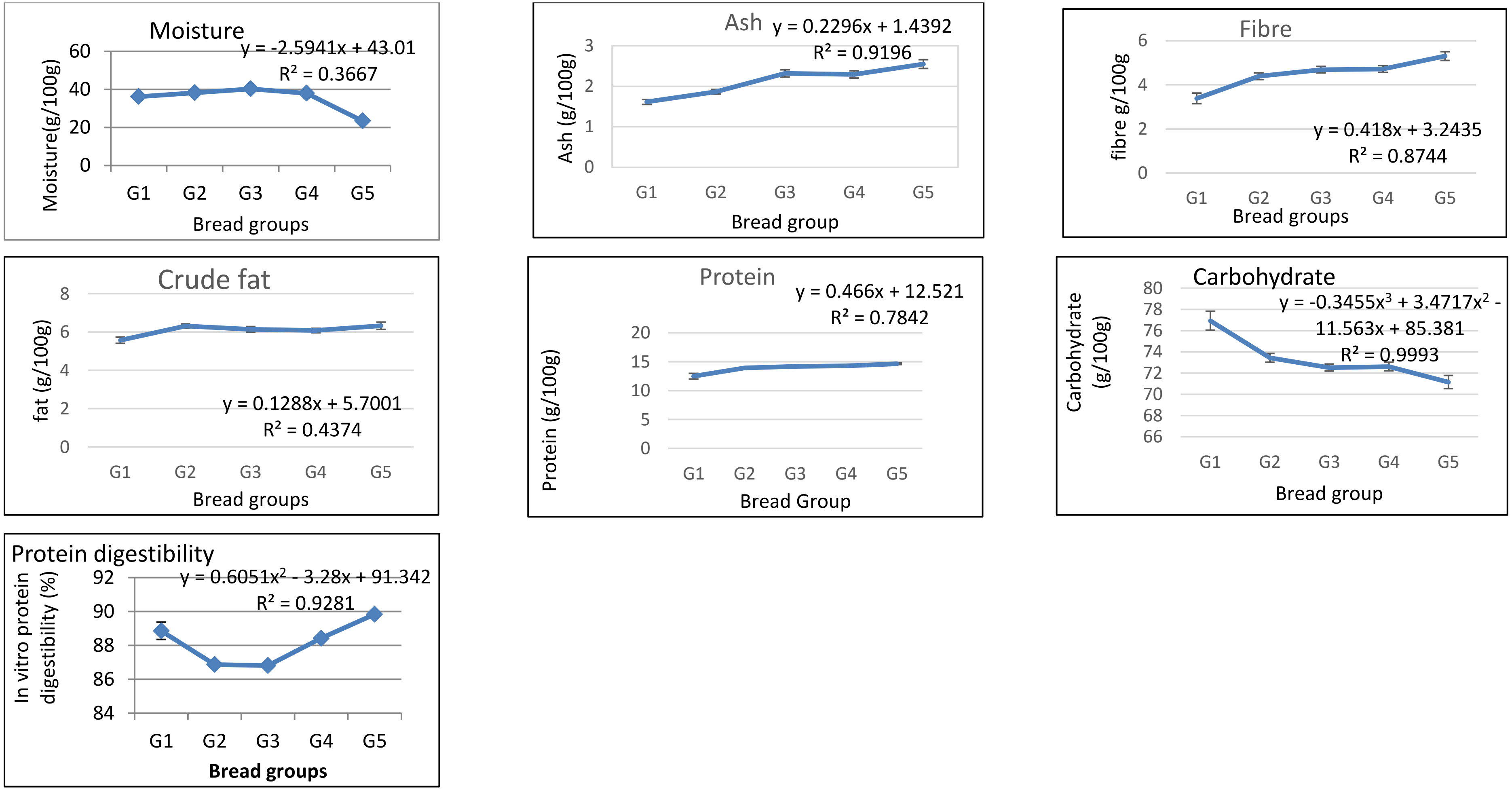

Figure 5 Nutrient content of chickpea enriched sorghum-wheat composite bread.

Appendix VII: Publication

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DOI: http:/dx.doi.org/10.14303/ajfst.2017.118
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Full Length Research Paper

Development of sorghum-wheat composite bread and evaluation of nutritional, physical and sensory acceptability

[1]Mariera Lucy., [2]Owuoche James O., [1]*Cheserek Maureen

[1] Department of Human Nutrition, Egerton University P.O Box 536 Egerton, Kenya.
[2]Department of crops, Horticulture and Soil Science, Egerton University P.O Box 536 Egerton,Kenya.
.*Corresponding Author's E-mail:mjcheserek@yahoo.co.uk/mcheserek@egerton.ac.ke.com, +254-0701-885509

ABSTRACT

Increase in bread consumption, health awareness and demand for nutritious foods has necessitated research on composite bread to meet these needs. Sorghum (Sorghum bicolar L.) is nutritious, this study used a new sorghum genotype EUS130 in development of sorghum composite bread in the proportions wheat: sorghum flour 100:0 (Control), 96:4, 92;8, 88: and 84:16. Baking was done using Straight dough method. Nutrient content, shelf life, physical properties: height (dough strength), length (dough resistance to extensibility) and W (deformation energy), and baking properties: specific loaf volume, P/L ratio, loaf weight were determined. Sensory acceptability was done using 50 semi-trained panellists. The study observed that protein content was highest in 8% but decreased ≥ 12% sorghum, fat was higher in 8% sorghum bread compared to control. Dough height and P/L ratio were highest in 8% sorghum while length was highest in control. At 16% sorghum, loaf texture, crumb colour, mouth feel and general appearance decreased. Microbial count was highest in wheat bread while shelf life increased with increase in sorghum. In conclusion, 8% sorghum flour can be partially substituted with wheat flour to develop bread with improved nutritional and sensory quality.

Keywords: Physical properties, shelf life, composite bread, nutritional quality, sensory quality.

Appendix VIII: Authorization

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471, 2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/18/10161/21633**

Date: **30th October, 2018**

Lucy Naisianoi Mariera
Egerton University
P.O. Box 536-20115
NJORO.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Development and evaluation of nutritional and sensory quality of Chickpea enriched sorghum-wheat composite bread,"*** I am pleased to inform you that you have been authorized to undertake research in **Nakuru County** for the period ending **30th October, 2019.**

You are advised to report to **the County Commissioner, the County Director of Education and the County Director of Health Services, Nakuru County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

DR. STEPHEN K. KIBIRU, PhD.
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nakuru County.

The County Director of Education
Nakuru County.

National Commission for Science, Technology and Innovation is ISO9001:2008 Certified

Appendix IX Research Permit

THIS IS TO CERTIFY THAT:
MS. LUCY NAISIANOI MARIERA
of EGERTON UNIVERSITY, 536-20115
NJORO,has been permitted to conduct
research in *Nakuru County*

on the topic: *DEVELOPMENT AND EVALUATION OF NUTRITIONAL AND SENSORY QUALITY OF CHICKPEA ENRICHED SORGHUM-WHEAT COMPOSITE BREAD*

for the period ending:
30th October,2019

Permit No : NACOSTI/P/18/10161/21633
Date Of Issue : 30th October,2018
Fee Recieved :Ksh 1000

....
Applicant's Signature

....
Director General
National Commission for Science, Technology & Innovation

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013

The Grant of Research Licenses is guided by the Science, Technology and Innovation (Research Licensing) Regulations, 2014.

CONDITIONS

1. **The License is valid for the proposed research, location and specified period.**
2. **The License and any rights thereunder are non-transferable.**
3. **The Licensee shall inform the County Governor before commencement of the research.**
4. **Excavation, filming and collection of specimens are subject to further necessary clearance from relevant Government Agencies.**
5. **The License does not give authority to transfer research materials.**
6. **NACOSTI may monitor and evaluate the licensed research project.**
7. **The Licensee shall submit one hard copy and upload a soft copy of their final report within one year of completion of the research.**
8. **NACOSTI reserves the right to modify the conditions of the License including cancellation without prior notice.**

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TEL: 020 400 7000, 0713 788787, 0735 404245
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Website: www.nacosti.go.ke

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National Commission for Science, Technology and Innovation

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CONDITIONS: see back page