OPTIMIZATION OF GLUTEN-FREE BREAD PREPARED FROM GREEN BANANA, PUMPKIN SEED AND CASSAVA COMPOSITE FLOURS

BY

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DECLARATION

Declaration by the Candidate

I declare that this thesis is my original work and has not been presented for a degree in any other university or institution of higher learning. No part of this thesis may be reproduced without prior consent of the author and/or University of Eldoret, Kenya

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DEDICATION

This thesis is dedicated to:

My loving dad and mum, Mr. and Mrs. Jacob Songok for their selfless sacrifice towards giving me a strong foundation in life through education. I am very proud of you and appreciate the sacrifices that you made.

My dear husband Dr Gregory Kerich, my lovely daughter Janelle Jepkosgei and son Jesse Kigen, you are my pillars in life, you give me a reason and inspiration to be the best I can. I appreciate your unending and unconditional support.

I love you all.

ABSTRACT

Awareness and increased diagnosis of celiac disease and gluten intolerance in African countries has created the need for developing innovative and improved quality and gluten free breads. The locally available food products such as, green banana, pumpkin seed and cassava flours which are gluten-free and have ideal baking qualities are underutilized in commercial bread production. The main objective of this study was to formulate, develop and determine the physico-chemical and sensory properties of gluten-free bread made from green bananas, pumpkin seed and cassava composite flours. Mixture design experiment was used to formulate seven variations of bread that included 100% each of banana, pumpkin seed and cassava flours, composites with 50**:**50 Banana: Pumpkin seed, Banana: Cassava and Pumpkin seed: cassava, one sample with ⅓ of banana: pumpkin seed: cassava, while the eighth 100% wheat was the control. To investigate the chemical characteristics, proximate composition including moisture, protein, fat, ash carbohydrate and energy and mineral content including zinc, iron, phosphorous and copper were determined according to standard AOAC International Methods. Physical characteristic (specific volumes) were established using AACC seed displacement method. The sensory characteristics of gluten-free bread were evaluated for hardness, springiness, cohesiveness, chewiness and resilience using a descriptive panel Acceptability was evaluated by 55 consumers using a 9 point hedonic scale for appearance, smell, flavor and texture. Results for the flour were reflected in gluten-free bread blends. Compositing flours with PSF significantly increased ash by 21-50%, lipids by 69-81%, proteins by 50-90% and energy by 46-57% compared to all other breads. Green banana bread had highest (1.51 mg/100 g) phosphorus content.. Pumpkin seed bread had the highest levels of manganese, copper, zinc and iron of 0.15, 0.95, 2.52 and 2.57 mg/100 g respectively. Gluten-free breads were close to wheat bread in specific volume with a difference of 16% in overall centroid green banana pumpkin seed cassava bread and in 24% in binary combination of green banana cassava bread. . Green banana bread proved to be the hardest with 11.07 N compared to wheat bread (control) with 4.31 N. Cassava bread was only 6% and 8% less springy and cohesive respectively than wheat bread. All the gluten-free breads and wheat bread recorded the same in chewiness with a range of 2.53 to 5.52 with green banana bread on higher side. Principal Component Analysis (PCA) explained 86% of the total variation in bread samples, of which 57% separated wheat from gluten-free breads, while 29% separated bread types with pumpkin seed and those without. All the gluten-free breads were liked by consumers with scores ranging from 70-76%.Combination of pumpkin seed cassava bread was the highest ranked gluten-free bread by consumers. Optimization results for combined proximate, physical and consumer acceptability of gluten-free breads scored pumpkin seed bread at 72% with overall desirability was 89%. Pumpkin seed flour produced the most nutrient dense bread with increased levels of ash (minerals), fiber, protein and fat content. Pumpkin seed flour will serve as a vehicle in food fortification for both celiac patients and gluten sensitive individuals. The best physical characteristics are imparted by cassava flour. It is recommended that locally available food products like green banana, pumpkin seed and cassava be promoted for use in production of gluten-free bread and other baked products in Kenya and other developing countries.

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ACRONYMS

AACC	American Association of Cereal Chemist
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists'
CB	Cassava Bread
CD	Celiac Disease
CMC	Carboxy-methylcellulose
CRD	Completely Randomized Design
DATEM	Diacetyl Tartaric Acid ester of Monoglyceride
GBB	Green Banana Bread
GBCB	Green Banana Cassava Bread
GBPSB	Green Banana Pumpkin Seed Bread
GBPSCB	Green Banana Pumpkin Seed Cassava Bread
GFB	Gluten Free Bread
GFD	Gluten Free Diet
HPMC	Hydroxypropyl methylcellulose
HLA	Human Lymphocyte Antigen
IITA	International Institute of Tropical Agriculture
ISO	International Organization Standardization
KIRDI	Kenya Industrial Research and Development Institute
NACOSTI	National Commission for Science, Technology, and Innovation
NICE	National Institute for Health and Care Excellence
PSB	Pumpkin Seed Bread
PSCB	Pumpkin Seed Cassava Bread
RCBD	Randomized Complete Block Design

SSL	Sodium Steoroyllactylate
TPA	Texture Profile Analysis
UNICEF	United Nations Children’s Emergency Fund
USDA	United States Department of Agriculture
WHO	World health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Celiac disease (CD) is a systemic immune-mediated disorder which affects 1% to 6% of the global population (Fesano and Catassi, 2012). The disease primarily damages the small intestinal mucosa in response to the gliadin fraction of wheat gluten (Deora, Deswal, Dwivedi and Mishra, 2015) and related storage proteins (prolamines) from barley, rye and oats (Tsatsaragkou, Yiannopouloss, Kontogiorgi, 2012). A significant consequence of celiac disease is villous atrophy of the small intestines leading to nutrient malabsorption (Fesano and Catassi, 2012), chronic diarrhea, abdominal distension, weight loss and malnutrition (Cenit, Olivares, Codoner, Sanz, 2015).

Previously, CD was considered a disorder affecting people of European ancestry (Cook and Holmes, 1984). However, recent studies have revealed an increase in global prevalence (Fesano and Catassi, 2001) affecting people in India, the Middle East and North Africa among others. For instance, a study of school children in India showed a prevalence of 1 in 310 children (Sood, MidhaSood, Avasthi and Sehgal, 2006). In Africa, children of the Saharawi people from Western Sahara have a 5.6% prevalence rate, the highest worldwide and five times higher than developed countries (Ratschi and Catassi, 2001). Another study also confirmed that CD was the cause of nutrient malabsorption in Sudanese children (Mohammed, Karrar and Safi, 2006).

The increased prevalence of CD in Africa and other developing countries is attributed to change from traditional to western diets with high wheat and barley consumption (Cataldo, 2007). Consumption of wheat and gluten based products such as flat breads, biscuits, cookies, pasta and beer has increased incidences of celiac disease (Cureton, Pamela & Fesano, Alessio, 2009). The only known treatment for CD is lifelong adherence to a gluten-free diet (Arendt, Moroni and Zannini, 2011). Consequently, due to the increasing prevalence, there is a growing demand from consumers, for palatable, nutritious and gluten-free products.

Bread, traditionally made from wheat is one of the most widely consumed staple foods by humanity (Cauvain, 2007). Non-wheat flours used for production of gluten-free bread are selected on the basis of availability, quality attributes of the final product and price (Litwinek, Ziobro, Gambus, and Sikora, 2014). Additionally the type of bread which most resembles traditional products available on the local market is most preferred by consumers. Gluten free flours which have been used by researchers to produce bread include maize (Schober, Messerschmidt, Bean, Park and Arendt, 2005), rice (Kawamura Konishi, Shuda, Koga, Honda, 2013), sorghum (Schober et al, 2005), finger millet (Taylor and Emmambux, 2008), quinoa (Makinen, Zannini & Arendt, 2013) amaranth (Mlakar, Turinek, Jakop, Bavec, Bavec, 2009), buckwheat (Mariotti, Pagani and Lucisano, 2013) and oats (Huttner and Arrendt, 2010)

The quality characteristics of bread are derived from the unique properties of wheat proteins to form gluten whose visco-elastic properties enable dough to maintain desirable volume, texture and retain gas (Zannini, Millerjones, Renzetti, Arendt, 2012). Gluten free breads lack the gluten matrix resulting in poor technological quality with low specific volume, high crumb hardness and high staling rate (Onyango, Mutungi, Unbehend and Lindhauer, 2011). To compensate for the absence of gluten, higher fat, pre-gelatinized starch, stabilizers and varied hydrocolloids have been used as a means of trapping and stabilizing gas bubbles in bread (Couvain, 2007). Xanthan gum is one of the most effective additives in improving dough structure, while obtaining the best bread firmness and specific volume values (Dermirkesen, Behic,Gulum& Serpil, 2010).

Implementation of a gluten-free diet has to take local foods and dietary habits into account to be effective. Studies on gluten-free bread production have used locally and naturally available gluten-free flour ingredients such as rice, maize, sorghum, soy (Sciarini *et al,* 2010), buckwheat (Krupa-Kozak *et al,* 2011) and maize, potato, cassava, or rice starches (Onyango *et al,* 2011). There is limited documented evidence of gluten-free bread made from composites of green banana, pumpkin seed and cassava. Therefore the aim of the current research was to optimize the production of gluten free bread using these crops.

1.2 Statement of the Problem

Among the African population, there is increased prevalence of CD in North African countries including Morocco, Algeria, Tunisia, Libya Egypt and Sudan (Barada, Bitter, Mokadem, Hashash and Green, 2010). This has been attributed to high wheat and barley consumption as major food staples. The only treatment is strict adherence to a gluten free diet which can heal and reverse the intestinal damage (Green and Celler, 2007). In Kenya, shift towards the Western diet and a growing tourism industry have resulted in increased consumption of wheat-based products (Navnet*et al*., 2014) which is likely to increase the prevalence of CD in the country.

Gluten-free bread is very expensive to produce as lack of gluten affects dough rheology and overall quality requiring more advanced technologies and complex formulations, compared to other traditional breads, which are easy to handle (Sing and Whelan, 2011). Studies show limited availability in the local shops and supermarkets.

Challenges encountered by consumers include poor quality and high cost of gluten free cereals, limited availability and short shelf life of gluten-free breads (Stevens and Rashid, 2008). To reduce the cost, producers should use locally available food products including maize (Lambert *et al*., 2009) such as bananas, pumpkin-seed and cassava, which are widely accepted, frequently consumed and affordable. These foods could play a crucial role in food security, nutrition and income generation for the rural poor (Magbagbeola, Adetoso and Owolabi, 2010) in Kenya. There is no gluten-free bread on the Kenyan market made from these food products. This study was conducted to utilize green banana, cassava and pumpkin seed to produce gluten-free bread.

1.3 Objectives of the Study

Broad Objectives

To formulate, develop, determine and optimize physico-chemical and sensory properties of gluten-free bread made from green banana, pumpkin seed and cassava composite flours.

Specific Objectives

1. To formulate and develop gluten- free bread from green banana, pumpkin seed and cassava composite flour at varying ratios.
2. To determine the proximate composition of green banana, pumpkin seed and cassava composite flours and breads
3. To determine the physical properties of green banana, pumpkin seed and cassava bread and their composites.
4. To evaluate the sensory characteristics of gluten free bread made from green banana, pumpkin seed and cassava composite flours.

5. To optimize gluten free bread made from green banana, pumpkin seed and cassava composite flours

1.4 Hypotheses

H_1: There is a significant difference between physico-chemical properties of bread made from green banana, pumpkin seed and cassava composite flours and wheat bread.

H_1: There is significant difference between the sensory characteristics of bread made from green banana, pumpkin seed and cassava composite flours and wheat bread.

$H_{1:}$ There is significant difference between nutrient composition and textural characteristics of optimized gluten-free breads and their composites.

1.5 Justification of the Study

Bread is an important breakfast item mainly produced using wheat flour. Gluten-free bread has been a challenge to produce because substitute gluten-free flours can be more expensive as their demand in the market is lower compared to other flours. Enrichment of flour through compositing is one possible alternative to improving quality and increasing availability of gluten-free bread on the market. There is therefore a need to create this demand in food industries by using locally available, affordable and healthier gluten free food products such as green banana, pumpkin seed and cassava. This study utilized these food products to produce alternative bread that people suffering from celiac disease can consume. This is beneficial to them in meeting their bread intake. The study will also be beneficial to bread manufacturers as the market for utilization of green banana, pumpkin seed and cassava flours to produce gluten free bread has not been fully exploited in Kenya. The general population who consume bread will also benefit from this study as they can prepare the bread from alternative green banana flour, pumpkin seed flour and cassava flour. The bread will assist the Ministry of Health in managing people with celiac disease as a strategy in health intervention.

CHAPTER TWO

LITERATURE REVIEW

This review highlights the challenge of celiac disease by looking at its origin, prevalence and its symptoms. It also gives an insight into the strategies adopted in dealing with the condition through use of various food additives such as hydrocolloids, development of gluten-free bread using locally available food products such as cassava, green banana and pumpkin seeds and their composites. It concludes by giving the importance of these food products as functional ingredients in food industry and how they can be utilized to produce nutritious gluten-free bread that is affordable.

2.1 Definition and Origin of Celiac Disease

Celiac disease (CD) is a lifelong autoimmune disease, characterized by an inappropriate immune response to dietary protein fractions glutenin, gliadin, hordein and secalin found in wheat, rye and barley, respectively (Niewinski, 2008). Celiac disease, also known as gluten sensitivity enteropathy or celiac sprue, originated from the word *koiliakos (Greek)* which means suffering in the bowel (Cataldo and Montalto, 2007). Large proportions of these protein fractions resist digestion by proteases once inside the intestinal tract, remaining intact in the lumen (Boswel, 2010). The condition exclusively affects genetically pre-disposed individuals who carry the human lymphocyte antigen (HLA) either DQ2 or DQ8 (Trynka, Wijmenga, and van Heel, 2010). Celiac disease is activated by consumption of prolamines (glutenin gliandin and secalin) which are storage proteins present in cereal as they are soluble in 70-90 % alcohol (Darewiez, Dziuba and Minkiewiez, 2008; Shan, Molberg, Parrot, Harusch, Filiz and Gray, 2002).

2.2 The Prevalence of Celiac Disease

According to the National Institute for Health and Care Excellence (NICE, 2009a), the prevalence of CD in the population has been underestimated in the past because many CD patients are either asymptomatic or they experience mild symptoms which are never investigated among Americans and Europeans. Previously, celiac disease was thought to have the highest prevalence in people of European origin (Cataldo and Montalto, 2007) mainly Northern Europe and Australia. In a review, Kang *et al.* (2013) reported that CD was rare in sub-Saharan Africa and the orient. This author further reported that of the 266 studies only six biopsy-proven cases in ethnic Japanese and eighteen cases among ethnic Chinese were reported. In the United Kingdom (UK), the prevalence of CD is estimated to be around 1% (Aggarwal, Lebwohl and Green, 2012). Fifteen studies using serological tests on adult populations confirmed prevalence of 0.07% to 1.9% (NICE, 2009a), of those conducted in the UK and showed a prevalence of 0.8 – 1.9%.

New evidence now shows that CD is common across many ethnic groups with prevalence ranging from 2-5% (Schuppan, Zimmer, 2013)). Populations with high exposure to dietary gluten, such as the Italian population, tend to have a higher prevalence (Volta *et al*, 2001). The highest prevalence of CD (5.6%) was identified in a North African tribal population who consume a wheat-based diet (Barada *et al*, 2010). Over-exposure to gluten could account for the high prevalence of CD identified in this tribe. Additionally increased rates of CD have resulted increased CD diagnosis in many countries, (Violato, Gray, Papanicolas & Oullet, 2012). These have been attributed to improvements in the accuracy of diagnostic testing and better awareness of the wide-ranging symptoms (Loftus and Murray, 2003). Despite the improvement in diagnostic testing, CD remains undiagnosed or misdiagnosed in the majority of cases (Lohi *et al*, 2007; NICE, 2009a). This may be due to the proteins found in dietary cereal grains such as wheat, rye and barley which involved gluten activating CD symptoms and the fact that approximately 50% of people with CD are asymptomatic (Tursi, Elisei, Giorgetti, Brandimarte and Aiello, 2009).

In the past, it was thought that CD was curable (Hopman *et al*, 2008). However, currently it is recognized as a life-long condition that needs to be treated through permanent elimination of gluten in the diet. This is because gluten has been isolated as the compound responsible for the development of CD (Rubio-Tapia, and Murray, 2010).

2.3 Symptoms of Celiac Disease

Some of the symptoms of CD include indigestion, abdominal pain, bloating and gas production, bulky fatty bowel motions that are sometimes pale and offensive smelling, failure to thrive, vomiting, muscle wasting and hypoproteinaemia including possible ascites (Maureen *et al,* 2014). In their review, Catassi, Fornaroli and Fesano (2002) reported that other common symptoms of CD include intestinal malabsorption, such as chronic diarrhea, weight loss, abdominal distension and anemia. Among these conditions others such as muscle cramps due to low calcium levels, and blistering, itchy or painful rashes particularly above the knees, elbows, buttocks and back (dermatitis herpetiformis) may be realized (Di Martino Ortiz B *et al*, 2018). In addition, nervous system damage can result in advanced untreated conditions resulting in symptoms such as numbness and ‘pins and needles’ in limbs, and changed behavior (Shannahan and Leffler, 2017).The disease may also present itself in a milder form with non-specific symptoms such as fatigue, vague abdominal pain in adults (Shannahan and Leffler, 2017). Untreated CD can lead to long-term risks such as osteoporosis, anemia and gastrointestinal malignancy (Hamer, 2005).

2.4 Solution to Celiac Disease

The only treatment for CD is strict adherence to a gluten-free (GF) diet throughout the patient's lifetime, which results in clinical and mucosal recovery. Currently gluten free products are mainly starch based containing rice and corn flour rich in carbohydrates with reduced amounts of protein, dietary fiber, vitamins and minerals which are nutritionally required by celiac patients (Xingli *et al*., 2017).According to Matos and Rosell., 2011 there is a growing concern on nutritional adequacy of the gluten free dietary patterns for celiac and non celiac since they always are involved in excessive consumption of fats with reduced intake of complex carbohydrates, dietary fiber, vitamins and minerals. However one of the current strategies is increasing nutritional value of gluten free breads by incorporating food products such as green banana, cassava and pumpkin seed which have additional functional properties. These raw food products are often presented as new crops but they have been in use by local populations in traditional ways for many years. This therefore calls for new innovations on composite enrichment of gluten free (GF) baked products with dietary fibre in order to ensure that CD patient consumes the recommended 25-38 g of fibers per day (Grehn *et al*., 2001).According to Tsatsaragkou *et al.,* 2016 dietary fibers have been widely used due their nutritional and functional benefits in gluten free bread formulations since they play a crucial role in water binding capacity, gel formation as well as textural thickening effect. Insoluble fibers have been used in testing their effect on texture and sensory acceptability of gluten-free bread (GFB) improving particle size (Martinez *et al*., 2014). Fiber addition has been confirmed to greatly influence dough cohesion and starch pasting properties i.e pea fiber and oat bran (Aprodu and Banu., 2015).

Resistant starches in green banana and soluble fiber enrichment have proved to decrease glycemic responses of GFB which finally give desirable qualities in individuals with celiac disease and insulin dependent diabetes (Gunness and Gidley., 2010). Resistant starch plays several functional roles. It reduces energy of food and enhances digestive functions thereby in improving bread quality (Tsatsaragkou *et al*., 2014). To bring about good gluten free bread qualities additives such as gums, protein and enzymes have been experimented in gluten free bread making and this have resulted in improved elasticity and porosity of bread (Wang *et al*., 2017)

2.5 Gluten Formation, Structure and Matrix

Gluten constitutes 80-85% of the total protein in wheat (Dermirkesen*et al.*, 2013).These proteins, which are found in the endosperm of wheat grain form a continuous three dimensional matrix in starch granules when mixed with water (Van Der Borght *et al.*, 2005). The cohesive viscoelastic dough formed retains carbon dioxide gas produced during fermentation and oven rise making baked products achieve high volume and soft texture (Claire, 2014; Dermirkesen *et al.*, 2013). The main gluten proteins, gliadins and glutenins are long chains of amino acids. Gluten is only formed after the hydration of gliadin and glutenin in wheat flour and kneading and pumping of the dough (Figure 2.1). The proteins change their shape, move closer to each other and form bonds (Gambus*et al*, 2009). The gliadin chains fold onto themselves to form weak bonds with each other and whereas the glutenin bonds forming extensive tight networks.

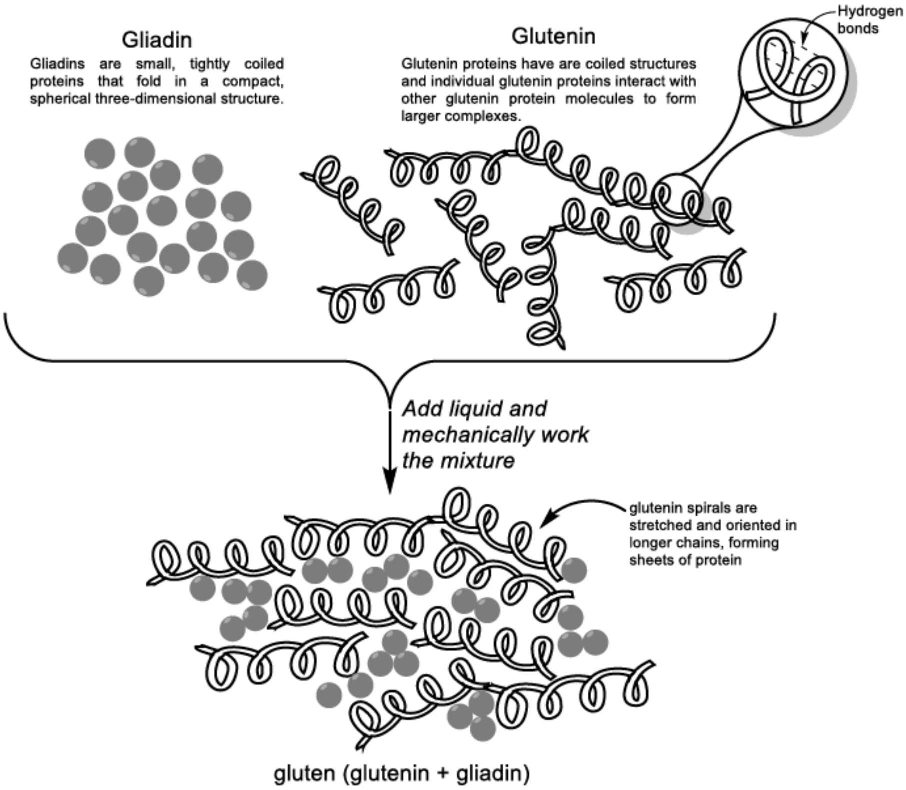


Figure 2.1: Gluten Formation following hydration based on (Wieser, 2007)

2.6 Role of Gluten in Baking

The absence of gluten can be a problem to bakers and cereal researchers since it can cause the texture of bread to be crumbly an unpleasant in, color after baking (Houben, Hochstotter, and Becker, 2012; Matos *et al*., 2013). A study by Houben *et al.,* (2012) on possibilities to improve the quality of gluten free bread, established that lack of gluten leads to a less cohesive dough that lacks elasticity, is difficult to handle and gives bread with a crumbly texture, poor color with a series of defects including short shelf life, rough and dry mouth feel and non-satisfying after taste) (Houben *et al*., 2012). Gluten is also responsible for holding carbon dioxide during proofing and without gluten the bread has low volume (Matos *et al,* 2012). Matos *et al.,* (2012) further established that the gluten free bread available in the market has poor technological quality and low volume, Therefore studies carried out should look at ways of improving the quality of gluten free products such as baked bread

2.7 Development of gluten free bread

Traditionally, most gluten-free products were produced by using native and modified starches that were blended with different hydrocolloids (Matos *et al.*, 2012). Currently the market for gluten free bread has grown and several other ingredients and additives are currently being used with the aim of initiating viscoelastic properties of gluten and produce bread with sensory properties similar to wheat bread. Starches and hydrocolloids are the most widely used in bakery product formulations since they have unique qualities in texture and appearance (Anton and Artifield, 2008: Dermirkesen et *al.,* 2010). Rice starches have been used widely in gluten free bread production to assist in structure formation and in mimicking gluten network resulting in improved quality of the final product (Mariotti *et al*., 2009). For hydrocolloids or gums to function well several factors play a role including chemical nature of the gum, temperature, PH range, electrolyte concentration, particle size, thermal treatment, chelating agents and storage ability (Dermirkesen *et al*., 2013).

Gums such as xanthan, guar, locust bean and tragacanth have been used as binding agents in gluten free corn starch bread production and the bread has shown increased volume but with decreased crumb structure (MollakhaliMeybodi *et al*., 2015) .Hager *et al.* (2012) showed that hydroxypropyl methylcellulose (HPMC) had the greatest potential in improving bread specific volume in maize-teff bread. Eduardo *et al*., (2014) also demonstrated that use of carboxymethyl cellulose in composite flours of cassava-maize-wheat bread increased loaf volume, crust color and crumb texture. Xanthan gum improves batter consistency and quality of gluten free bread thus leading to high volume, increased average cell size, lower crumb firmness and improvement in overall gluten free bread appearance (Shittu *et al*., 2009).

Emulsifiers normally referred to as surfactants or surface acting agents have been used together in gluten free bread (GFBS) production Steven and Baker, 2010). In bread making emulsifiers play a role in final product quality and functional attributes since they stabilize the dough and reduce the rate of retro gradation (Gomez *et al*., 2013).Commonly used commercial emulsifiers in bakery industry include polysorbate 80(PS80), sodium steoroyllactylate (SSL) and diacetyl tartaric acid ester of monoglycerides (DATEM) since they serve as dough strengtheners, crumb softeners and increases the volume of crumb structure of bread (Xiujin *et al*., 2007: Gomez *et al*., 2013).Emulsifiers play a role at the beginning of baking, during fermentation, mixing, mechanical handling, moulding, proofing and during transport(Gomez *et al* 2003). Emulsifiers SSL and DATEM have been used in enhancing specific volume of cassava-maize and wheat composites (Eduardo *et al* 2014).Pectin has been found to help in dough gas retention , volume increase, improved crumb and retardation of bread staling process (Kenljz *et al*., 2013). Eggs have been utilized over time as natural emulsifiers since they act as binders in baked products thus aiding in emulsification properties.

2.8 Gluten-free bread making

Production of gluten free bread differs greatly from the normal wheat bread recipe. This is attributed to the fact that gluten free bread follows a liquid batter recipe resulting giving it weak, unstable and porous matrix leading to shorter mixing and proofing bread time (Arendt *et al.,* 2008.Several studies indicate that gluten free products exist in the market but scientific literature on production of gluten free bread in developing countries is still limited due to commercial secrecy (Hroyuki *et al.,* 2017).Therefore gluten free bread alternatives should be appetizing to celiac sufferers who cannot consume gluten and people who wish to leave gluten in their diets.

Adherence to gluten-free diet by patients with celiac disease has been a hurdle since available bakery products in the market have limitations associated with high cost, low quality and limitations on availability of the food products (Engleson and Atwell, 2008). A number of consumers together with bakers have been faced with several challenges when wheat is substituted with other gluten free flours. For example a study by Arendt, (2009) indicated that using gluten free ingredients leads to product having poor quality due to lack of gluten network. Different types of flours present in the market have been used and of special interest are those rich in starch such as rice and corn flour, which have been considered to be the best (Farage,VillasBoas, Gandolf, Pratesi, Zondanadi, 2014). Historically, maize was used in gluten-free bread production since it was locally available, affordable and cheap, followed by rice flours which were combined with corn, potato, or cassava starch with proteins and hydrocolloids acting as binding agents (Matos *et al*, 2013).Currently alternative there is increased use of local food products that are composited with cereal flours to improve the nutrition quality (Milde *et al*, 2012). Fruit flours have been of interest to most bakers and one of them is the use of chestnut flour which is gluten free. A study by Chenlo *et al*, 2007 evaluated the rheological behavior of chestnut flour and found out that the GF bread product was nutritionally rich, but the sensory characteristics were poor due to low volume of the product resulting in a dark colour and the final product generally had undesirable hardness. Current findings on fermented chestnut flour sourdoughs have been seen to have improved specific volume and crumb hardness in bread(Aguilar *et al* 2016).On nutritional enrichment fruit ingredients such as unripe bananas (Sarawong *et al* 2014) and orange pomace (O'shea *et al*., 2015) have also been tasted in gluten free bread production. A study by Batista et al (2018) showed that replacement of wheat with 50% pumpkin seed flour and carob flours in cupcakes did not alter the quality.

2.8.1 Cassava and its Nutritional Importance

Cassava (*Manihot esculenta*Crantz) a perennial drought resistant crop that grows well in poor tropical soils is also known as Yucca, Manioc and Mandioc in various parts of South America (Hauze *et al* 2016) and belongs to Euphorbiaceae family (Hauze *et al* 2016). Cassava is a staple food in most parts of tropical Africa.). However peak starch yield differs between cassava varieties as observed by Apea –Bah, Oduro, Ellis, and Safo-Kantanka, 2011. The composition of cassava root changes slightly with increasing age and maturity as it becomes more fibrous and the starch content declines. Cassava is a poor source of protein as it contains only 1-3% protein on dry matter basis (Montagnac, *et al*., , 2009) and has low essential amino acids such as methionine, lysine, tryptophan, phenylalanine and tyrosine (Falade and Akingbala, 2010). Cassava roots can be processed into granulated products such as gari, meat cakes, chips, relish, cookies and strips (Cardoso et al., 2005; Onabulu, 2010; IITA, 2006). Cassava flour is also one of the major gluten free food products on the world market today (Ogunjobi and Ogunwolu, 2010). This flour has other applications in foods, feeds and chemical industries (Balagopalan, 2002). People suffering from CD, can use products made from cassava flour (Sciarini, *et al*., 2008). Due to this fact researchers have used cassava root flour and starch composites to produce other bakery products (Nweke *et al*, 2002) such as pastries (Oladunmoye*et al*, 2004) as well as confectionaries (Fiiro, 2006) and pastes (Nwabueze and Anoruoh, 2009).

Though cassava flour has been of interest, it is limiting in almost all essential nutrients and its continuous use can result in malnutrition due to micronutrient deficiencies such as iron, vitamin A and Iodine (UNICEF, 2004). Therefore, to solve this problem, a food based approach may be to composite cassava flours with nutrient dense flours. Locally available food products such as green banana and pumpkin seeds which normally are thrown as waste have been neglected and underutilized though they are nutrient dense. Therefore inclusion of green banana flour and waste pumpkin seed flour in formulation of food products is an alternative to providing nutritional enrichment and reducing costs in waste management.

2.8.2 Green Banana and its Nutritional Importance

Green banana (*Musa*spp) is the world fruit crop that is largely grown in tropical and subtropical regions (Daniells, 2003). It is rich in carbohydrates, fiber, vitamins A, B, C as well as calcium and iron (Kolawole, Falade, Samson, Oyenyinka, 2014; Daniells, 2003). Some hybrid cultivars have high carotenoid content and can be used in supplementing vitamins in diets of populations dependent on banana (Englberger, Darnton-Hill, Coyne, Fitzgerald and Marks, 2003). Green banana flour is of great interest to researchers due to its functional or medicinal components that include resistant starch and dietary fiber which have been reported to play a key role in human health (Bello-Perez *et al* 2011; Rabbani *et al*, 2010). Green banana flour also is rich in polyphenols and antioxidants (Ovando-Martinez *et al*, 2009). These antioxidants are catechin, epicatechin and gallocatechin (Krishnam and Prabhasankar, 2010). Hence green banana provides resistance to chronic diseases such as cardiovascular dysfunction and muscular degeneration at old age and muscle cramp for athletes (Mohapatra *et. al*, 2010). Additionally, immunity defending proteins (lectins) in green banana help provide a defense mechanism and boost immune responses. Dried green banana pulp powder is anti-ulcerogenic against aspirin induced ulceration and, therefore, effective in prophylactic treatment and healing ulcers (Nurul, 2013). Bananas are also useful for the treatment of infant diarrhea, celiac diseases and colitis

(Kang *et. al*, 2013). Green bananas are helpful in culinary production and consumption especially snacks and pre-cooked products. A study by Zondanadi (2012) used green banana flour in production of gluten-free pasta where sensory tests showed 84.5% acceptance by celiac patients against 61.2% acceptance for non- celiac individuals. Green banana flour therefore has shown great potential in improvement of nutritional quality of products.

2.8.3 Pumpkin Seed and its Nutritional Importance

Pumpkins the *Cucubitaceae* family are herbaceous annual crops which contain edible fruits and include pumpkin, squash, cucumber, musk melon and watermelon (Leffingwell *et al*., 2015). The *Cucubitaceae* family, in addition to beneficial pulp contains numerous seeds which are considered by-products (Batista *et al.,* 2018). The food industry is utilizing these plant parts that are thrown as wastes such as peels and seeds in production of products that are rich in fiber (Ambroio *et al* 2006; Tavares *et al*, 2016). Pumpkin seeds are boiled, roasted or baked into snacks (Dietmar., 2005). These seeds have high oil content 47% (Shaban and Sahu, 2017). According to (Shaban and Sahu, 2017) the oils extracted from pumpkin seeds are essential for wellbeing and health among individuals. Pumpkin has received considerable attention in the past few years because of the nutritional and health protective values of its seeds. The seed is an excellent source of protein and has some pharmaceutical activities such as anti-diabetic, antifungal, antibacterial, anti-inflammation activities and antioxidant effects (Nkosi and Apaku, 2006). Pumpkin seeds provide protection against internal worms and are recommended for diarrhea in addition to being good sources of protein and minerals such as iron, copper and phosphorous (Amara *et al* 2008). These seeds are rich in medicinal and nutritive components and are used for therapeutic purposes worldwide (Revathy and Sabitha, 2013).One of the ways in which pumpkin seeds can be utilized is through dehulling to produce flour. According to Stevenson (2007) pumpkin seeds offer a nutritious, sweet, somewhat soft and chewy snack or food additive. A study carried out by Gorgonio,Pumar and Mothe (2011) in Brazil on macroscopic and physiochemical characterization of sugarless and gluten free cake enriched with fibers made from pumpkin seed flour and cornstarch revealed that the cake displayed satisfactory macroscopic and chemical characteristics, rich in soluble fiber and less calories compared with standard cake. These seeds have been found to have the highest levels of antioxidants than any other nut seed or food (Amara et al 2008). They are also rich in vitamins and minerals that the body needs (Dhiman, 2009) and also have been proved and confirmed to supply iron, protein and unsaturated oils (Elinge *et al*., 2012). Pumpkin seed flour fortified complementary food mix is also economical and nutrient dense source, with highly acceptable sensory qualities and rich nutritive value (Dhiman, 2009). A study by Fu *et al* (2006) found that pumpkin seeds have been utilized widely as flavor enhancers in gravies and soups and can be used in cooking and baking and as a nutrient supplement and functional agent.

Green banana, pumpkin seed and cassava composite flour blends can be a viable option in development of gluten-free products for the local market. Thus there is need to investigate the potential ingredients together with additives and other technological aids such as xanthan gum in developing high quality gluten free product at an affordable price (Blanco *et al,* 2011).

Table 2.1 Food composition table of green banana, pumpkin seed and cassava flours per 100 g dwb

		Green banana flour	Pumpkin seed flour	Cassava flour
Nutrient	**Unit**			
Proximate				
Energy	Kcal	92	429	89
Protein	G	2.65	17.86	1.43
Total lipid	G	0	17.86	0
Carbohydrates	G	39.82	53.57	27.88
Total fiber	G	0.9	35.7	4.1
Minerals				
Calcium(ca)	Mg	0	0	18
Iron(Fe)	Mg	0	3.86	1.29
Sodium (Na)	Mg	0	679	0

USDA Food products database 2016

2.9 Xanthan gum and its technological importance

Xanthan gum was first used in production of gluten free starch based breads in 1974 and has been in use since then (Anton and Artfield, 2008). It is a polysaccharide derived from an organism *Xanthomonas campestris*, industrially from carbon sources through microbial fermentation (Palaniraj and jayaraman, 2011). When mixed with water it forms a gel that mimic the structure of gluten in baked food products (McFadden *et al*, 2011). It is used to improve the visco-elastic properties of gluten free dough and batters more than carboxy methylcellulose (CMC), pectin, agarose, and B- glucan (Lazaridou, *et al*., 2007). It is a good emulsifier since it has the ability to blend disparate ingredients such as water and oil into a cohesive blend (McFadden *et al.*, 2011). Gambus *et al. (*2007) compared addition of xanthan gum and guar gum in gluten free breads and found that dough mixed for 10 minutes using xanthan gum had higher loaf volume. A higher amount of xanthan gum maintains a softer texture after 72 hours. These findings support the idea that longer mixing periods (10 minutes) with addition of xanthan gum in gluten free breads could improve loaf volume.

Xantham gum contributes several positive qualities to baked products that include smoothness, air incorporation and retention and recipe tolerance to batters (Rashidat *et al.*, 2009). It also improves the volume and texture, and reduces the calorie content of GF breads (Hager & Arendt., 2013). An image analysis by Dermirkesen *et al.(* 2009) using electron scanning microscope showed high pore area fraction values in bread of 46% rice flour replacing chestnut flour using xanthan guar gum blend-DATEM mixture and baked in infrared microwave combination oven. Rice bread with no additive and no chestnut flour had the lowest pore fraction. Consequently, due to formation of high-viscosity xanthan gum is very common in commercial gluten free products. This behavior of xanthan gum is important in bakery products during dough preparation, i.e., pumping, kneading and rolling and thus resulting to improved quality of the final product (Lorenzo *et al.*, 2008).Quality of a product and its related characteristics such as flavor and texture affects food purchasing and consumer decision in the market (Farnakalidis, 1999). Consumers emphasize sensory experiences relating to appearance, texture, flavor, aroma and taste which can motivate them when eating food (consumer acceptability (Westenhoefer and Pudel, 1993)**.**

2.10 Descriptive Sensory Evaluation

Descriptive sensory evaluation is a scientific discipline used in food industry that provides a complete measure, analysis and interpretation of reactions in characterizing food products with materials that can be perceived through senses of sight, smell, taste, touch and hearing (Lawless and Heymann, 2010).These attributes of food include appearance, odor, taste, texture, flavor and sound (Gramatina, *et al*., 2012). In the food industry, companies usually use sensory tests which include descriptive analysis and consumer affective tests to analyze the effects of ingredients on processing and change of products during storage (Stone and Sidel, 1993). Human subjects are used as tools to do descriptive tests where 6-15 panelists who have undergone a screening and selection process are trained (Meilgaard *et al*, 1999).

Sensory profile attributes include odour, appearance, texture, sound and taste based on the five senses (ISO, 11036). Sensations are based on attributes which are coded according to their intensities on a sensory scale (ISO, 11037) referred to as hedonic scale. Sensory panelists are responsible for generating descriptors for each attribute (ISO, 5496).The work of assessors is to understand the scale of evaluation during training and actual sensory testing (ISO 5496; ISO 4121). Assessors also come up with references and definitions used in sensory evaluation (ISO, 4121). The primary goal of descriptive sensory evaluation is to conduct valid and reliable tests and to come up with data that will provide basis for product identification (Meilgaard, *et al*., 1999). A well trained descriptive panel is used to analyze and identify quality attributes as well as use preference tests on what might influence consumers' decision in a given product (Dzung, *et al*., 2004). Descriptive sensory evaluation has been used in bread samples manufactured in Nordic countries and these have been reported in different publications (Kihlberg *et al*, 2004, 2005, 2006; Heinio2003; Pohjanheimo *et al*, 2006, 2010). For example in a study by Baba, Mangla, Daniel, Danrangi, 2015 on sensory evaluation toasted bread fortified with banana flour the sensory results showed that there was no significant difference ($p>0.005$) among all the toasted samples in terms of taste, aroma, appearance and texture however the toasted bread with 30% banana flour recorded the highest mean value. In another study by Chung and Noor (2008) on influences of partial substitution of wheat flour with banana flour on physico-chemical and sensory characteristics of doughnuts revealed that doughnuts with 20% banana flour was the most acceptable.

According to a study by Laura,*et al*., (2013) optimization of composite flour biscuits by mixed response surface methodology the sensory results showed that the overall centroid with 33.33%cocoyam flour,33.33%sorghum flour, 33.33%pigeon pea flour received higher rating in all the sensory attributes. Among the binary combinations 50% cocoyam flour and 50%sorghum flour was the most acceptable. Among the pure blends the biscuits with 100% pigeon pea flour had the least scores of ≤6.5 for texture, taste, crispness and general acceptability. The panelist termed the biscuits with highest pigeon pea flour as having a bitter aftertaste. The binary combination of 50:50 cocoyum sorghum flour biscuit was the most acceptable. Another study by Nuno *et al., (*2011) on bread with and without gluten revealed that gluten-free bread was the most acceptable with score between 6.1 to 7.1. According to Udeme,*et al.,(* 2014) on microbiological, nutritional and sensory quality of bread produced from wheat and potato flour blends showed that the color of bread baked from wheat-Irish potato flour blend(95:5%) was most preferred to 100% wheat flour while wheat - sweet potato (90:10%) and wheat-irish potato flour blend (90:10%) aroma was the most liked.

2.11 Texture Profile Analysis of Bread

Texture profile analysis is an instrumental test developed to provide objective measurements of texture parameters (Scheuer *et al*., 2016). In sensory analysis in the mouth, characteristics attributed to texture include mechanical attributes (applied force), geometrical attributes (that relates to the shape, size and particle orientation inside the food) and other attributes relating to perception of moisture or fat content (Szczesniak, 2002)

Parameters observed in the texture profile analysis include hardness, adhesiveness and cohesiveness, springiness and resilience. These are widely used and compare both sensory attributes and rheological properties of various foods (Scheuer *et al*., 2016).

2.12 Summary and Knowledge in Gaps

Based on available information it is not only the West but also developing countries Kenya included that are affected by CD. The gluten free diet restrictions bring a lot of changes into the patient's life since gluten free food substitutes are more expensive and difficult to find. Thus global market needs to utilize locally available food products to fill this gap. There is need to develop gluten free food products even for those who do not suffer from any gluten digestion problems, and having the knowledge that consuming gluten free food products can have additional nutritional benefits including relief from symptoms of gluten sensitivities and boosting levels of protein, fiber, vitamins and minerals found in gluten-free food products than those found in wheat flour. Therefore this study seeks to fill gluten-free bread market and understanding on conditions associated to celiac disease prevalence. Kenya is a major international tourist destination, and therefore the tourists who visit should find these foods in the Kenyan market Banana, pumpkin seed and cassava in Africa are under-utilized in the making of bakery products because they are regarded as foods of low commercial value with little industrial use and termed as 'poor man's foods' yet they are rich in nutrients contributing to good health. Therefore, the goal of this study was to produce gluten-free bread from locally available underutilized food products for the management of celiac disease. Hence, green bananas, pumpkin seed and cassava were composited to improve the nutrient content of bread and to increase their consumption among the population.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Bread ingredients

Three food products cassava (*Manihote sculenta*), green banana (*Musa acuminata*) and pumpkin (*Cucurbita pepo L*) seed were purchased from the market in Eldoret, Kenya. Additional ingredients were instant dry yeast (Saf-instant®-lesaffre), Prestige margarine (Bidco Africa Ltd, Thika, Kenya), skimmed milk powder "Miksi®", Promasidor Ltd, Nairobi, Kenya), baking powder "chapa mandazi®" (Kapa oil Refineries Ltd, Nairobi, Kenya), Xanthan gum -Pradip enterprises (EA) Ltd PEL®) and eggs were available in the local market .

3.2 Location of the study

Chemical analyses of moisture, crude protein, crude oil, crude ash and mineral content were conducted in the Chemistry laboratory of the University of Eldoret. Development of the product and sensory evaluation were conducted in the food preparation laboratory in the same University. Physical analyses of specific loaf volume, electronic image scanning and texture profile analysis were carried out at the Kenya Industrial Research and Development Institute (KIRDI) in Nairobi, Kenya.

3.3 Experimental design

The three component simplex centroid design of Scheff (1965) was used to formulate seven variations of flour blends for the gluten free bread preparation using cassava, green banana and pumpkin seed flours.

The physicochemical analyses which included four physical (weight, volume, specific loaf volume and texture) six chemical (moisture content, crude protein, crude fat, ash, energy contents and carbohydrate) and five elementals (potassium, manganese, Copper, zinc and Iron) parameters were all carried out using the Randomized

Complete Block Design (RCBD). Descriptive sensory evaluation also based on RCBD involved the assessment of eight types of bread that included seven variations of gluten free bread and 100% wheat as control evaluated by a panel of twelve individuals. Each treatment (8 samples) was randomly assigned to each unit (12 panelists) within each block (3 sessions) to evaluate all samples in triplicate.

The consumer acceptability was based on a Completely Randomized Design (CRD). Randomized three digit codes were used to blind each bread sample and sample arrangement on trays randomized for the panelist. The evaluation process was also randomized where consumers came to the evaluation room at random to evaluate the samples for acceptability.

3.4 Preparation of Cassava, Banana and Pumpkin seed flours

All the food products, cassava tubers, green bananas and fresh pumpkin were cleaned to remove dirt and soil. Cassava tubers were peeled manually using a knife, washed, chipped to 2 cm thickness and dried in an oven at 50^0C until completely dry within 48 hours using the method described by Nwosu, Owuomanam, Omere and Eke (2014).

Green bananas were processed according to the procedure described by (Aurore *et al*, 2009) with slight modifications. The banana peels were removed using a clean sharp knife, soaked in 0.5% concentrated citric acid solution for 10 minutes, drained, sliced into 0.5 cm thick pieces and placed on a tray before drying in an oven at a temperature of 50^0C for 48 hours.

Processing of pumpkin was conducted using the method described by Revalthy and Sabitha (2013). Fresh pumpkin was cut to pieces. The seeds were removed, washed in clean water, put on a clean tray in the sun to dry, then roasted for five minutes and cooled. The dried cassava chips, green banana and roasted pumpkin seeds were each milled into flour using a commercial electric hammer mill (Powerline^R, BM-35, Kirloskar, India) in Eldoret town fitted with 2.00 mm sieve opening screen. The flours were sieved to remove extraneous materials using 75 um mesh, kept in air tight plastic containers and stored at ambient temperature until required for chemical analysis and bread production.

3.5 Flour formulations

The first three were pure blends consisting of 100% green banana flour, 100% pumpkin seed flour and 100% cassava flour. The next three composite blends in the ratio 50:50 consisted of green bananas: pumpkin seed, green banana: cassava and pumpkin seed: cassava flours. The seventh variation was composited in the ratio 33:33:33 composed of blended green banana; pumpkin seed: cassava flours. An eighth variation of 100% wheat flour was added as the control. The flour formulations are shown in table 3.1.

Table 3.1: Percentage flour blends for gluten free bread preparation

Flour blends	Green Banana(GB)	Pumpkin Seed(PS)	Cassava(C)	Total %
GBF	100	0	0	100%
PSF	0	100	0	100%
CF	0	0	100	100%
GBPSF	50	50	0	100%
GBCF	50	0	50	100%
PSCF	0	50	50	100%
GBPSCF	33.33	33.33	33.33	100%
WF	100			100%

Pure blends: GBB (Green banana bread), PSB (Pumpkin seed bread), CB (Cassava bread) and WB (Wheat bread)
Composites: GBPSB (Green banana pumpkin seed bread), GBCB (Green banana cassava bread) and PSCB (Pumpkin seed cassava bread) GBPSCB (Green banana pumpkin seed cassava bread)

3.5.1 Ingredients for bread preparation

The formulated flour blends were incorporated in the basic procedure for bread preparation. For each of the flour formulations a constant amount of ingredients that included milk powder, brown sugar, xanthan gum, baking powder, egg white, and dry yeast were added in the proportions shown in table 3.2 for bread formulation, based on the method for gluten free bread production described by (Roshid, Wazed, Islam, Mohamud and Khatun, 2016). A constant amount of flour for bread production was added amounting to 200 g (32.52%) for the eight variations based on proportion on Table 3.1.The resulting dough weight for bread amounted to 615 g (100%).

Table 3.2 Formulations of gluten free and wheat bread dough's

Ingredients	Quantity of ingredients(g)	% Proportion of weight
Flour	200g	32.52
Milk powder	30g	4.88
Brown sugar	15g	2.43
Xanthan gum	7g	1.14
Baking powder	3g	0.49
Egg white	100g	16.26
Shortening	30g	4.88
Instant dry yeast	5g	0.81
Mixing water	225g	36.59
Total dough weight	615g	100%

The gluten free bread was prepared using the method described by Mir *et al* (2016) with slight modifications. Dry ingredients of flours, brown sugar, milk powder, xanthan gum, baking powder, and instant dry yeast were weighed, mixed and sieved together into a bowl. The remaining ingredients such as egg white, shortening and water for mixing were added according to the formulation in Table 3.2 and mixed using straight dough method where water was added gradually until the batter was even. Mixing was done using the KMix Kenwood bread mixer at a speed 3 for 2 minutes. The preparation procedure included two minutes of mixing all the ingredients, a spatula was used to collect all the ingredients in the middle of the bowl. A minute of mixing aided in forming a batter which was then poured into non-stick baking tins of 22.5 x 8 x 7cm. The loaf batter for each formulation weighed 550 g. The breads were placed in an electric prover for 45 minutes at 90^0F (32.22^0C) with humidity of 80-90%. The proved breads were then transferred to a preheated electric oven and baked for 30 minutes at 180^0C top and bottom heat.

The baked bread was cooled on racks for 2 hours then put into zip lock polythene bags and stored for 24 hours at ambient temperature before further tests. For chemical analyses the gluten free was sliced into 1cm thick and cut into smaller pieces and dried in an oven at 50^0C for 2 hours then ground into a powder using mortar and pestle and stored at 4^0C in an airtight plastic container until required. Figure 3.1 shows the preparation processes of both wheat and gluten-free breads.

3.6 Physical analyses

The physical analyses were conducted 24 hours after baking. The parameters measured included specific volume and instrumental texture analyses.

3.6.1 Determination of specific loaf volume

Bread was weighed on a digital scale ACS 30 India and weight for each loaf recorded in grams. Specific loaf volume was determined by the seed displacement method (AACC International 2000) Method 10-05-01 with slight modifications. Briefly, millet seeds were poured into a 2000 ml measuring cylinder to the top, then poured out. The bread was weighed, placed in the centre of the same cylinder, all spaces around the bread were then completely filled with millet and the top of the cylinder leveled. The volume of the remaining millet seeds were measured in a different measuring cylinder and that amount gave the volume of bread. Loaf volume was then determined using the formula:

Loaf volume (cm^3) = volume of bread – volume of millet seeds in the measuring cylinder

Specific volume was calculated as:

Specific volume (g/cm^3) $= \frac{\textbf{volume (cm3)}}{\boldsymbol{weight(g)}}$

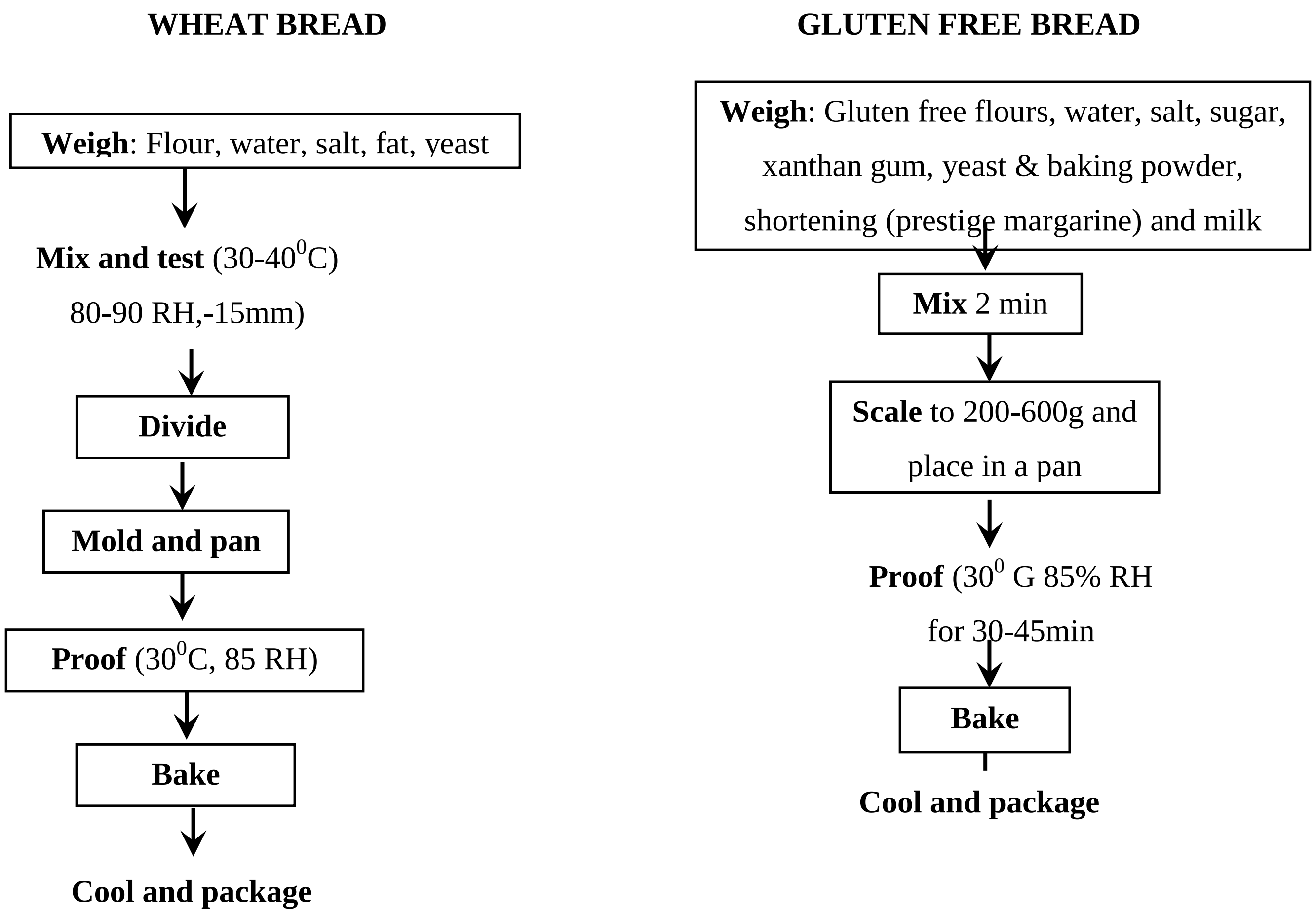


Figure 3.1: Procedures for wheat and gluten free bread baking process adopted from Arendt *et al* 2008

3.6.2 Instrumental texture analyses of gluten free bread

Texture profile Analysis (TPA) of bread made from green banana, pumpkin seed and cassava was conducted using a TA-XT plus texture analyzer (Stable Microsystems Ltd Godalming Surrey, UK) using AACC 1995 modified method 74-09 (TPA). The analyzer was fitted with a 75 mm compression platen cylinder (probe) to measure hardness, cohesiveness, chewiness, springiness and resilience. The bread was cut into 10 mm thick slices using an electric bread slicer (Ayres Jones- Mono equipment®). Rings of 30 mm diameter were punched out from two slices selected from the center of each loaf and stacked on top of each other to give a total of 20 mm thickness at the centre of the texture analyzer equipped with 50 Kg load cell. The height was set at 40 mm and force at 5 g. Pretest speed was 1mm/sec and test speed was 5mm/sec when in contact with bread. The posttest speed was 5mm/sec. The distance it moved inside the materials (penetration) was 10mm/sec.

3.7 Proximate analyses

3.7.1 Moisture Content

Moisture content of flours and gluten free bread were determined using the oven drying procedure (AOAC International 1995) Method 934.01. Samples of 2 g weight were dried in an oven (Memmert, UNB 300, Schutzart, Germany) at 105^0C for 4 hours. The moisture content was obtained by calculating loss in weight as a percentage of the original weight.

3.7.2 Ash content

Ash (mineral) content was determined using (AOAC International, 1995) Method 923.03. A sample of 2 g of the bread was heated at 600^0C for 6 hours in a muffle furnace (Carbolite® 530 2 AU, Bamford, Sheffield, England) to constant weight. The ash content was obtained and weight of the residue expressed as a percentage of the initial weight of the sample.

3.7.3 Crude oil

Crude fat content was determined using the Soxhlet extraction method (AOAC International, 1995) Method 920.29. Two grams of the samples were weighed into a thimble and oil extracted using petroleum ether solvent for 8 hours. The extract was then oven dried at 105^0C for 30 minutes, cooled in desiccators and weighed. Crude oil content was determined by calculating the change in weight of the flask and expressed as a percentage of the initial weight.

3.7.4 Crude Protein

Crude protein was determined by the Micro kjeldahl method (AOAC International, 1995) Method 984.13. Samples of 0.5 g from each of the flours and bread were digested in a heating block (Digester system 20, type 115, Milano, Italy) at $370\text{-}400^0$C for about 60-90 minutes or until the content become clear. To 0.2 mls of the digested sample, 5 ml of a previously prepared NI mixture was added and allowed to stand for about 15 minutes before 5 ml of N2 was added. The resulting mixture was allowed to stand for one hour during which it developed a blue color. The absorbance was read in a spectrophotometer (Spectronic 2ID, Milton Rod, AKiu®, and Germany at 650 mm).The absorbance values were used to read the % N from a graph plotted using standard (Okalebo, Gathua, and Woomer 2002).The % N in each sample was calculated using the formula

$$\%\ \text{Nitrogen} = \frac{(a-b)\times v\times 100}{1000\times w\times al\times 1000}$$

Where a= Concentration of N in the solution b = concentration of N in the blank

v = Total volume at the end of analysis procedure

w = Weight of the dried sample

al = Aliquot of the solution taken

The crude protein content was achieved by multiplying the % Nitrogen by a factor (6.25).

3.7.5 Carbohydrate content

The carbohydrate content was calculated by difference (FAO, 2003) using the following formula:

% Carbohydrate = 100 – (% fat + % moisture + % ash (mineral + % protein).

3.7.6 Energy Content

Energy content was calculated using Atwater conversion factors (FAO, 2003) where mean value was multiplied for crude protein, crude fat and total carbohydrate of 16.736 kJ, 37.656 kJ and 16.736 kJ, respectively. Results were presented as kilojoules per 100 g sample.

3.7.7 Mineral analysis

Manganese (Mn), Copper (Cu), Zinc (Zn) and iron (Fe) were analyzed using the atomic absorption spectrophotometer (AAS) (AOAC International, 1995 Method 985.35). Briefly, Samples were digested, atomized and their concentration recorded against the standards for each mineral. For phosphorus determination, the flame photometer Jen way PFP 7-UK was used. The sample, digested for the other minerals was used against a standard concentration. The solution concentration for each unknown blank was determined then the mean blank value was subtracted from the unknown.

The formula used was

P in Sample (%) $= \frac{c \times v \times f}{w}$

Where c = the corrected concentration of P in the sample

v= Volume of the digest

f= dilution factor

w= weight of the sample

Two milliliters aliquot was used therefore

P in Sample (%) $= \frac{c \times 0.125}{w}$

3.8 Descriptive sensory analysis

3.8.1 Recruitment and screening

Students from the University of Eldoret who normally consume bread and did not suffer from allergies or celiac disease were invited to apply for participation on a descriptive sensory panel through advertisement on notice boards, phone calls and emails. Of the thirty eight (38) applicants who responded, 20 attended an introductory session where they were subjected to three different types of screening tests to determine their sensory acuity. Before the tasting exercise the panelist filled in a consent form that informed them about the nature of the samples they would evaluate. The first test was the basic test to identify, sweet, sour, bitter, salty and umami tastes as described by Lawless and Heymann (2010), presented to panelists as filter papers of different shapes impregnated with the taste solution. The aroma identification test was second and panelists identified pineapple, caramel, passion, banana, lemon, vanilla, chocolate and strawberry aroma. The last was an exercise to describe differences in attributes related to taste, flavor, texture and appearance among different types of bread. The final panel of 12 selected constituted five 5 men and 7 women aged between 19 to 28 years.

3.8.2 Training of the descriptive panel

The 12 panelists were trained in 15 sessions of 2 hours each for three consecutive weeks using the generic descriptive method described by (Einsteine, 1991) to conduct the sensory profiling of eight types of bread. During training the panelist were familiarized with the bread samples and identified differences in attributes that existed among the samples with reference to appearance, texture, flavor and aftertaste. To clarify the sensory attributes of the bread among panelists, food items (Table 3.3) were used as reference samples. Panelist agreement was evaluated through several tests during training. The panelists generated and reached a consensus for 34 descriptors for gluten free breads and the 100% wheat bread control with their definitions, reference standards to anchor the scale ends and the order of descriptors on the ballot (Table 3.3). Figure 3.2 shows panelists in one of training session.

Figure 3.2: Descriptive sensory panel during a training session evaluating the bread samples

Evaluation of gluten free bread

Evaluation of gluten free breads and control was carried out over a period of three days in three sessions of 1 hour each a day following a randomized complete block design. During each session all the eight breads were randomly presented to each panelist. To avoid fatigue panelists first evaluated a set of four breads followed by a 20 minute break before evaluating a second set of 4 breads. Each sample was presented as ¼ bread showing both the crust and crumb in a transparent polyethylene zip lock type bag of 10 cm x 5 cm identified with random three digit codes arranged randomly on a white tray. Panelists assessed the samples seated in individual stations where they could not see each other. Each panelist was provided with a plastic tumbler filled with distilled water and carrots slices for cleansing the palate before and between tasting of samples, a serviette and toothpick. Additionally the panelists received a ballot for assessment, a list of descriptors with definitions, a pencil and a rubber. Reference samples were available throughout the evaluation sessions. Sensory evaluation research room at the University of Eldoret was well ventilated and lit for evaluation to take place at ambient temperature. Using 34 descriptors each of the 8 bread samples were rated for appearance, aroma, flavor, texture and aftertaste on a (0-10) scale. Responses were entered manually on the ballot (Appendix 4)

3.9 Consumer Evaluation

Sample preparation

The gluten free breads and control samples used by the consumer panel were prepared in the same way as those for the descriptive panel (chapter 3 section 3.6)

Recruitment and Screening

Recruitment of consumer panel was through an advertisement on the University of Eldoret notice board, to select a sample of 55 consumers among the staff and student population who were regular consumers of bread. Those who responded were asked to fill a consent form (Appendix 2) informing them about the samples and to ascertain their personal commitment in participating in consumer panel to evaluate the eight samples of bread. At the end of screening session a random number of twenty four males and thirty one females aged between 19 and 50 years were selected.

Table 3.3: Descriptive sensory attributes used by trained panel to evaluate gluten free and wheat breads

Attribute/descriptors	Definition	References	Rating scale
Appearance (crust)			
Surface color intensity	Color intensity of crust ranging from light brown to dark brown	White bread[1] crust(light)=0 Brown bread[2] crust(dark)=10	Not dark=0 Very dark brown=10
Evenness of surface	Degree of evenness on top surface	Bread crust(even)=0 Hard dry mandazi[3] =10	Even=0 Uneven=10
Surface shine	Light reflection on the surface	White bread(not shiny) =0 Tea scones[4](very shiny) =10	Not shiny=0 Very shiny=10
Appearance crumb			
Surface color intensity	Color intensity of crumb ranging from light cream to dark brown	White bread (light)=0 Brown bread(dark)=10	Light =0 Dark=10
Yellow surface color	Intensity of crust surface color associated with egg yellow	Pancake[5] (light yellow)=0 Scones(dark yellow)=10	Light yellow=0 Dark yellow=10
Roughness of top surface	The degree to which roughness could be perceived on the top surface of crumb	White bread(not rough) =0 Whole meal bread[6](very rough) =10	Not rough=0 Very rough=10
Pore size	Size of the holes on the crumb surface	White bread crumb (small) =0 Whole meal bread(big) =10	Small=0 Big=10
Pore regularity	Homogeneity of pores in the crumb	White bread (regular)=0 Hard dry mandazi (irregular) =10	Regular=0 Irregular=10
Compact	Degree of denseness of particles on top surface	White bread(not compact)=0 Hard dry mandazi (very compact) =10	Not compact=0 Very compact=10
Spongy	Extent of air pockets contained in sample	White bread(very spongy)=10 Hard dry mandazi (not spongy)=0	Not spongy=0 Very spongy=10
Fine	Degree of smallness of particles on surface perceived by sight	Brown sugar[7](not fine)=0 Icing sugar[8] (very fine)=10	Not fine=0 Very fine=10

Table continued

Attribute/descriptors	Definition	References	Rating scale
Damp/moist	Perception by sight of surface water on crumb	Hard dry mandazi (not damp) =0 Stiff porridge[9] (very damp) =10	Not damp=0 Very damp=10
Aroma/smell(crumb)			
Stale bread aroma	Intensity of aroma associated with stale bread	Fresh baked bread(no stale bread aroma) =0 Stale bread(intense stale bread) =10	No stale bread aroma=0 Intense stale bread aroma=10
Sour milk aroma	Intensity of aroma associated with sour milk	Fresh milk[12](No sour milk aroma) =0 Sour milk[13] (intense sour milk aroma) =10	No sour milk aroma=0 Intense sour milk aroma=10
Fermented aroma	Intensity of aroma associated with fermented yeast	Pancake(no fermented aroma)=0 Fermented yeast(intense fermented aroma)=10	No fermented aroma=0 Intense fermented aroma=10
Cooked banana aroma	Intensity of aroma associated with cooked banana	White bread(no cooked banana aroma)=0 Boiled banana unsalted(intense cooked banana aroma)=10	No cooked banana aroma=0 Intense cooked banana aroma=10
Cooked cassava aroma	Intensity of aroma associated with cooked cassava	White bread(no cooked cassava aroma)=0 Boiled cassava unsalted(intense cooked cassava aroma)=10	No cooked cassava aroma=0 Intense cooked cassava aroma=10
Flavor (crumb)			
Sweet flavor	Fundamental taste sensation associated with sugars	Spring water without sucrose (no sweet taste)=0 5% sucrose solution in spring water (intense sweet taste)=10	No sweet taste=0 Intense sweet taste=10
Fermented maize meal flavor	Intensity of flavor associated with fermented maize meal	Stiff maize meal porridge=0 Fermented maize meal snack [15]=10	No fermented maize meal flavor=0 Intense fermented maize meal flavor=10

Table continued

Attribute/descriptors	Definition	References	Rating scale
Cooked banana flavor	Intensity of flavor associated with cooked banana	White bread (no cooked banana flavor)=0 Boiled banana unsalted(intense cooked banana flavor) =10	No cooked banana flavor=0 Intense cooked banana flavor=10
Cooked cassava flavor	Intensity of flavor associated with cooked cassava	White bread (no cooked cassava flavor)=0 Boiled cassava unsalted(intense cooked cassava flavor)=10	No cooked cassava flavor=0 Intense cooked cassava flavor=10
Cooked pumpkin flavor	Intensity of flavor associated with cooked pumpkin	White bread (no cooked pumpkin flavor)=0 Boiled pumpkin unsalted(intense cooked pumpkin flavor)=10	No cooked pumpkin flavor=0 Intense cooked pumpkin flavor=10
Bland flavor	Degree of mild sensation of taste no bland taste, intense bland taste	Pancake (no bland flavor)=0 Stiff maize meal(intense bland flavor)=10	No bland flavor=0 Intense bland flavor=10
Texture (crust)			
Crusty texture	Noise made in the first bite of the sample between the molars(auditory assessment)	White ugali[10] (not crusty) =0 Whole meal bread (very crusty) =10	Not crusty=0 Very crusty=10
Chewy texture	Toughness of the sample perceived during mastication	Cassava bread(not chewy) =0 Pumpkin seed bread[18] (very chewy) =10	Not chewy=0 Very chewy=10
Texture (crumb)			
Rough texture	Degree of abrasiveness of products surface perceived by the lips and tongue during mastication	White bread(not rough)=0 Whole meal bread(very rough) =10	Not rough=0 Very rough=10
Soft texture	Amount of force required to first bite through the sample with molars	Hard dry mandazi (not soft)=0 Pancake (very soft)=10	Not soft=0 Very soft=10

Table continued

Attribute/descriptors	Definition	References	Rating scale
Crumbly texture	Ease with which the sample is broken into smaller particles when chewed	Pancake (not crumbly) =0 Rich cake =10	Not crumbly =0 Very crumbly=10
Slimy texture	Degree to which a sample slides over the tongue during mastication	Hard dry mandazi (not slimy) 0 Jute mallow(very slimy)	Not slimy=0 Very slimy=10
Plastic texture	Degree to which the sample retains shape and does not return	Hard dry mandazi (not plastic)=0 Stiff porridge(very plastic)=10	Not plastic =0 Very plastic=10
Damp texture	Perception of surface water on crumb felt by touching with the finger	Hard dry mandazi (not damp) =0 Stiff porridge (very damp) =10	Not damp=0 Very damp=10
After taste (crumb)			
Fermented aftertaste	Intensity of flavor associated with fermented yeast	Pancake(no fermented taste)=0 Fermented yeast (intense fermented taste)=10	No fermented taste=0 Intense fermented taste=10
Gritty(grainy) residue in mouth	Degree to which mouth contains small particles after all of the sample has been swallowed	Pancake(not gritty)=0 Roasted fermented maize meal flour (very gritty)=10	Not gritty=0 Very gritty=10
Fibrous after taste	Degree to which the mouth contains fiber like particles after the sample has been swallowed	Pancake(not fibrous)=0 Pumpkin seed bread(very fibrous) =10	Not fibrous=0 Very fibrous=10

White bread[1], brown bread[2,] tea scones[4], whole meal bread[6] and rich cake[14] brands from super loaf mini baker's ltd, Kenya). Hard dry mandazi[3] (kaangumu) prepared from flour, salt, baking powder, butter sugar and egg mixed to hard dough then deep fried. Pancake[5]- prepared from starch based batter containing eggs, milk and butter. Brown sugar[7] and sucrose[11] (Nzoia company (k) ltd. Icing sugar[8] used was a product of Tri-clover Industries (k) ltd. White ugali[10] andstiff porridge[9]-a type of stiff porridge made from maize or corn meal flour eaten with vegetable. Fresh milk[12] and sour milk[13] (Brookside Dairy (k) ltd).Fermented maize meal flour[15]- a type of snack locally known as 'mkarango' prepared from roasted fermented maize meal flour.

Figure 3.3 Tray set up for descriptive sensory evaluation of gluten-free bread

Evaluation session

Evaluation was carried out in one day in the Food Preparation Laboratory of the University of Eldoret. The panelists were presented with eight samples, each with three digit blinding codes. They were instructed to take a sip of water before starting to taste and in between tasting the different samples. Carrot sticks were also offered for cleansing their palate. The ballot had three sections. Section A (Appendix 5) entailed scoring the degree of liking or disliking a bread sample on a 9 point hedonic scale with (dislike extremely – 1; neither dislike nor dislike -5: and like extremely – 9 (Peryam and Pilgrim, 1957). The parameters evaluated were (appearance, aroma/smell, flavor and texture. In section B (Appendix 5), panelists ranked the 8 samples from the most liked at 1 to the least liked at 8. Section C addressed the intent to purchase evaluation on a 5 point scale with 1, as least likely and 5 being most likely. Each session lasted 45 minutes.

3.10 Data analysis

All the chemical and physical properties were analyzed by one way analysis of variance (ANOVA). The statistical software used was SAS version 9.01. All means were compared using fisher's least significant difference test.

For the descriptive sensory analysis, mean scores by panelists for the sensory attributes were determined by two-way ANOVA with samples as fixed effects and panelists as random effects, the software used was Statistica Version 8.0 (Statsoft, Tulsa, OK). A correlation matrix with bread samples in rows and descriptors in columns was used to perform Principle Component Analysis (PCA) of the significant sensory attributes obtained from means across panelists.

Box and whisker plots were used to illustrate consumer hedonic score distribution for the gluten free bread and control. Optimization was done to produce the best optimal through maximizing and minimizing attributes of proximate mineral and physical characteristics using design expert version 11 for the various bread blends. Significant differences were considered at $p \leq 0.05$.

3.11 Ethical considerations

Permission to carry out the research was granted by the National Commission for Science, Technology and Innovation (NACOSTI) permit number Nacosti/p/16/21631/11478. A letter of permission to carry out the research was sought from ministry of education science and technology both in Uasin Gishu and Nairobi countries. An informed consent of the descriptive and consumer panelists was sought before involving them in the study.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Proximate composition of flours

The proximate composition of three basic flours, green banana (GBF), pumpkin seed (PSF) and cassava (CF) used to formulate composite flours for the preparation of gluten free bread is shown in Table 4.1. The fourth sample, wheat (WF) was the control. The moisture content of the four flours ranged between 5.6 g/100 g to 13.1 g/100 g for pumpkin seed and cassava flour respectively. The moisture values were ≤ 14%, recommended for storage of flours (Butt *et al*, 2004; Ojo *et al.*, 2017). High moisture levels in flours encourage growth of microorganisms, leading to microbial spoilage (Oduro *et al.*, 2009) and reduced shelf life.

Pumpkin seed had significantly higher ash, fat, protein and energy content, making it the greatest contributor of these nutrients to the composite flours. The ash content of PSF was higher by 42, 76 and 94% than GBF, CF and WF, respectively. Similar studies have reported ash contents for PSF ranging between 4.4 g/100g (Costa *et al* 2018) and 5.5 g/100g (Elinge *et al.*, 2012), all higher than wheat, banana and cassava in this study. High ash content is an indicator of high mineral content (Hamed *et al.*, 2008) therefore PSF was an important source of minerals in the composite flours. Nutrient therapy has been confirmed as the only way to deal with celiac disease and its associated symptoms, and mineral supplementation is advocated for gluten free diets (Kupper, 2005)

Table 4.1: Proximate composition of green banana, pumpkin seed, cassava and wheat flours*g/100 g dmb

	Moisture	**Ash**	**Lipids**	**Protein**	**Carbohydrate[1]**	**Energy KJ[2]**
FLOURS						
Green banana	$11.40^{b}\pm0.18$	$3.13^{b}\pm0.15$	$0.93^{c}\pm0.08$	$2.66^{c}\pm0.04$	$83.87^{b}\pm0.36$	$1449.90^{c}\pm5.01$
Pumpkin seed	$5.60^{d}\pm0.10$	$5.37^{a}\pm0.08$	$36.22^{a}\pm0.19$	$20.07^{a}\pm0.05$	$32.74^{d}\pm0.30$	$2247.72^{a}\pm3.44$
Cassava	$13.12^{a}\pm0.13$	$1.30^{c}\pm0.10$	$0.40^{d}\pm0.05$	$1.21^{d}\pm0.01$	$84.97^{a}\pm0.12$	$1440.69^{d}\pm1.80$
Wheat	$7.10^{c}\pm0.10$	$0.32^{d}\pm0.09$	$1.68^{b}\pm0.08$	$12.71^{b}\pm0.11$	$78.1^{c}\pm0.16$	$1584.58^{b}\pm2.02$

Values are means± standard deviation. Values with the same superscript letters on the same column are significantly different at (P<0.05) as assessed by Least significant difference.

[1]Calculated using difference method (FAO, 2003) where % carbohydrates=100-(% fat+ % moisture+ % ash+ % protein).

[2]Calculated by multiplying with Atwater's factors (FAO, 2012) where energy (Kj) = (%cabohydrates×16.736) + (%protein×16.736) + (% oil×37.656)

Pumpkin seed flour also had the highest (36.2 g/100 g) crude fat content, which was 90 times higher than CF which had the lowest (0.40 g/100g). These results are consistent with those of Karanja *et al* (2013). Elinge *et al* (2012), and Habib *et al* (2015), who reported that pumpkin seed flour has 32-41% fat content,. Higher crude fat content is an indicator that pumpkin seeds contain a substantial amount of edible oil compared to cotton seed oil (22-27%) sunflower (30-35%) soybean (18-22%) and olive (12-50%) (Owen *et al.*, 2000).Plant oils are good sources of fat soluble vitamins. Studies have reported deficiencies of vitamins A (Effiong *et al.,* 2009; Elinge *et al.,* 2012), and E (Hozyasz *et al*, 2003; Trabert 1992) in untreated CD-patients. Pumpkin seed oil provides vitamins and a concentrated source of energy in the diet of people with CD.

The protein content for PSF (20.07 g/100 g) was notably higher by 86.8, 93.9 and 36.7% than GBB, CF and WF, respectively. Elinge *et al*, (2012) and Costa *et al.*, (2018) recorded slightly higher values of 27 and 28.8g /100 g respectively for unshelled pumpkin seed flour. In contrast, a study on dried flour from pumpkin seed grown in 13 different regions of Kenya by Karanja *et al.*, (2013) found protein content ranging from 14 to 30 g/100 g. The lower protein content in this study may be attributed to varietal differences of the pumpkin seeds. Low protein value of cassava flour is expected (Salcedo *et al* 2010, Somendrika *et al*, 2016) due to variation in soil and the fact that most studies reported protein values on wet basis but this study protein value was on dry matter basis . Compositing PSF with the other flours increases protein content. Recent study on assessment of chemical composition, physical and sensory properties of biscuits produced from yellow yam, unripe plantain and pumpkin seed flour blends by Bellen *et al.* (2018) confirmed a significant increase in protein in flour bends of 80%: YYF, 10%: UPF, 10%: PSF. A study by

Adelekan *et al. (*2013) reported similar increase in protein content of trifoliate yam when fortified with pumpkin seed flour. Similar findings have been reported by Igbabul *et al* (2015) and Okpala *et al* (2013). This study therefore pumpkin seed flour has an advantage in serving as a food fortificant in this study. Increased protein content has been shown to reverse growth retardation in CD patients (Farrell, 2002). Additionally, proteins contained in pumpkin seed have been found to also increase secretion of serotonin in brain cells thus fighting depression, a common disorder among celiac patients (Gentile, *2012*).

Cassava flour recorded the highest carbohydrate levels compared to the other flours with a small but significant percentage difference($p < 0.05$) of 1.29% from WF but higher for PSF and GBF of 8.09and 61.47% respectively, Table 4.1. The high carbohydrate content can be explained by high starch content (Lebot, 2009) the form in which cassava stores energy in the root. Similar results (86 g/100 g) were reported by Kamau *et al* (2017), though other researchers, Ojo *et al* (2017) and Sikuku *et al* (2018) reported slightly higher values of about 90 g/100 g. The carbohydrate value obtained in this study was higher for pumpkin seed flour compared to that obtained by Elinge *et al* (2012) who reported a level of 28.03g/100g and 31.50 g/100 g by Mohaamad *et al (*2014). Carbohydrates provide calories, promote utilization of dietary fats and prevent overuse of protein as an energy source rather preserving it for its function in growth and development (Balogun *et al.,* 2012). According to Eleazu and Ironua (2013) carbohydrate/dietary fiber is important in controlling oxidation of food products to reduce absorption of cholesterol from the intestines by converting starch into simple sugars, thus help celiac patients in weight management and reduce chances of them developing diabetes.

Pumpkin seed flour had higher (2247.72kJ) energy content compared to GBB, CF and control (WF), by 35.5, 35.9 and 29.5%, respectively. As mentioned earlier, pumpkin seed stores most of its energy in the form of fat compared to green banana, wheat and cassava that store energy in the form of starch (Karanja *et al*, 2013). Energy value for PSF in this study was slightly lower than 2578.72 Kj/100 g of PSF results reported by Mohaamad *et al* (2014) and 2359.77 Kj /100 g obtained by Elinge *et al* (2012).

4.2 Proximate composition of bread blends and control

The results for proximate composition of the formulated gluten-free breads and control are presented in Table 4.2. Moisture content for the gluten-free bread blends ranged between 37.51 to 51.65 g/100 g compared to wheat bread which was 21.72 g/100 g. Higher moisture levels have been reported for gluten free breads with different formulations, for instance rice based bread enriched with proteins attained a value of 41.66 to 46.13 g/100 g (Marco and Rossel, 2008). Enrichment of gluten free breads with fiber possibly also increased moisture levels, similar to a study by Sabanis *et al*., (2009) who found values ranging from 49-53 g/100 g).

The high moisture content may be due to large amount of water used during product formulation (Sandri et al., 2017) thus causing less air entrapment resulting in heavy dough (Mir *et al.,* 2016: Cervenka *et al*., 2008).

In this study, high moisture content may also be explained by the use of xanthan gum which has been associated with higher crumb moisture retention due to its water binding capacity (Poonnakasem *et al.,* 2015). In addition, xanthan gum has the ability to transform free water to bound water in food products (Maleki & Milani, 2013). The control (WB) had a lesser amount of moisture possibly due to the presence of gluten protein which enables the dough to rise, form air pockets and water to evaporate, hence less bound water in bread (Yaseen *et al.,* 2010)

Table 4.2: Proximate composition of gluten free bread made from green banana, pumpkin seed, cassava and their composites*g/100 g dmb

BREAD	**Moisture**	**Ash**	**lipids**	**Protein**	**Carbohydrate**[1]	**Energy KJ**[2]
GBB	$51.65^{a}\pm0.05$	$4.72^{c}\pm0.08$	$1.20^{e}\pm0.05$	$2.62^{e}\pm0.06$	$39.82^{d}\pm0.16$	$755.35^{h}\pm1.36$
PSB	$22.67^{g}\pm0.21$	$6.62^{a}\pm0.10$	$31.77^{a}\pm0.15$	$24.70^{a}\pm0.10$	$14.25^{h}\pm0.23$	$1848.07^{a}\pm1.72$
CB	$37.51^{f}\pm0.49$	$1.62^{g}\pm0.08$	$1.00^{f}\pm0.05$	$1.25^{f}\pm0.05$	$58.62^{a}\pm0.47$	$1039.64^{c}\pm10.29$
WB (control)	$21.72^{h}\pm0.20$	$0.65^{h}\pm0.05$	$3.88^{d}\pm0.08$	$17.44^{b}\pm0.11$	$56.15^{b}\pm0.24$	$1380.79^{b}\pm4.00$
GBPSB	$50.22^{d}\pm0.10$	$5.23^{b}\pm0.15$	$11.57^{b}\pm0.08$	$12.33^{c}\pm0.06$	$20.66^{g}\pm0.11$	$987.56^{e}\pm5.19$
GBCB	$50.50^{c}\pm0.05$	$3.28^{f}\pm0.08$	$1.12^{f}\pm0.02$	$2.46^{e}\pm0.06$	$42.63^{c}\pm0.02$	$796.98^{g}\pm0.67$
PSCB	$51.25^{b}\pm0.10$	$4.07^{e}\pm0.07$	$10.60^{b}\pm0.05$	$11.17^{c}\pm0.09$	$22.90^{f}\pm0.05$	$969.46^{f}\pm2.45$
GBPSCB	$48.25^{e}\pm0.25$	$4.23^{d}\pm0.13$	$9.42^{c}\pm0.08$	$10.93^{d}\pm0.03$	$27.17^{e}\pm0.16$	$992.24^{d}\pm4.92$

Values are means± standard deviation. Values with the same superscript letters on the same column are significantly different at ($P<0.05$) as assessed by Least significant difference

[1]Calculated using difference method (FAO, 2003) where % carbohydrates=100-(%fat+ % moisture+ %ash+ %protein)

[2]Calculated by multiplying with Atwater's factors (FAO, 2012) where energy (Kj) = (%cabohydrates×16.736) + (%protein×16.736) + (%oil×37.656)

GBB (Green banana bread), PSB (Pumpkin seed bread), CB (Cassava bread) and WB (Wheat bread), GBPSB (Green banana pumpkin seed bread), GBCB (Green banana cassava bread) and PSCB (Pumpkin seed cassava bread) GBPSCB (Green banana pumpkin seed cassava bread)

Pumpkin seed bread had the highest ash content of 6.62 g/100 g which was 76% higher than CB the gluten free bread with the lowest ash content and 90% higher than WB, the control. Hence, all the composite breads with PSF had significantly higher ash content than those without. For instance, GBPSB with 5.23 g/100 g ash content was 37% and 22% higher than GBCB and PSCB, (Table 4.2). Costa *et al* (2018) and Silva (2012) also reported increased ash content of foods with added pumpkin seed flour.

Crude fat levels of the gluten free bread blends ranged between 1.00 to 31.77 g /100 g for CB and PSB, respectively while WB had 3.88 g /100 g. Higher crude fat levels are desirable for supplementing oil in products with low fat content such as CB.

In this study PSB had the highest protein content (24.70 g/100 g) compared to other gluten free breads; and 9 and 10 times higher than GBB and CB, respectively (table 4.2). The WB was 29.39% in relation to PSB. Compositing flours significantly increased protein content among bread blends with GBPSB recording higher protein value of 12.33 g/100 g. This however was lower than PSB by 80.24%, 9.41% and 11.35% in GBCB, PSCB and GBPSCB compared to PSB. It is likely that the higher protein content in all the breads compared to the basic flours was due to addition of PSF and use of egg white in the bread formulations. The lower protein levels in other bread blends can be attributed to mixing with starch based flours with lower protein content which reduced levels of protein (Turkit *et al.,* 2016). Further, the high protein content of PSF adds unique functional properties such as high lysine, improving the protein quality of bread (El-Soukkary, 2001).

Cassava bread had the highest (58.62 g/100 g) carbohydrate content though this was only 4% higher than WB. This may be attributed to the higher carbohydrate content of the cassava tuber compared to the other flour sources (Nwosu *et al.,* 2014) such as

GBF and PSF with 76% and 32% lower carbohydrate content, respectively. Further blending cassava with flours less in carbohydrate such as PSF and GBF reduced its content in bread. For example, the carbohydrate content in GBCB was 27% lower than 100% cassava bread. Other researchers have also reported the same results. Alves *et al.,* (2012) produced bread with PSF and found a reduction in carbohydrate content when the level of PSF was increased. Similarly, Gorgonio *et al.,* (2011) evaluated cakes made with PSF and starch blends and observed a reduction in carbohydrate content.

Pumpkin seed bread had the highest (1848 KJ) energy content, 25% higher than WB. Additionally, all breads made with PSF among the blends had significantly higher energy content than those without. For example, GBPSB had 19% higher energy than GBCB. This is attributed to the higher fat content of PS, a concentrated energy source which translates to high energy using Atwater's factor (FAO, 2003).

4.3 Mineral composition of flours

The results of mineral contents of gluten free and wheat flours are presented in Table 4.3. Phosphorus content of the four flours ranged between 0.26 to 0.61 mg/100g. The phosphorous content was highest in GBF which was 54, 57 and 90% higher than PSF, CF and WF, respectively. The phosphorous content in green banana is consistent with USDA (2018) values. High phosphorus levels in GBF is useful in maintaining bone growth in celiac patients, reducing chances of osteoporosis, proper kidney function and cell growth (Mohammed *et al.,* 2014). Abnormalities in electrolyte balance among celiac patients including hyper and hypophoshatemia have been reported in patients lacking phosphorus (Sullivan *et al.,* 2009) thus celiac patients will utilize phosphorus in balancing these electrolytes in their bodies.

Green banana flour further had the highest (0.35 mg/100g) manganese content, 80%, 63% and 20% higher than PSF, CF, and the WB control, respectively. The manganese content in GBF is within the range documented by USDA (2018) and Elinge *et al.*, (2012) for PSF.

Table 4.3: Mineral composition of green banana, pumpkin seed, cassava and wheat flours*mg/100 g dmb

Flours	Phosphorus	Manganese	Copper	Zinc	Iron
Green Banana	0.61^{a}±0.09	0.35^{a}±0.02	0.04^{b}±0.02	0.73^{b}±0.11	0.60^{d}±0.12
Pumpkin Seed	0.28^{b}±0.00	0.07^{c}±0.02	0.23^{a}±0.02	3.00^{a}±0.01	3.91^{a}±0.09
Cassava	0.26^{b}±0.05	0.13^{b}±0.00	0.24^{a}±0.04	0.02^{c}±0.01	0.82^{c}±0.05
Wheat	0.06^{c}±0.02	0.15^{b}±0.01	0.28^{a}±0.02	0.08^{c}±0.02	1.06^{b}±0.12

Values are means± standard deviation. Values with the same superscript letters on the same column are significantly different at (P<0.05) as assessed by Least significant difference

Manganese serves a useful purpose in nutrient utilization by celiac patients because it activates enzymatic reactions associated with carbohydrate, fat and protein metabolism (Payne, 1990).

Green Banana had the lowest (0.04 mg/100 g) copper content, 88% lower than wheat flour (control) with the highest. According to Botero *et al.*, (2011), copper deficiency has been observed in 6.8-33% of patients with celiac disease. Copper stimulates the immune system in fighting infections, repairing injured tissues (Mohammed *et al.,* 2014) in addition to supporting growth, production of bones, teeth, hair, blood, nerves, skin, vitamins and hormones (Reddy and Love, 1999). Copper deficiency in the body can result in complications associated with microcytic anemia, neutropena and thrombocytopenia (Halfdanarson *et al.*, 2009). Thus using PSF and CF in the development of gluten free bread will contribute a substantial daily requirement amount of copper equivalent to that in WF Table 4.3 thus meeting the requirement for celiac patients.

The zinc concentration was highest in the PSF (3.0 mg/100 g), this was significantly higher by 76% in relation to GBF. Zinc levels in WB and CB were not significantly different but low. The low concentration of zinc in wheat flour may be attributed to the milling process, which separates the bran and germ, which contain the minerals and other micronutrients are removed (Mellen *et al.*, 2008; Heshe *et al.*, 2016). Among patients with coeliac disease, zinc deficiency has been linked to endogenous loss rather than malabsorption (Crofton *et al.*, 1990). Patients suffering from skin lesions have associated this to zinc deficiency (Topal *et al.*, 2015). Zinc plays an important role in proper functioning of sense organs such as taste and smell (Payne, 1990). It also aids in protein and carbohydrates metabolism and mobilization of vitamin A from its storage site in the liver and synthesis of DNA and RNA necessary for cell production (Gitririe, 1989).Thus PSF can serve as a vehicle in supplementing zinc reserves in celiac patients.

Iron was highest in PSF with mean value of 3.91 mg/100g and lowest in GBF with a mean value of 0.06 mg/100 g. Wheat flour and CF had 73 and 79%, lower iron contents respectively, compared to PSF. Iron deficiency anemia is one of the symptoms in undiagnosed celiac disease (Goddard *et al,* 2005). Celiac disease has also been linked with continued damage of the small intestine resulting in iron deficiency anemia among newly diagnosed patients (Halfdanarson *et al.,* 2007; Claudia *et al.,* 2016). Gluten free bread developed from PSF formulation in this study will have higher iron content than the conventional wheat breads will have the potential in improving intake of iron.

4.4 Mineral composition of bread blends and control

Table 4.4 shows the mineral content of the gluten free bread made from the unblended flours, composite flours and from WF. Phosphorus content was highest (1.51 g/100 g) in GBB; all composites with GBF had significantly higher phosphorous content than WB which was 86% lower than the GBB. This is explained by GBF having the highest quantity of phosphorus compared to all other flours (Muzanila *et al.,* 2003)

The 100% PSB, GBB and CB had the same quantities of manganese ranging from 0.12-0.15 mg/100 g. Blending reduced manganese content in all the composite breads. This may be due to dilution by blending with flours with less manganese. The WB had content 86% lower than PSB with the highest content.

It was also evident that PSB still maintained an appreciable amount of copper; zinc and iron table 4.4 recording 0.95 g/100 g, 2.52 g/100 g and 2.57 g/100 g, respectively. This strongly correlates with higher ash content in pumpkin seed flour in this study as earlier explained.

TABLE 4.4 Mineral composition of gluten free bread made from green banana, pumpkin seed, cassava and their composites*mg/100 g dmb

BREADS	Phosphorus	Manganese	Copper	Zinc	Iron
GBB	$1.51^{a} \pm 0.08$	$0.14^{ba} \pm 0.01$	$0.28^{b} \pm 0.01$	$0.57^{c} \pm 0.03$	$1.02^{c} \pm 0.14$
PSB	$0.32^{de} \pm 0.00$	$0.15^{a} \pm 0.01$	$0.95^{a} \pm 0.03$	$2.52^{a} \pm 0.02$	$2.57^{a} \pm 0.49$
CB	$0.33^{de} \pm 0.05$	$0.12^{bc} \pm 0.01$	$0.26^{b} \pm 0.01$	$0.01^{f} \pm 0.00$	$0.90^{c} \pm 0.16$
WB	$0.21^{f} \pm 0.02$	$0.05^{d} \pm 0.02$	$0.14^{c} \pm 0.02$	$0.06^{f} \pm 0.01$	$1.00^{c} \pm 0.06$
GBPSB	$0.96^{b} \pm 0.03$	$0.11^{bc} \pm 0.01$	$0.13^{c} \pm 0.02$	$2.34^{b} \pm 0.09$	$1.90^{b} \pm 0.09$
GBCB	$0.42^{c} \pm 0.02$	$0.07^{d} \pm 0.01$	$0.12^{c} \pm 0.02$	$0.15^{d} \pm 0.00$	$0.83^{c} \pm 0.07$
PSCB	$0.26^{fe} \pm 0.05$	$0.10^{c} \pm 0.04$	$0.12^{c} \pm 0.03$	$0.19^{d} \pm 0.01$	$1.83^{b} \pm 0.02$
GBPSCB	$0.38^{dc} \pm 0.02$	$0.07^{d} \pm 0.00$	$0.12^{c} \pm 0.02$	$0.09^{e} \pm 0.01$	$0.89^{c} \pm 0.08$

Values are means± standard deviation. Values with the same superscript letters on the same column are significantly different at (P<0.05) as assessed by Least significant difference

GBB (Green banana bread), PSB (Pumpkin seed bread), CB (Cassava bread) and WB (Wheat bread)

GBPSB (Green banana pumpkin seed bread), GBCB (Green banana cassava bread) and PSCB (Pumpkin seed cassava bread)

GBPSCB (Green banana pumpkin seed cassava bread)

4.5 Physical properties of gluten free bread

Pure wheat bread (control) had significantly higher specific volume (2.80g/100g) compared to all samples of gluten free breads. Specific volume of GFB did not differ from each other ($p>0.05$). The higher specific volume for wheat bread may be attributed to the gluten network responsible for visco-elasticity in the dough enabling it to rise during fermentation and proofing (Oladunmoye *et al.*, 2010) together with wheat dough extensibility and elasticity (Nkhabutlane *et al.*, 2014). Gluten free flours are more hydrophobic and insoluble in nature thus giving them a characteristic low specific loaf volume due to inability to form the gluten network and hold air (Nkhabutlane *et al.*, 2014).According to Mc Carthy *et al* (2005) high specific volume of bread is associated with a softer crumb and higher overall quality.

Table 4.5 Physical characteristics of gluten free bread made from green banana, pumpkin seed, cassava and their composites

Bread treatments	Weight (g)	Volume (cm^3)	Specific volume (cm^3/g)
GBB (200g)	466.33^{a}	996.70^{a}	2.14^{b}
PSB (200g)	457.97^{ba}	1056.70^{a}	2.30^{b}
CB (200g)	459.87^{ba}	953.30^{a}	2.08^{b}
WB (200g)	426.83^{b}	860.40^{a}	2.80^{a}
GBPSB (100g+100g)	465.20^{a}	1063.30^{a}	2.29^{b}
GBCB (100g + 100g)	477.17^{a}	1016.70^{a}	2.13^{b}
PSCB (100g+100g)	454.33^{ba}	993.30^{a}	2.19^{b}
GBPSCB 67g+67g+67g)	466.97^{ba}	1050^{a}	2.35^{b}

Values are means± standard deviation. Values with the same superscript letters on the same column are significantly different at ($P<0.05$) as assessed by Least significant difference

Pure blends: GBB (Green banana bread), PSB (Pumpkin seed bread), CB (Cassava bread) and WB (Wheat bread)

Composites: GBPSB (Green banana pumpkin seed bread), GBCB (Green banana cassava bread) and PSCB (Pumpkin seed cassava bread) GBPSCB (Green banana pumpkin seed cassava bread)

4.6 Instrumental Texture analysis

The instrumental texture characteristics of gluten free breads made from GBF, PSF, CF and their composites is shown in Table 4.6. Hardness of breads ranged from 4.31 N (WB) to 11.07 N (GBB). Green banana bread was significantly different in terms of hardness and chewiness from WB and other gluten free breads. In texture profiling hardness refers to the maximum force required to deform the product to given distance i.e force to compress between molars, bite through with incisors, compress between tongue and palate (www.stablemicrosystem.com, 2016). According to Osella *et al* (2005) bread hardness is related to moisture content, moisture migration and its redistribution. The findings of this study were in agreement since GBF (Table 4.1) and GBB (Table 4.2) had the highest moisture content compared to all other samples with higher highest hardness Table 4.6. In contrast, WB (control) with the lowest moisture content was the least hard. It is also possible that higher hardness in GBB may be due to incomplete gelatinization of starch, lack of gluten matrix and less expansion of gas cells (Loong and Wong, 2018).

Springiness or elasticity indicates the elastic recovery that occurs when the compressive force is removed (Abdelghafor *et al.*, 2011). The values for springiness obtained in this study ranged between 0.77 (PSCB) and 0.94 (WB). Wheat bread again had significantly higher springiness than all the other breads. This may be explained by the absence of gluten which reduced the ability of the gluten free bread batter to hold gases, leading to elastic reduction in breads (Mahmoud *et al*, 2013). Springiness in gluten free breads is affected by moisture content, moisture redistribution and retrogradation of starch (Osella *et al* 2005; Lazaridou *et al* 2009). Higher values of springiness have been reported by Cornejo and Rosell (2015) to be more desirable on bread freshness and elasticity.

Table 4.6 instrumental Texture characteristics of gluten free breads made from green banana, pumpkin seed, cassava and their composites

	Bread	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)	Resilience
1	GBB(pure)	11.07^{a}	0.82^{cbd}	0.62^{ed}	5.52^{a}	0.30^{cd}
2	PSB(pure)	9.26^{ba}	0.78^{d}	0.59^{e}	4.10^{ba}	0.26^{d}
3	CB(pure)	5.37^{bc}	0.88^{b}	0.73^{b}	3.31^{b}	0.36^{bc}
4	WB (Control)	4.31^{c}	0.94^{a}	0.81^{a}	3.24^{b}	0.47^{a}
5	GBPSB(binary)	7.38^{bac}	0.82^{cd}	0.62^{ed}	3.71^{ba}	0.29^{cd}
6	GBCB(binary)	6.96^{bac}	0.83^{cb}	0.70^{cb}	4.04^{ba}	0.41^{ba}
7	PSCB(binary)	6.77^{bac}	0.77^{d}	0.69^{cb}	2.53^{b}	0.34^{bcd}
8	GBPSCB(centroid)	8.87^{bac}	0.80^{cd}	0.66^{cd}	3.99^{ba}	0.30^{cd}

Values are means± standard deviation. Values with the same superscript letters on the same column are significantly different at ($P<0.05$) as assessed by Least significant difference

Pure blends: GBB (Green banana bread), PSB (Pumpkin seed bread), CB (Cassava bread) and WB (Wheat bread)

Composites**:** GBPSB (Green banana pumpkin seed bread), GBCB (Green banana cassava bread) and PSCB (Pumpkin seed cassava bread) GBPSCB (Green banana pumpkin seed cassava bread)

Cohesiveness depicts the strength of internal bonds and characterizes the extent to which a material can be deformed before it raptures (Onyango *et al.,* 2011). Bread with higher cohesiveness is considered more desirable since it forms a bolus rather than disintegrates during mastication while bread lows in cohesiveness will easily crumble. Wheat bread was the most cohesive with a mean value of 0.81while the least cohesive was 100% PSB with 0.58, indicating that pumpkin seed bread will easily crumble. The crumbly texture of bread with high pumpkin seed content may be attributed to presence of grainy/coarse particles associated with whole meal flours due to retainment of bran during milling (Suba *et al.,* 2013; Drakos *et al.,* 2017).

Notably, among gluten free bread blends, CB and its composites, GBCB, PSCB and GBPSCB depicted more cohesiveness with a mean of 0.73, 0.70, 0.69 and 0.66, respectively. This is consistent with the findings of Taylor & Belton (2002) who suggested that cassava starch forms more cohesive gels due to high concentration of amylopectin to amylose resulting into a more cohesive food product. More cohesive products retain more gas and produce a higher bread specific volume (Tess *et al.,* 2015). In this study, Wheat bread also recorded higher bread volume table 4.5.

Chewiness is the product of firmness, cohesiveness and springiness and is defined as energy required masticating solid food to a simple soluble product ready for swallowing (Tess *et al.,* 2015) or the hardness behavior of bread (Liu *et al.,* 2015). Bread samples had chewiness values ranging from 2.52-5.52 N. Green banana bread was the most chewy (5.52 N) while WB was the least (2.52 N). Majority of gluten free breads have been reported to record chewiness values of 2.33 to 5.77 N (Matos and Rosell 2012). Lower chewiness is associated with products that easily break in the mouth such as biscuits (Matos and Rosell, 2012).

Resilience values showed that WB had the highest elasticity value of 0.48 while PSB was the least with 0.26. It has been suggested that reduced resilience and springiness is due to loss of elasticity (Onyango *et al.*, 2011) This is, attributed to use of xanthan gum instead of gluten, lowering the dough's ability to hold gases in gluten free bread (Pyler, 1973). The more resilient and elastic characteristics of WB may be explained by interaction between starch and gluten in the dough, causing the dough to be more elastic thus forming a continuous sponge structure of bread after heating (Hoseney *et al.,* 1994)

4.7 Descriptive sensory evaluation

Descriptive profiling of the 9 types of bread scored by a trained sensory panel yielded 34 attributes (chapter 3 Table 3.3). Analysis of variance (ANOVA) F-values were significant for 24 attributes at $p \leq 0.05$ between the bread types (Table 4.7).

The data were further analyzed by Principle Component Analysis (PCA) a multivariate data analysis model to summarize the variation in the bread attributes. The first two principle components explained 86% of the total variation in bread samples shown in Figure 4.1. Principle component (PC1) expressed 57% of the total variation and separated wheat bread to the right from gluten free breads to the left (Figure 4.1a). Wheat bread was characterized by big and regular pores, shiny and spongy surface, crumbly, hard and chewy texture and sweet flavour. In contrast, the gluten free breads had damp/plastic/ compact/ slimy texture, compact and moist appearance and fermented aroma Figure 4.1b. The second Principal Component (PC2) added 29% to the explanation of variation and separated all bread types with pumpkin seed on the top side of the plot from those without (i.e. banana and cassava breads) on the bottom side of the plot. The breads with pumpkin seed were characterized by crumb and crust roughness, fibrous, crusty and gritty residues which were depicted both in texture and aftertaste and were negatively correlated with even surface crust, fine appearance and soft texture of cassava bread.

Table 4.7: Mean scores for sensory attributes of gluten free blends as evaluated by a trained descriptive sensory panel (n=12)

Attributes	**GBB 100%**	**PSB 100%**	**CB 100%**	**WB 100%**	**GBPSB 50:50%**	**GBCB 50:50%**	**PSCB 50:50%**	**GBPSCB 33.33:33:33:33.33**	**F values**
Surface color crust	4.94^{cde} ± 2.6	6.18^{ef}±2.5	1.55^{a}±1.7	3.79^{bc}±3.3	6.64^{f}±2.5	5.45^{def}±2.9	3.52^{b}±2.7	4.30^{bcd}±2.6	*12.86
Even surface crust	5.91^{c} ± 2.2	3.55^{ab}±2.2	4.70^{b}±2.5	6.88^{c}±2.7	4.58^{ab}±2.3	4.61^{ab}±2.5	3.52^{a}±2.3	3.55^{ab}±1.9	*8.57
Surface shine crust	2.68^{a} ± 2.2	2.18^{a}±1.9	2.67^{a}±2.4	6.03^{b}±2.7	3.00^{a}±2.7	2.70^{a}±2.3	2.36^{a}±2.3	2.45^{a}±2.0	*9.19
Surface color intensity crumb	6.68^{d} ± 2.3	6.42^{cd}±1.9	1.45^{a}±1.0	2.06^{a}±1.5	6.42^{cd}±2.0	5.58^{cb}±2.2	5.15^{b}±2.2	5.79^{cdb}±2.2	*33.88
Rough crumb top surface	3.39^{a}±1.9	6.64^{d}±1.9	3.30^{a}±2.2	4.36^{ab}±3.1	6.21^{cd}±2.0	4.21^{a}±2.4	5.76^{cd}±2.1	5.45^{cd}±2.2	*10.37
Pore size crumb	2.03^{a}±1.9	4.79^{bc}±2.1	4.15^{bc}±2.0	6.33^{d}±2.6	3.88^{b}±2.0	4.27^{bc}±2.2	5.06^{c}±2.1	4.76^{bc}±2.1	*10.53
Pore regularity	3.70^{a}±1.7	4.55^{cba}±1.8	4.12^{ba}±2.1	5.09^{ca}±2.2	4.58^{cba}±2.0	5.00^{cb}±2.0	4.70^{cb}±2.0	5.36^{c}±1.6	*2.44
Compact Appearance	6.45^{d}±2.0	4.27^{bc}±1.6	4.12^{bc}±2.0	2.85^{a}±1.8	4.18^{bc}±2.1	4.85^{c}±1.7	3.58^{ba}±1.6	4.30$^{bc±}$1.83	*10.30
Spongy surface	2.45^{a}±1.9	4.06^{bc}±2.5	5.33^{d}±2.4	7.64^{e}±2.4	3.61^{b}±1.9	4.58^{bcd}±2.1	4.40^{bcd}±2.6	4.88^{cd}±2.0	*14.23
Fine appearance	5.70^{c}±2.5	3.64^{a}±2.2	5.45^{cb}±2.1	5.70^{c}±3.1	3.67^{a}±2.1	4.30^{a}±2.0	3.48^{a}±2.2	4.55^{ab}±2.1	*5.29
Damp/moist appearance	7.12^{c}±2.2	6.00^{bc}±2.2	6.00^{bc}±2.3	3.27^{a}±2.6	6.30^{bc}±2.1	6.18^{bc}±2.1	5.36^{ba}±2.3	5.24^{b}±2.5	*7.81
Stale bread aroma	4.79^{b}±2.7	5.24^{b}±2.5	4.67^{b}±2.8	2.15^{a}±2.7	4.88^{b}±2.6	4.64^{b}±2.7	4.39^{b}±2.9	4.52^{b}±2.8	*3.81
Sour milk aroma	3.52^{a}±3.2	3.64^{a}±3.2	4.00^{a}±3.2	2.73^{a}±3.1	3.36^{a}±3.0	3.48^{a}±2.9	3.94^{a}±3.2	3.73^{a}±3.2	0.52ns
Fermented aroma	5.12^{b}±2.9	5.58^{b}±2.9	4.79^{b}±3.0	2.52^{a}±2.9	4.82^{b}±3.0	4.76^{b}±2.8	5.00^{b}±2.7	4.61^{b}±3.0	*3.20
Cooked banana aroma	3.97^{b}±2.3	2.82^{a}±2.0	3.40^{ab}±2.4	3.09^{ab}±2.3	3.61^{ab}±2.2	3.61^{ab}±2.3	3.45^{ab}±2.4	2.70^{a}±2.3	*1.15ns
Cooked cassava aroma	3.48^{ab}±2.0	3.27^{ab}±2.0	4.30^{b}±2.4	3.03^{a}±2.2	3.33^{ab}±2.2	3.33^{ab}±2.4	3.91^{ab}±2.5	2.73^{ab}±2.2	*1.05ns
Sweet flavor	3.18^{a}±1.6	3.33^{a}±2.2	3.21^{a}±1.7	5.76^{b}±2.3	2.76^{a}±1.5	3.45^{a}±1.7	3.27^{a}±2.2	3.24^{a}±2.0	*7.38

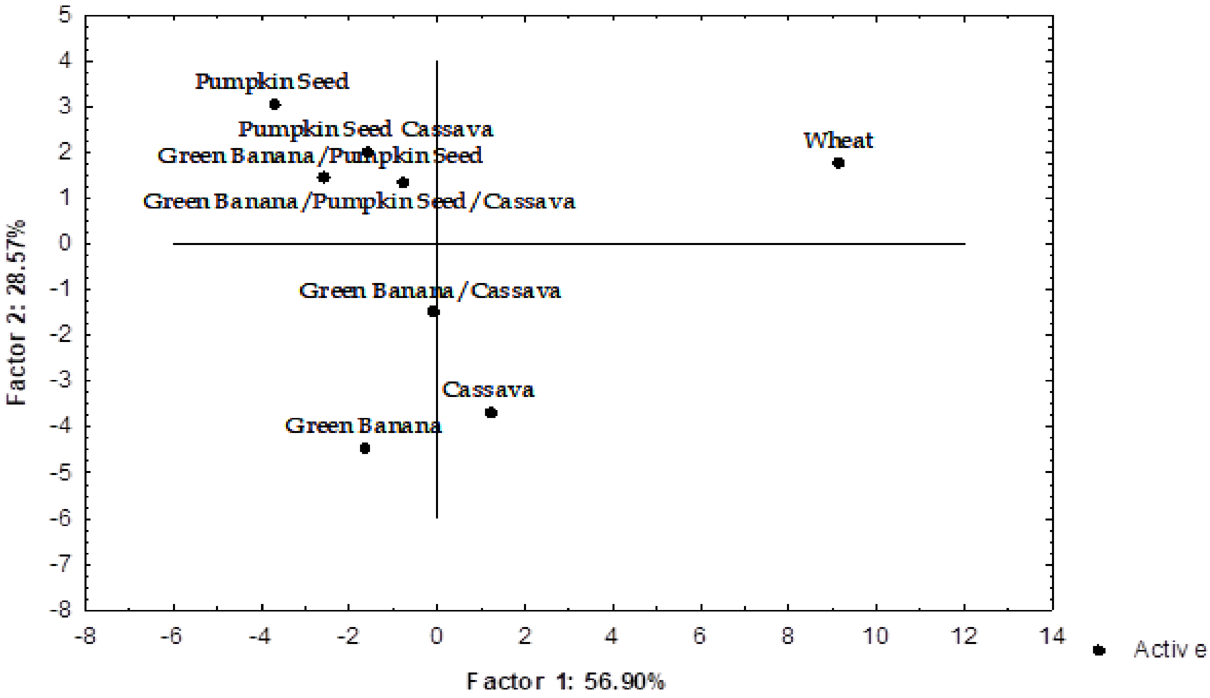
Values are means± standard deviations. Values in a row followed by different letter notations ($^{a-e}$) are significantly different at p≤0.05,* p≤0.05 ns= not significant

Table 4.7 Continued

Attributes	GBB 100%	PSB 100%	CB 100%	WB 100%	GBPSB 50:50%	GBCB 50:50%	PSCB 50:50%	GBPSCB 33.33:33:33:33.33	F value
Fermented maize flavor	5.03^{b}±2.6	4.82^{b}±2.7	4.06^{b}±2.8	1.97^{a}±1.9	5.00^{b}±2.8	4.33^{b}±2.5	4.36^{b}±2.8	4.27^{b}± 2.9	*4.40
Cooked banana flavor	3.94^{a}±2.5	3.00^{a}±2.2	3.64^{a}±2.3	3.18^{a}±2.3	3.42^{a}±2.5	3.74^{a}±2.2	3.61^{a}±2.4	3.09^{a}±2.3	0.64ns
Cooked cassava flavor	3.94^{a}±2.2	3.64^{a}±2.4	4.00^{a}±2.2	3.36^{a}±2.6	3.09^{a}±2.3	3.45^{a} ±2.2	3.82^{a}±2.4	3.76^{a}±2.1	0.58ns
Cooked pumpkin flavor	2.82^{cbb}±2.4	3.87^{c}±2.5	2.33^{b}±2.0	2.79^{cbb}±2.7	3.82^{c}±2.5	2.45^{bb}±2.0	3.52^{cb}±2.0	3.54^{cb}±2.4	*2.19
Bland flavor	2.88^{ab}±2.9	3.42^{ab}±2.9	3.64^{b}±3.0	2.12^{a}±2.9	3.33^{ab}±2.9	3.21^{ab}±2.8	2.94^{ab}±2.8	3.52^{ab}±3.0	0.89ns
Crusty texture crumb	2.79^{cab}±2.2	4.58^{d}±2.8	2.27^{a}±1.9	2.55^{ab}±2.7	3.88^{cd}±2.8	3.27^{cab}±2.5	3.97^{cd}±2.6	3.64^{cbd}±2.8	*3.10
Chewy texture crumb	4.18^{bc}±2.9	7.09^{e}±2.6	4.00^{b}±2.7	2.00^{a}±2.8	6.21^{de}±2.6	4.24^{bc}±2.6	6.00^{de}±2.2	5.33^{cd}±2.9	*11.64
Rough texture crust	3.33^{ba}±2.2	6.03^{d}±2.5	4.21^{cb}±2.6	2.30^{a}±2.7	5.00^{cd}±2.0	4.18^{cb}±2.2	5.64^{d}±2.3	5.03^{cd}±2.5	*8.39
Soft texture	5.76^{d}±2.3	3.12^{a}±2.3	6.15^{d}±2.5	6.39^{d}±3.2	4.36^{bc}±2.2	5.54^{dc}±2.5	3.73^{ba}±2.4	4.48^{bc}±2.4	*7.20
Crumbly texture	3.61^{bc}±2.7	4.58^{c}±2.6	2.27^{a}±1.6	7.76^{d}±2.4	3.76^{bc}±2.6	3.09^{ba}±2.2	3.36^{ba}±2.4	3.82^{bc}±2.7	*14.66
Compact texture	6.15^{c}±2.6	4.18^{b}±2.2	4.24^{b}±2.4	1.30^{a}±1.4	4.73^{b}±2.4	4.94^{b}±2.4	3.82^{b}±2.3	4.09^{b}±2.4	*11.58
Slimy texture	3.82^{c}±2.4	2.58^{ba}±2.0	5.00^{d}±2.9	2.24^{a}±2.4	3.55^{cb}±2.2	4.30^{cd}±2.7	3.58^{cb}±2.3	3.67^{cb}±1.8	*4.38
Plastic texture	6.27^{b}±2.3	5.82^{b}±2.6	6.12^{b}±2.5	3.30^{a}±3.0	5.12^{b}±2.5	5.27^{b}±2.4	5.27^{b}±2.4	5.30^{b}±2.4	*4.27
Damp texture	6.27^{b}±2.5	6.09^{b}±2.4	5.91^{b}±2.7	2.42^{a}±2.3	5.42^{b}±2.4	5.76^{b}±2.2	5.58^{b}±2.2	5.30^{b}±2.4	*8.33
Fermented after taste	5.21^{d}±2.6	5.12^{cd}±2.4	3.64^{b}±2.6	1.40^{a}±1.4	4.85^{cd}±2.6	3.94^{cb}±2.6	4.27^{cdb}±2.5	4.18^{cdb}±2.6	*7.95
Gritty residue in mouth	2.76^{a}±2.1	7.70^{c}±1.8	1.94^{a}±2.2	1.70^{a}±2.6	6.12^{b}±2.3	2.76^{a}±2.6	5.94^{b}±2.5	5.12^{b}±2.6	*28.66
Fibrous aftertaste	2.24^{a}±1.7	7.73^{d}±2.0	1.76^{a}±1.9	1.61^{a}±2.5	6.61^{c}±2.5	2.36^{a}±2.0	6.12^{bc}±2.5	5.18^{b}±2.7	*38.52

Values are means ± standard deviations. Values in a row followed by different letter notations ($^{a-e}$) are significantly different at p≤0.05,* p≤0.05 ns= not significant\

a)



b)

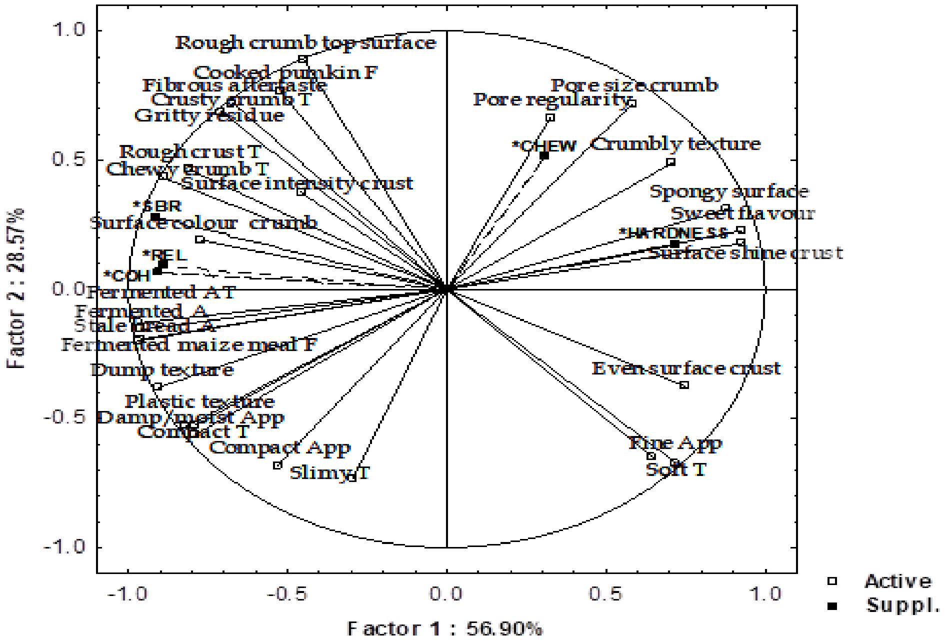


Figure 4.1: Principle component analysis (correlation matrix) of variation of gluten free bread blends in relation to control. (a) Plot for the first two principle components of gluten free breads (b) plot for the loading projections for the different significant sensory attribute

*A- Aroma, APP-Appearance, AT- Aftertaste, CHEW-Chewiness, COH-Cohesiveness F-Flavor REL-Resilience SBR-Springiness, T-Texture

The existence of visible and regular pores in WB may be attributed to glutenin and gliadin which when hydrated is responsible for the elastic and cohesive properties of gluten (Claire, 2014; Dermirkesen *et al.,* 2013). Gluten influences gas retention during dough expansion resulting in the formation of pores creating a spongy crumb, shiny surface and crumbly texture (Wieser, 2007: Biesiekjerski, 2017).

Open cell structured porous food materials consisting of pores that form an interconnected network are comparatively softer than closed cell networks (Rathnayake *et al.,* 2018). Additionally nutrients in wheat flour together with other ingredients such as fat, sugar, minerals, starch and protein results in the final characteristics of taste and texture of WB such as crumbly texture (Pangal *et al.,* 2006).

Lack of gluten matrix in the gluten free bread resulted in damp /moist, compact and plastic appearance and texture due to closeness of the pores and high moisture content. Excessive moisture in gluten free breads as explained earlier is attributed to slower hydration (high moisture content in the batter) and less air entrapment in bread thus leading to heaviness in bread (Ameh *et al.,* 2013). The sweet flavor in WB may also be attributed to the use of sugar and yeast through the process of fermentation resulted to formation of 3 methyl-1-butanol the major volatile compound resulting in flavor and aroma in bread crust and crumb (Birch *et al,* 2014). Due to use of lipid these results to formation of volatile compounds originating from lipid oxidation and millard reaction resulting to formation of 2 acetyl-pyroline, 4 hydroxy-2,5 Dimethyl-3(2H) furanone responsible for flavor and aroma in bread (Moskowitz *et al* 2012:Pico *et al*, 2015). However 2-E-nonenal has been identified from miallard reaction causing strong aroma in WB (Moskowitz *et al*, 2012).

Cassava bread in the same quadrant with wheat bread figure 4.1a is associated with evenness of the surface, fine appearance and soft texture. This may be attributed to cassava flour having binding characteristics similar to those of wheat such as being crunchy and crumby texture (Pasqualone et al., 2010).Current innovations in gluten-free product development have proved that cassava flour possesses positive bakery characteristics such as acceptable texture, mild taste and fineness, giving it higher advantage in replacing wheat. Eleazu *et al* (2014) studied effect of partial replacement of wheat flour with high quality cassava flour on chemical composition, antioxidant activity, sensory quality and microbial quality of bread concluded that substitution of wheat flour with varying levels of cassava flour(ie10%,20%,30% and 40%) produced bread with acceptable sensory attributes similar to those of 100% wheat.

Pumpkin seed had the greatest influence on the descriptive sensory properties of gluten free breads (Figure 4.1b). This could be attributed to fiber that was present in the husk (Bowman, 2011), giving the rough, fibrous, crusty and gritty texture and aftertaste and affecting the chewing process. Pyrazins and aldehydes formed during roasting of pumpkin seeds have been shown to contribute to desirable roasted flavor, sweetness and chewy texture in pumpkin seed bread (Ramli *et al.*, 2006: Bowman, 2011).

Green banana bread was located on the same quadrant with PSB and was associated with fermented, damp compact and slimy texture and appearance (Figure 4.1b). This could be attributed to GBB having higher moisture content diluting the protein network making bread to have lower specific volume resulting in hardness in bread due to closed structure of the dough (Noort *et al.*, 2010).

4.8 Consumer acceptability

The acceptability of bread by consumers evaluated across the different blends for appearance, smell, flavor, texture and total quality is shown in Table 4.8. Color, texture and aroma attributes show consumer preference of a given product and higher mean imply better acceptability (Taghdir *et al.*, 2017).New products success in the market with rising competition rate in food industry is determined by consumer acceptability(Siro *et al.*, 2008).

Table 4.8 shows that consumers' rating for all the attributes (appearance, smell, flavour and texture).Wheat bread was significantly higher in all the attributes in relation to gluten free bread. Hedonic score for Appearance of WB by consumers was (8.31). This is probably due to the brown crust colour of wheat bread (de Graaf *et al.*, 1999) and smooth texture, in addition to consumers' familiarity with wheat bread in the market (Hatdman*et al.*, 2011) compared to gluten-free bread which was new to them. Green banana bread was scored lowest (6.11) and was significantly different from all other breads except GBCB.

Bread composited with banana flour had compact, damp and moist appearance, which imparted unappealing appearance characteristics to consumers compared to the other breads figure 4.1b.

Consumers preferred WB aroma which scored 7.85 while gluten-free breads were statistically the same with exception of CB which scored (6.00). Presence of volatile compounds attributed to baking in the oven and Maillard reaction that take place in heating of wheat bread may have resulted in a strong smell (Farah, 2012) making WB smell more preferred than other gluten free breads.

Dilution of starch, contribute to appealing aroma in baked products are additional factors which interfered with sensory rating of aroma (Wirtz, 2003) for gluten free breads. In addition presence of high moisture content of gluten-free breads in this study could have weakened the aroma in these breads.

Flavor in bread could be influenced by ingredients used such as flour, salt, sugar, milk and fat. Wheat bread flavor was liked most rating (7.85) liking. This could be attributed to presence of volatile flavor protein compounds pyrazines and aldehydes released during Maillard reaction resulting in desirable flavor, sweetness and chewy texture in wheat bread. Additionally fermentation due to the action of baker’s yeast with other ingredients generated new flavor components improved WB flavor (Martin, 2013).

Table 4.8: Consumer acceptability of gluten free breads made from green banana, pumpkin seed cassava and their composites

Bread	Appearance	Smell	Flavor	Texture
GBB	$6.11^{d}\pm1.93$	$6.55^{bc}\pm 1.58$	$6.31^{bc}\pm1.75$	$6.73^{bc}\pm1.56$
PSB	$7.02^{bc}\pm1.65$	$6.63^{b}\pm1.71$	$6.70^{b}\pm1.43$	$7.02^{b}\pm1.51$
CB	$7.01^{b}\pm 1.54$	$6.00^{c}\pm1.92$	$6.18^{bc}\pm1.60$	$6.80^{bc}\pm1.68$
WB	$8.31^{a}\pm 1.00$	$7.85^{a}\pm1.30$	$7.85^{a}\pm1.30$	$8.33^{a}\pm0.90$
GBPSB	$6.85^{bc}\pm1.50$	$6.25^{bc}\pm1.83$	$6.09^{c}\pm1.64$	$6.55^{bc}\pm1.66$
GBCB	$6.72^{cd}\pm1.63$	$6.15^{bc}\pm1.58$	$6.35^{bc}\pm1.52$	$6.31^{c}\pm1.49$
PSCB	$7.15^{b}\pm 1.15$	$6.15^{bc}\pm1.70$	$6.41^{bc}\pm1.33$	$6.85^{bc}\pm1.52$
GBPSCB	$7.18^{b}\pm1.35$	$6.23^{bc}\pm1.78$	$6.67^{b}\pm1.50$	$6.89^{b}\pm1.46$

Values are means± standard deviation. Values with the same superscript letters on the same column are significantly different at ($P<0.05$) as assessed by Least significant difference

GBB (Green banana bread), PSB (Pumpkin seed bread), CB (Cassava bread) and WB (Wheat bread)

Composites: GBPSB (Green banana pumpkin seed bread), GBCB (Green banana cassava bread) and PSCB (Pumpkin seed cassava bread) GBPSCB (Green banana pumpkin seed cassava bread)

The liking of pumpkin seed bread reduced significantly when GB was added. This could be attributed by increased dietary fibres which compete with starch for water thus causing low gelatinization (Zhou and Therdthal, 2007) diluting the starch thus resulting in reduced flavor components making this bread undesirable. Wheat bread texture again was the most liked with (8.33).This could be as a result of consumer familiarity with wheat bread .This could be attributed to protein gliandin and glutenin present giving WB providing viscoelastic properties in bread thus giving it good structure and desirable textural characteristics such as fines, sponginess, and crumbly texture associated to WB. Pumpkin seed bread however received considerable texture liking of (7.02). Consumers may not have penalized the rough surface parameter crust and cumb in PSB thus higher acceptability towards it due to their knowledge that it is fibre enriched product and it gives certain health benefits (Ares *et al.,* 2008).This behavior however did not interfere with consumer's judgments.

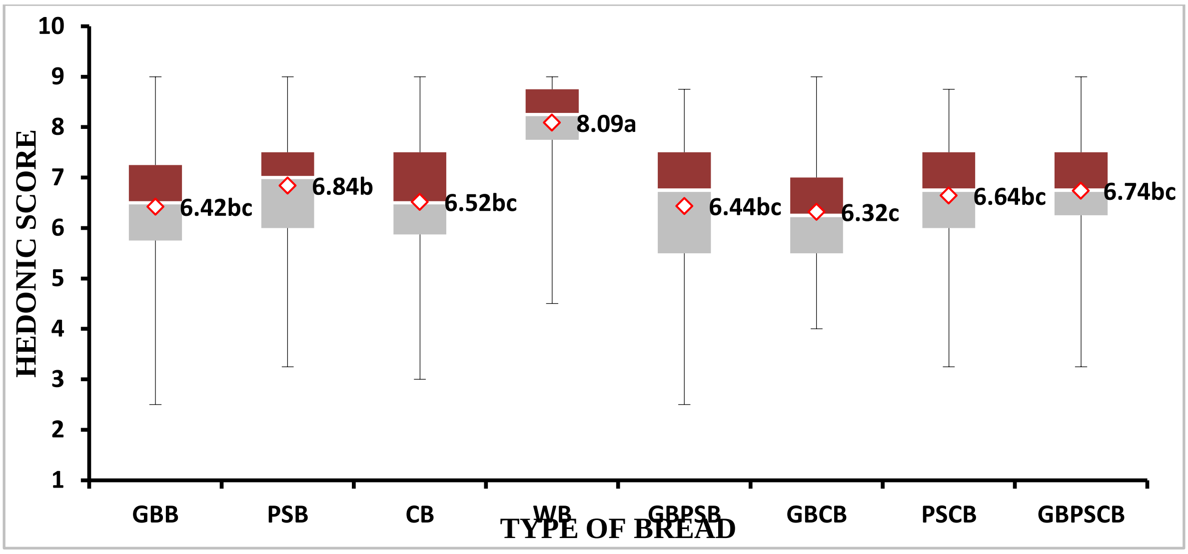


Figure: 4.2 showing hedonic score for total quality of gluten free bread and control

(GBB (Green banana bread), PSB (Pumpkin seed bread), CB (Cassava bread) and WB (Wheat bread) Composites: GBPSB (Green banana pumpkin seed bread), GBCB (Green banana cassava bread) and PSCB (Pumpkin seed cassava bread) GBPSCB (Green banana pumpkin seed cassava bread.

Total quality of WB was favored by consumers rating 90% in relation to other gluten free bread blends. This could be attributed to availability and variety of baked products made from wheat and its positive attributes such as smell/aroma, flavor and texture mentioned above. This reflected higher score rating for smell, flavor and textural attributes mentioned above. Total quality of GBB, CB, GBPSB, PSCB and GBPSCB were statistically similar with slight liking. However PSB among gluten-free bread recorded 76% in terms of total quality. The results also connected well with ranking, intent to purchase and optimization where PSB received higher rating showing potential of pumpkin seed in food product development.

Figure: 4.3 showing pictorial diagram of pure bread GBB (Green banana bread), PSB (Pumpkin seed bread), CB (Cassava bread) and WB (Wheat bread) **Composites**: GBPSB (Green banana pumpkin seed bread), GBCB (Green banana cassava bread) and PSCB (Pumpkin seed cassava bread) GBPSCB (Green banana pumpkin seed cassava bread)

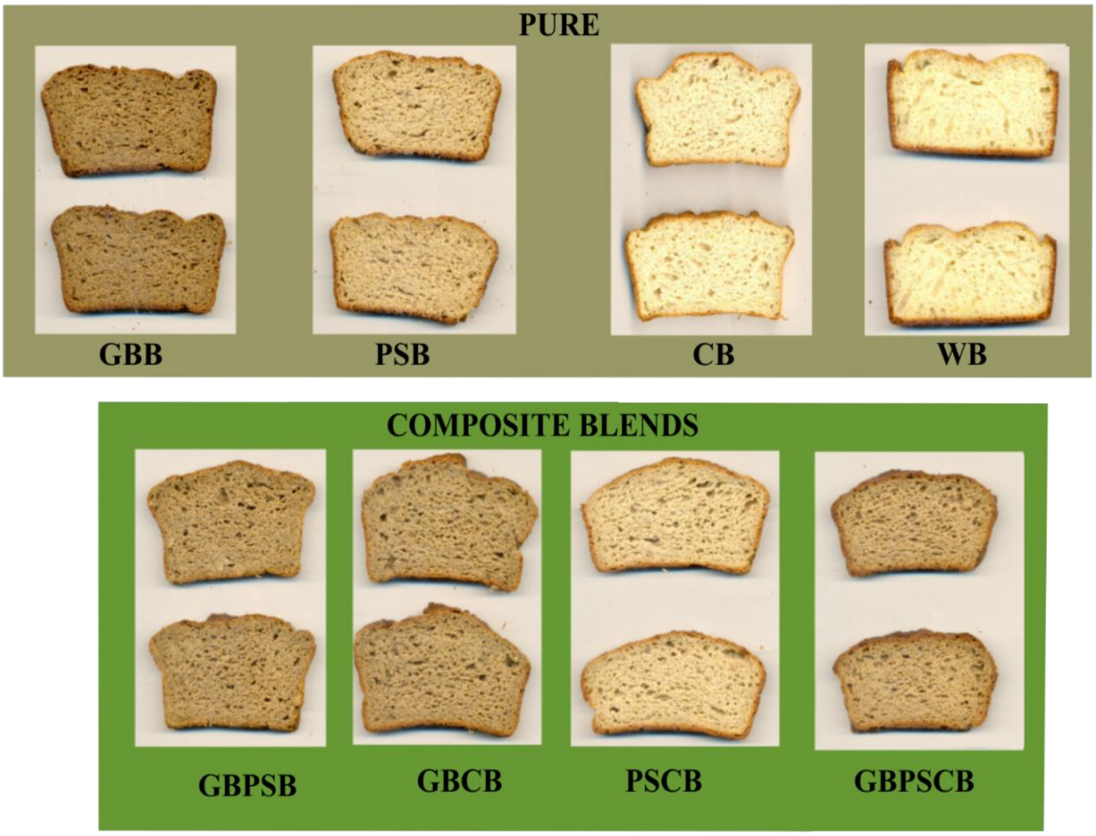


Figure: 4.4 showing slices of pure bread GBB (Green banana bread), PSB (Pumpkin seed bread), CB (Cassava bread) and WB (Wheat bread) Composites: GBPSB (Green banana pumpkin seed bread), GBCB (Green banana cassava bread) and PSCB (Pumpkin seed cassava bread) GBPSCB (Green banana pumpkin seed cassava bread)

4.9 Ranking of gluten free bread in relation to control

The consumers were told to rank the eight samples of bread in a scale of 1-8 where 1-most liked and 8-least liked. Wheat bread was ranked at 1 meaning it was the most liked by consumers this was as a result of consumers' familiarity with the product Table 4.9. This was further supported by the findings on descriptive data where WB had acceptable appearance, texture, aroma, flavor and acceptable total quality. Among the gluten-free bread PSCB was ranked at 2 this could be attributed to the fact that the flour was composited in 50:50% thus contributing characteristics texture, taste, fines and chewiness making this bread acceptable in relation to wheat bread. The least ranked bread was GBCB which was ranked at 8 this could be as a result of GBB hardness, damp, compact and plastic texture due to higher water holding capacity which was earlier explained in descriptive characteristics. Other breads were ranked as PSB-3, CB-4, GBPSB-5, GBPSCB-6, and GBB-7.

Table 4.9: Ranking of gluten free breads made from green banana, pumpkin seed cassava and their composites

Bread	Mean ± SD	Rank Order
GBB	5.44±1.99	7
PSB	4.60±2.21	3
CB	4.64±2.08	4
WB	1.40±1.23	1
GBPSB	4.80±1.98	5
GBCB	5.49±2.24	8
PSCB	4.56±1.84	2
GBPSCB	5.07±1.91	6

GBB (Green banana bread), PSB (Pumpkin seed bread), CB (Cassava bread) and WB (Wheat bread) Composites: GBPSB (Green banana pumpkin seed bread), GBCB (Green banana cassava bread) and PSCB (Pumpkin seed cassava bread) GBPSCB (Green banana pumpkin seed cassava bread)

4.10 Intent to purchase of gluten free bread in relation to control

Quality of a product and its related characteristics such as flavor and texture affects food purchasing and consumer decision in the market (Farnakalidis, 1999). Hence, the intent to purchase was conducted after the consumers had evaluated acceptability. Intent to purchase was assessed in a scale of 1-5 (1 being the least likely, and 5 being the most likely) the likelihood of purchase of each product if it were available for purchase in the market. Wheat bread again was rated at 4.09 in terms of likelihood of purchase. This shows that consumers were well versed with wheat bread. In terms of gluten free bread CB and GBCB had the same rating in terms of likelihood of purchase. This could be attributed to consumers liking of cassava and attributing cassava characteristics such as smooth texture and fine appearance to those of WB. Other breads recorded the likelihood of purchase in order of PSB- 3.51; GBPSCB- 3.45 the least likelihood was in GBB-2.95 (figure 4.7.)

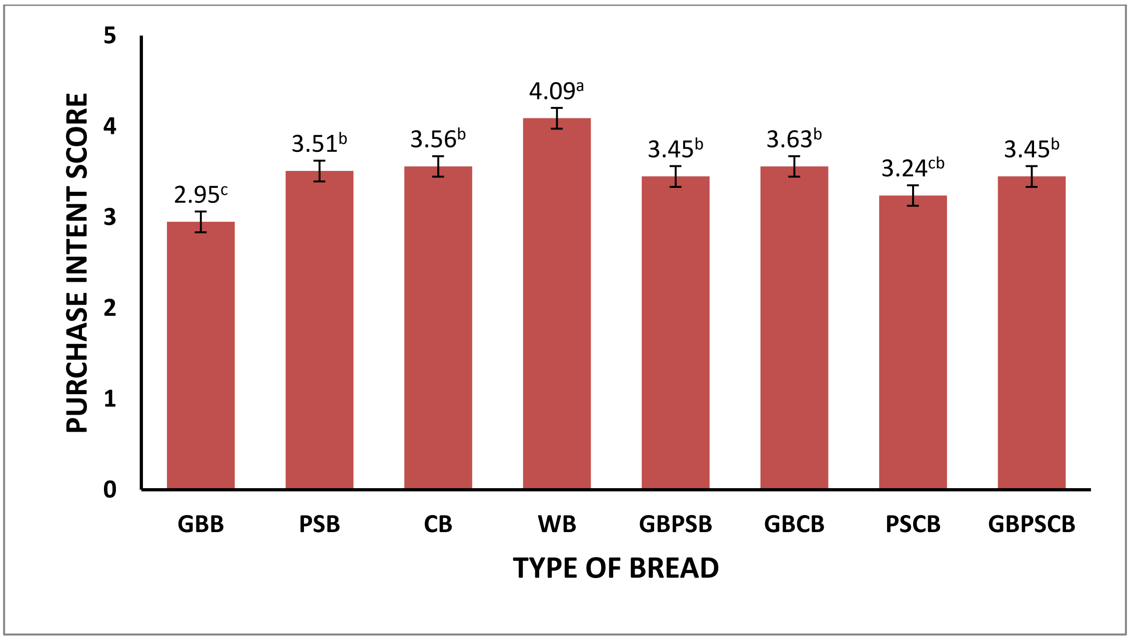


Figure 4.5: Intent to purchase of gluten free bread and control in a scale of 1-5
GBB (Green banana bread), PSB (Pumpkin seed bread), CB (Cassava bread) and WB (Wheat bread) Composites: GBPSB (Green banana pumpkin seed bread), GBCB (Green banana cassava bread) and PSCB (Pumpkin seed cassava bread) GBPSCB (Green banana pumpkin seed cassava bread)

4.11. Optimization of gluten free bread

Design expert software (version 11 State-Ease Inc, 2019) was adopted to determine the workable optimum conditions for mixture blends of X1-GBF, X2- PSF and X3-CF. Contour plots have been used because they give a response towards independent variables through getting the optimum points or desirability levels in selected specific parameters(Liu *et al* 2011).The main criteria for selection of optimization were on the basis of proximate, mineral textural and consumer characteristics using both graphical and numerical methods. The overall desirability was constraints as the response subject to maximization of proximate and mineral (Ash, lipids, proteins, carbohydrates, energy, phosphorus, manganese, copper, zinc and iron) minimization moisture. In the second case textural and consumer characteristics (specific volume, hardness, cohesiveness, chewiness, resilience, total quality, appearance, smell, flavor and texture) were maximized and while moisture and resilience were minimized. Optimum results for both numerical and graphical optimization were displayed using Table 4.10 and overlay diagrams. The contour plots for all the responses were superimposed and the regions that best satisfy all the constraints were selected as optimum points. Proximate and mineral results favored pumpkin seed and gave higher optimum value of 71% Figure 4.6 and overall desirability of 90% for the maximum constraints below and minimum moisture. This could be attributed to PSB having higher mineral content (Elinge *et al.*, 2012: Costa *et al.*, 2018) as explained earlier, good amounts of protein fats and energy and copper, zinc and iron Table 4.1 and 4.3.Textural parameters favored CB and gave a higher optimum value of desirability Table 4.10 and Figure 4.7 this could be attributed to uniqueness of cassava starch possessing characteristics similar to those of wheat bread (Ongunjobi *et al.,* 2016: Nweke *et al.*,2002) as explained earlier in PC1.

Table 4.10 Optimized proximate, mineral, physical and sensory characteristics of bread

Proximate and mineral characteristics		**Physical and sensory characteristics**	
Characteristics	**Optimum values**	**Characteristics**	**Optimum values**
X1-GBF	0.08	X1-GBB	0.17
X2-PSF	0.71	X2-PSB	0.36
X3-CF	0.21	X3-CB	0.47
Moisture	44.6	Moisture	48.17
Ash	5.32	Specific volume	2.28
Protein	17.78	Hardness	7.51
Lipids	20.78	Springiness	0.81
Carbohydrates	21.56	Cohesiveness	0.71
Energy	1439.21	Chewiness	3.32
Phosphorus	0.43	Resilience	0.33
Manganese	0.15	Total quality	6.64
Copper	0.29	Appearance	7.2
Zinc	1.65	Smell	6.15
Iron	1.99	Flavor	6.47
Overall desirability	0.90	Texture	6.83
		Overall desirability	0.92

Note: X1 (Green banana bread), X2 (Pumpkin seed bread), X3 (Cassava bread)

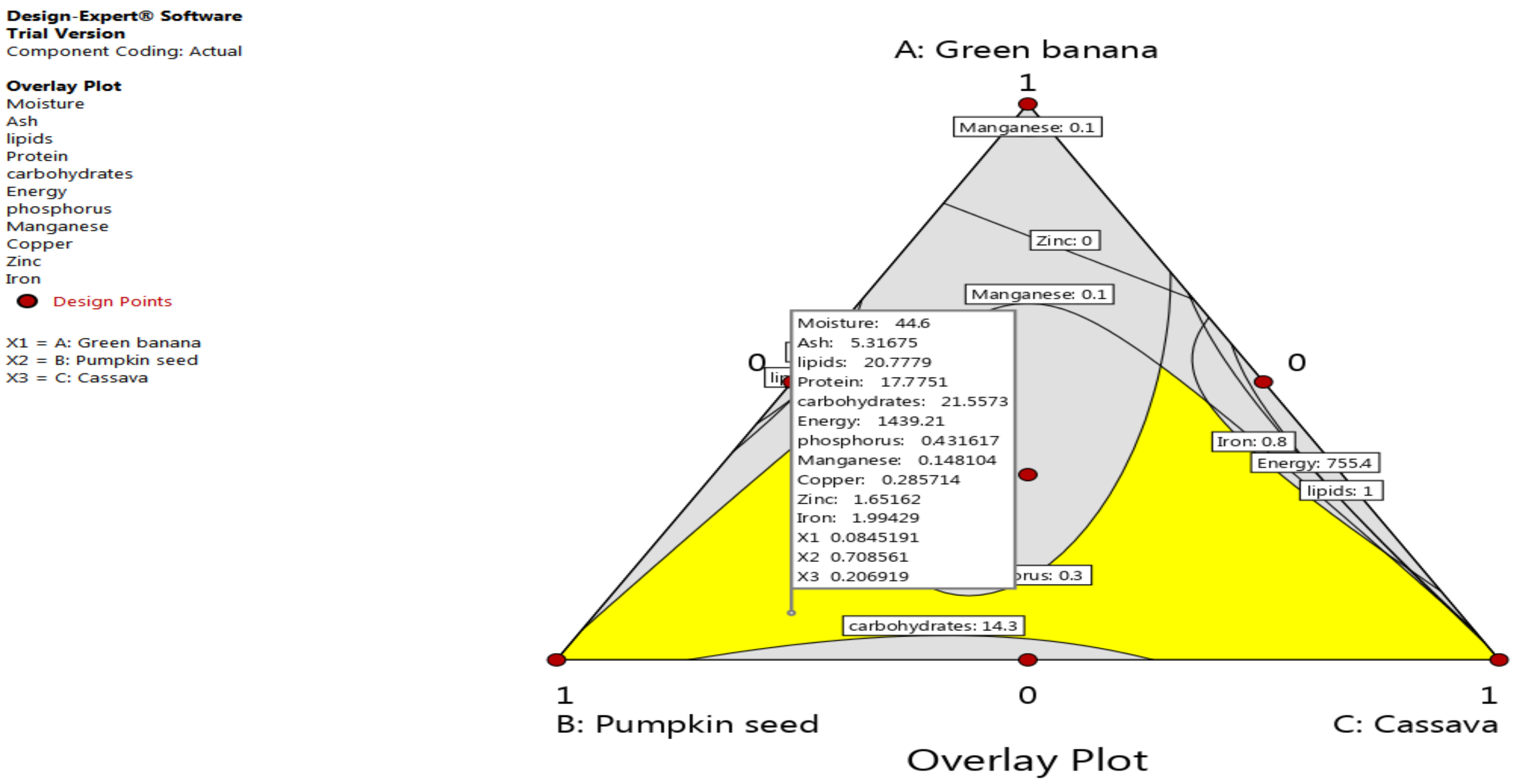


Figure 4.6 Optimum points for X1-GBF, X2-PSF, X3–CF moisture, ash, lipids, protein, carbohydrates, energy, phosphorus, manganese, copper, zinc and iron

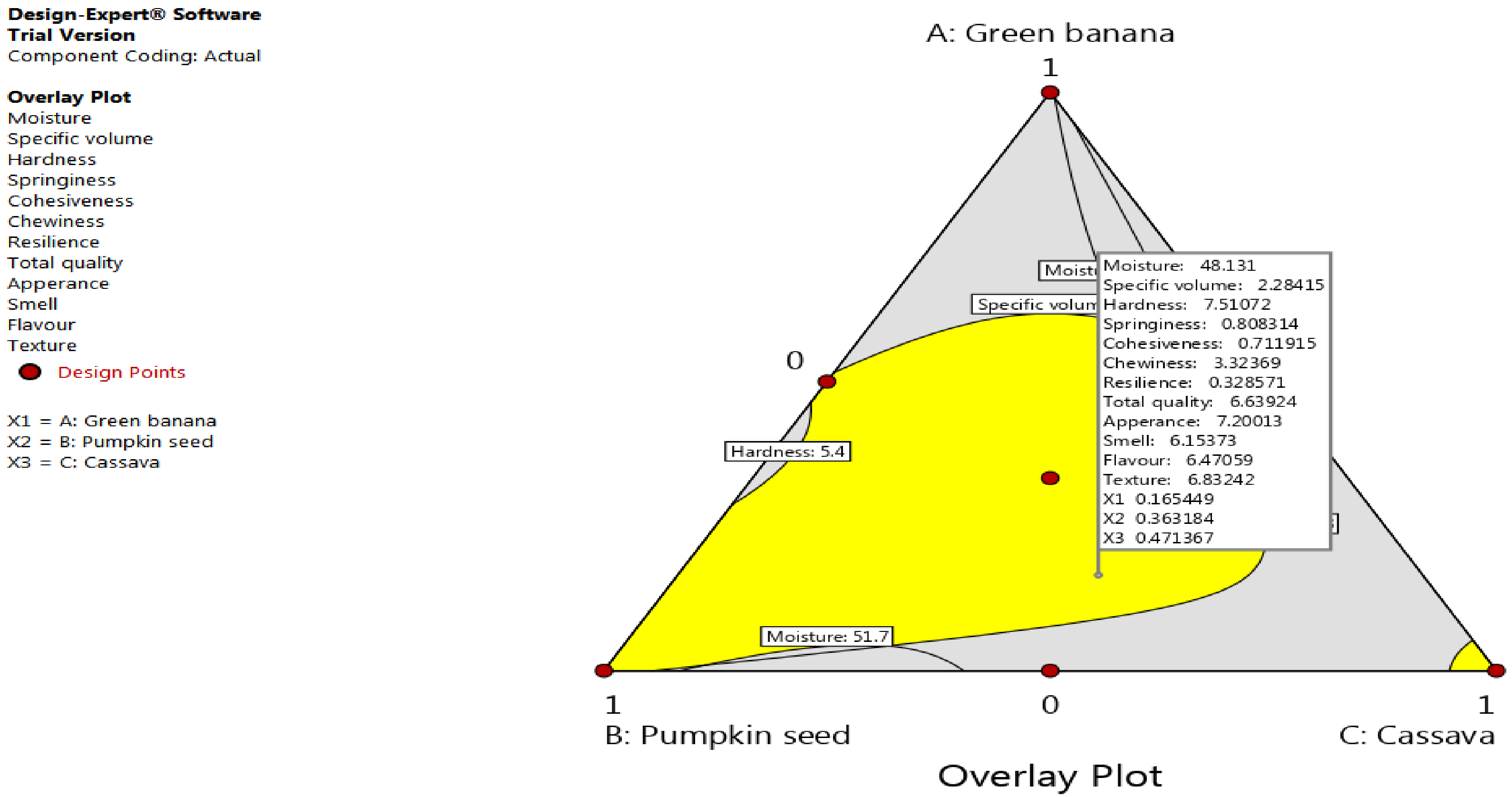


Figure 4.7 Optimum points for X1-GBF, X2-PSF, X3–CF Moisture, Specific Volume, Hardness, Springiness, Cohesiveness, Chewiness, Resilience, Total Quality, Appearance, Smell, Flavor, Texture and Overall Desirability.

Therefore the final optimized value would be recommended for preparation of gluten-free bread based on maximizing textural factors and minimizing moisture. Overlay plots for all the responses were presented in superimposed regions that best satisfy them according to table 4.10 and Figure 4.7 with optimum points for each textural and consumer characteristics.

The combined optimized characteristics for proximate, mineral, textural and consumer acceptability was also done by maximizing all the characteristics stated above and minimizing moisture and resilience. Overall Desirability was 89% summarized overall optimum responses were represented in overlay plot figure 4.5 below.

Table 4.11 Combined optimized proximate, mineral, physical and sensory characteristics of bread

Combined proximate, mineral, physical and consumer characteristics	
Characteristic	**Optimum value**
X1-GBF	0.27
X2-PSF-	0.72
X3-CF	0.013
Moisture	40.07
Ash	5.89
Protein	20.64
Lipids	18.01
Carbohydrates	16.27
Energy	1349.51
Phosphorus	0.61
Manganese	0.13
Copper	0.39
Zinc	1.85
iron	2.10
Specific volume	2.30
Hardness	8.94
Springiness	0.80
Cohesiveness	0.62
Chewiness	3.93
Resilience	0.29
Total quality	6.36
Appearance	6.96
Smell	6.36
Flavor	6.4
Texture	6.86
Overall desirability	0.89

Note: X1 (Green banana bread), X2 (Pumpkin seed bread), X3 (Cassava bread)

Through optimization of the gluten free bread both nutritional and functional properties of GBB,PSB and CB will be enhanced .The optimized gluten free bread in this study will give a suggestion of possible combination that would make this bread best accepted with most nutritive and therapeutic properties for celiac people and best sensory attributes. Pumpkin seed bread had higher contribution nutritionally there through optimization the results showed that it can be used in fortifying food products. Cassava on the other hand showed positive textural and consumer characteristics therefore the optimized bread will contribute positive characteristics similar to those of wheat. Optimization therefore is a tool that can guide celiac patients and those that are healthy conscious on nutrient blending and fortification in achieving the desired result. Further research should therefore be done to assess the efficacy of optimized bread on celiac patients.

4.12 General discussion

Despite wide array of locally available gluten free food products adherence to GFD remains a problem to both the healthy conscious individuals and those suffering from celiac disease. Removing gluten in diet might look simple but creates difficulty because wheat and other cereals containing gluten are widely consumed globally. There are available substitutes for CD patients but not all these substitutes contain all functional and technological characteristics that are available in wheat gluten. These substitutes include rice, potatoes, soybeans, maize, millet amaranth, green banana flour, sorghum and their derived products (Zondanadi *et al.,* 2012: Simpson and Thompson, 2012).

Nutritionally GFD may be healthy or unhealthy depending on the source, processing, storage and food choices the patient makes (Farage and Zondanadi, 2014). Alternative GFD in the market may exhibit negative characteristics such as being low in fiber, iron, folate, niacin, phosphorus and zinc since celiac patients have only one alternative of using refined grain foods and starchy products (Simpon &Thompson, 2012) which are readily available and cheap although whole grains should be recommended. High fatty foods is another unhealthy habit in GFD since these food products have unacceptable texture, firmness and other sensorial features as those containing gluten therefore fat helps to improve these features (Zondanadi *et al.,* 2014).

Locally available food products such as green banana, pumpkin seed and cassava flours can be used as alternative sources in GFD in dealing with celiac disease. This study established that compositing these flours with pumpkin seed flour in bread development lead to improvement in protein, fat, carbohydrates, energy, manganese, iron and zinc which was limiting in green banana and cassava flours. There has been growing interest in gluten free product development using various ingredients. Zondanadi *et al (*2009) used psyllium as a substitute in gluten free bread where chemical, nutritional and sensorial evaluations were performed and results showed revealed that bread from modified dough had less fat and fewer calories with good acceptance by individuals with or without celiac disease. Green banana flour has also been used in production of gluten free pasta. A study by Zondanadi *et al (*2012) performed nutritional and sensorial tests on gluten free pasta and results showed that modified samples had better acceptance (84.5%) for celiac individuals and (61.2%) for non- celiac. Gorgonio *et al* (2011) used pumpkin seed flour and corn starch in production of cakes where the cakes displayed satisfactory macroscopic and chemical characteristics with higher soluble fiber content and fewer calories in relation to standard cakes.

This study had two limitations. When gluten is removed the product should be modified and fabricated and other ingredients added which will make the final product cost higher. Another limitation was on information and education about celiac disease and diet maintenance. Healthy diet compliance and sufficient knowledge (Roma *et al.,* 2010) can assist celiac patients and those that are healthy conscious. Gluten free bread in this study will be available but if individuals are not educated on celiac disease and related conditions diet compliance would be a problem.

Therefore current study established that the gluten free bread from green banana, pumpkin seed and cassava composite flours will be technologically feasible and a good strategy in improving nutritional quality for celiac patients since pumpkin seed will serve as a vehicle in supplementing iron, protein and unsaturated oils which are essential.

4.13 Research Findings

In this study, eight variations of gluten free bread were prepared using green banana, pumpkin seed and cassava flours for potential introduction to the Kenyan market to alleviate celiac disease and gluten sensitivity. The study established that PSF is an important contributor of all nutrients including minerals, proteins and fats except carbohydrates and phosphorus based on results of proximate analyses. Other studies also established that fortification with pumpkin seed flour improved the nutrient density of pan bread (Costa et al., 2018) and cereal bars (Silva, 2012) with reference to minerals, protein and fat. Hence, pumpkin seed has the potential to be used as a fortificant for gluten free bread and baked products in the Kenyan market for health conscious people and those suffering from celiac disease.

This study also found that cassava is an important contributor of up to 47% of the physical and consumer characteristics in gluten-free bread based on optimization when moisture is minimized, though pumpkin seed is not bad either in these characteristics. The sensory characterization results showed that bread with cassava flour had even, fine and soft texture attributes. Cassava flour has been found to possess characteristics similar to wheat flour. For example Nwozu *et al* (2014) who substituted wheat with cassava flour found that bread had an acceptable texture. A similar study by Eliazu *et al* (2014) found that bread made from partial replacement of wheat with cassava had textural sensory attributes comparable to wheat bread.

A unique finding of this study is that compositing improved gluten-free bread, rather than each of the flours independently. Other researchers also demonstrated that blending flours improves chemical, physical and sensory properties. For instance, Bello *et al*. (2018) established that biscuits produced from blends of yellow yam, unripe plantain and 80 pumpkin seed improved the physico-chemical characteristics of biscuits. This may explain the improvement in the same characteristics of the products in this study.

This study also showed that among all the gluten free breads non was equal to WB in all the characteristics, though they were not very different as perceived by consumers based on the acceptability test. This is attributed to the gluten complex of wheat that confers the visco-elastic properties (Dermirkesen *et al*., 2013; Gambus *et al* (2009) enabling wheat dough to hold air and rise producing a texture appealing to consumers. In this study, xanthan gum was used to mimick the characteristics of gluten, hence the bread had almost similar characteristics to wheat bread. Further, the intent to purchase study demonstrated that all the variations of gluten free bread had an equal chance to be purchased if they were available in the market.

4.14 Hurdles

A factor that could have affected the results from the descriptive sensory evaluation was the possible differences among panelists. Psychological differences can cause panelists to vary on perception. For example, Brown and Braxton (2000) found that the perception of texture and preference for rich tea biscuits were affected by chewing duration and production of saliva among the panelist. In the present study, it was possible that the panelist may have differed in terms of surface colour of crust and crumb flavor and texture of breads. These errors were however minimized by giving them references samples throughout the evaluation period.

One of the hurdles in development of the gluten free bread was that the same amounts of ingredients including water were constant to the wheat and cassava mixtures to be too soft. This may have affected their textures. Nonetheless, there was a clear difference in bread types. Another challenge concerned browning in GBB and its level of stickiness during milling of flour and handling. The method of chipping documented by Aurore et 81 al (2009) was used in this study. It is possible that chipping to smaller pieces before drying would improve the milling process. However, the flour had the recommended moisture content for shelf stability.

4.15 Costing

Several food products found in the market including bread, customers rely on a product that has standards in terms of quality attributes and appearance at a perceived lower/affordable price.

Gluten free bread products demand is driven by real and perceived health benefits normally referred to by several names as natural, organic, green and health and therefore food manufacturing companies producing gluten free bread for celiac patients or wheat allergic consumers should meet these standards. Focus on baked food products and snacks should not compromise the core features of healthy nutritious food product since the existing gluten free products in the market are costly due to production and expensive gluten free ingredients.

In order for one to penetrate this market bakers and nutritionist should create strong ties between domestic and international celiac communities in supplying high quality gluten free bread using locally available food products i.e pumpkin seed, green banana and cassava at a better price to fulfill their needs since existing breads fail in quality and price. Therefore the gluten free bread produced should charge what the market will bear.

A rough estimate of production was prepared in table 4.12

From Table 4.12 it is evident that in case the consumer has to purchase the gluten free bread in their pure form PSB would be 1.02% more expensive in relation to WB. Wheat bread on the other hand would be 2.16%, 4.42%, 0.56%, 3.29%, 1.69%, and 1.78% expensive in relation to GBB,CB,GBPSB,GBCB,PSCB and GBPSCB respectively Table 4.12. Considering the benefits in pumpkin seed and market availability of gluten free breads price would not be an issue to consumers. Compositing these flours reduces the price of gluten free bread.

Optimized bread in this study therefore will be produced with the following ratios of flour combinations.

X1(GBF) -0.27-54g

X2(PSF) -0.72-144g

X3(CF) -0.013-2g

Considering the cost of ksh 72 per kg Table 4.12

Optimized gluten free bread produced in this study will cost ksh.98.48 which is cheaper with added functional and therapeutic benefits for celiac patients in relation to WB which cost ksh.106.4 Table 4.13.This bread will meet their cost constraints when locally available food products will be used.

Table 4.12: Ingredients cost for gluten free bread in relation to wheat bread

	GLUTEN FREE BREAD							WHEAT BREAD
INGREDIENTS AND COST	**GBB**	**PSB**	**CB**	**GBPSB**	**GBCB**	**PSCB**	**GBPSCB**	**WB**
GBF(200g)@ksh 70/Kg	14							
PSF(200g)@ ksh 85/Kg		17						
CF (200g)@ ksh 60/Kg			12					
GBPSB(200g)@ ksh 77.5/kg				15.5				
GBCB(200g)@ ksh 65/kg					13			
PSCB(200g)@ ksh 72.5/kg						14.5		
GBPSCB(200g)@ ksh 72/kg							14.4	
WF(200g)@ ksh 80/Kg								16
Sugar(15g)@ ksh 120/kg	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Milk powder(30g)@ ksh 550/400g	41.25	41.25	41.25	41.25	41.25	41.25	41.25	41.25
Yeast(5g)@ ksh 95/100g	4.75	4.75	4.75	4.75	4.75	4.75	4.75	4.75
Xanthan gum(7g)@ ksh 1250/1kg	8.75	8.75	8.75	8.75	8.75	8.75	8.75	8.75
Shortening(30g)@ ksh270/1kg	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1
Egg white(100g)@ ksh 10/g	10	10	10	10	10	10	10	10
Baking powder(3g)@ksh35/100g	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Total cost	**89.7**	**92.7**	**87.7**	**91.2**	**88.7**	**90.2**	**90.1**	**91.7**
Vat @16% INCLUSIVE	**104.1**	**107.5**	**101.7**	**105.8**	**102.9**	**104.6**	**104.5**	**106.4**

Table 4.13 Ingredients cost for optimized gluten free bread in relation to wheat bread

INGREDIENT AND COST	OPTIMIZED GLUTEN-FREE BREAD GBPSCB	CONTROL WB
GBPSCB(200g)@Ksh72/kg	9.20	16
Sugar(15g)@ ksh 120/kg	1.8	1.8
Milk powder(30g)@ ksh 550/400g	41.25	41.25
Yeast(5g)@ ksh 95/100g	4.75	4.75
Xanthan gum(7g)@ ksh 1250/1kg	8.75	8.75
Shortening(30g)@ ksh270/1kg	8.1	8.1
Egg white(100g)@ ksh 10/g	10	10
Baking powder(3g)@ksh35/100g	1.05	1.05
Total cost	**84.9**	**91.7**
Vat @16% INCLUSIVE	**98.48**	**106.4**

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1Conclusion

I. Compositing flours improve nutrient composition of GFB in terms of protein, fat, energy and ash (mineral) composition. Pumpkin seed proved to be the greatest contributor thus making it a potential material in fortifying food products in order to alleviate celiac disease.

II. Quality of GFB was improved by using xanthan gum in terms of weight and specific volume. Compositing flours decreased hardness, improved cohesiveness, chewiness, springiness and resilience.

III. Compositing flours with pumpkin seed imparted positive sensory characteristics such as crusty and chewy crumb, brown color of crumb and crust, texture of crumb, increased springiness, cohesiveness and resilience and reduced hardness and dense texture which are negative characteristics.

IV. Positive sensory characteristics of GFB such as appearance, aroma, flavor and texture improved the total quality of making them score above 6.00 making them desirable to consumers thus improving on the ranking of PSB and its composites thus giving higher rating in likelihood of purchase.

V. Gluten free bread showed slight difference in cost with wheat bread thus proving that the bread would be affordable but its benefits would surpass the cost.

5.2 Recommendations

I. As a way of controlling the associated symptoms and celiac disease public health workers and nutritionists should engage in awareness-raising on GFD thereby encouraging use of locally available food products through compositing them.

II. More studies should be carried out using different gums, formulations of flours and different fortifying agents that are GF to meet the requirements of celiac patients in order to meet the Codex Alimentarious Commission for gluten free products without altering organoleptic and physic-chemical properties of GFD.

III. Further studies should be conducted on clinical trials in using GFB to assess its effectiveness in curbing CD.

IV. Further studies should be carried out to develop GFB using composites of PSF as a fortificant with other cereals and indigenous African legumes. This will enable production of fortified bread for celiac disease patients.

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APPENDICES

APPENDIX I: Application Form For Serving In Trained Sensory Panel

APPLICATION FORM FOR SERVING ON A TRAINED SENSORY PANEL

1. Full name and surname ---

2. Your residential address? ---

----

3. Telephone or mobile cell No. --

4. E-mail address --

5. Your age? ---

6. Are you?	Male	Female	
7. Your occupation or main activity during 16/06/2016-05/07/2016 (e.g. student, house executive etc.)?			
8. Are a registered UoE student?		Yes	No
If yes ,state your student number, course and year of study			
9. Are you a UoE staff member?		Yes	No
If yes, state your personnel number and number of hour/week			

10. Please evaluate your ability to read, speak and write English on the following scale:

Poor ☐ Fair ☐ Average ☐ Good ☐ Excellent ☐

11. Are you allergic to anything?	Yes	No
If yes, give details.		

12. Please specify any specific food product/s that you prefer not to consume.		
13. Do you smoke?	Yes	No
If Yes, how many cigarettes a day?		
14. Will you be available for taste panels as explained during the introduction session on 16/06/2016 to 05/07/2016	Yes	No
15. Have you ever been on any sensory evaluation panel?	Yes	No
If yes, where/when/to evaluate what?		
16. Will you be able to attend the screening sessions on:		
Friday 17 JUNE 2016	Yes	No
Monday 20 JUNE 2016	Yes	No
20. If you are available for the screening sessions, would you attend at this time?		
10h30 -11h30	Yes	No
13h30 – 14h30	Yes	No
21 In no more than 20 words, write down why you think we should choose you for our sensory panel		

I declare that the information furnished above is correct and true to the best of my knowledge.

Signature Date

APPENDIX II: SENSORY EVALUATION CONSENT FORM

SENSORY EVALUATION OF GLUTEN FREE BREAD

Thank you for your willingness to potentially participate in a sensory evaluation project at the Department of Family and Consumer Sciences, University of Eldoret

Date of Participation: 16 June 2016 to 5th July 2016

Voluntary Nature of Participation: I understand that participation in this project is completely voluntary. I do not have to participate in this sensory project. If I do not agree to participate I can withdraw my participation at any time.

Risks to the individual: I understand that I will evaluate gluten free bread from green banana, pumpkin seed and cassava composites using descriptive sensory evaluation. The risk involved in eating the bread samples is no greater than that of eating bread purchased in the retail consumer market. I understand that the product samples may contain green banana, pumpkin seed, cassava, sugar, baking powder, vanilla, yeast, xanthan gum, vegetable oil and milk powder. I note that people who have lactose intolerant should avoid these products.

Confidentiality: participants are not required to reveal any confidential information. All responses to questions will be treated in a confidential manner. Responses to sensory questions via the evaluation form are tracked using numbers only. These numbers are not in any way related to the participant's name.

If you have any questions about this sensory project, contact Songok Lilian. Department of Family and Consumer Sciences, University of Eldoret 0725 325 228

I HAD THE OPPORTUNITY TO READ THIS CONSENT FORM, ASK QUESTIONS ABOUT THE SENSORY PROJECT AND I AM PREPARED TO PARTICIPATE IN THIS PROJECT.

Participant's Signature Date

Participant's Name *please print clearly*

Sensory Panel Leader Signature Date

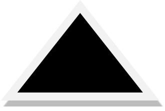
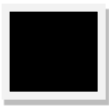
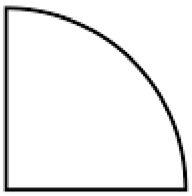
APPENDIX III: DESCRIPTIVE SENSORY EVALUATION

<u>SCREENING TESTS</u>

TEST 1

Name:____________________________ Date: 20-06-2016

Identify the taste on each of the papers

TEST 2

Name: ____________________________ Date: 20-06-2016

Identify the following flavours by smelling. Enter the code name of the sample you have identified against the flavour.

Perceived flavour	**Code**
Lemon flavour	
Banana flavour	
Chocolate flavour	
Pineapple flavour	
Caramel flavor	
Strawberry flavour	
Vanilla flavor	
Passion flavor	

TEST 3

Name:_____________________________ Date: 20- 06- 2016

You are provided with four samples of breads. Please take a sip of water before you start tasting and in between tasting the different samples. Using your own terms, show how the breads differ in taste, flavour, texture and appearance. Use the card provided to indicate the colour that fits each sample.

	501	629	730	150
Appearance				
Texture				
Odour				
Flavor				

APPENDIX IV: DESCRIPTIVE PANEL EVALUATION SHEET

WELCOME TO THIS TASTING SESSION

DEPARTMENT OF FAMILY AND CONSUMER SCIENCES

UNIVERSITY OF ELDORET

PANELIST CODE …………………………………………..

PANELIST NAME ……………………………………………

ENTER TRAY NO. ……………………………………………..

DATE: 8RD JULY, 2016/9th JULY 2016

Instructions

You are provided with (8) samples of bread. Please taste the samples in the order presented from left to right. Take a sip of water and eat a piece of carrot before you start tasting and in between tasting the different samples. Circle the relevant bar on the scale provided for each attribute.

Question 1:

Look at the sample **…………………………..** and rate the following appearance descriptors

Surface colour intensity of the crust

Light crust **DarkBrown crust**

Evenness of surface

Not even **Very even**

Surface shine

Not shiny **Very shiny**

Surface colour intensity of the crumb

Light crumb **Dark Brown crumb**

Roughness of crumb top surface

Pore regularity

Regular white **Irregular**

Compact

Not compact **very compact**

Pore size of crumb

Small pores **Big pores**

Spongy

Not spongy **very spongy**

Fine

Very fine

Not fine

Damp/moist

not damp **very damp**

Question 2:

Smell sample ………using short sniffs and rate the intensity of the following aroma descriptors

Stale bread aroma

No stale bread aroma **Intense stale bread aroma**

Sour milk aroma

No sour milk aroma **Intense sour milk aroma**

Fermented aroma

No fermented aroma **Intense fermented aroma**

Cooked banana aroma

No cooked banana aroma **Intense cooked banana aroma**

Question 3:

Cooked cassava aroma

Taste sample ………………and rate the intensity of the following flavour descriptors

No cooked cassava aroma **Intense cooked cassava aroma**

Sweet flavour

No sweet taste **Intense sweet taste**

Fermented maize meal flavour

No fermented maize meal flavour **Intense fermented maize meal flavour**

Cooked banana flavour

No cooked banana flavour **Intense cooked banana flavour**

Cooked pumpkin flavour

No cooked pumpkin flavour **Intense cooked pumpkin flavour**

Cooked cassava flavour

No cooked cassava flavour **Intense cooked cassava flavour**

Bland flavour

No bland flavour **Intense bland flavour**

Question 4:

Taste sample**……………**and rate the intensity of the following texture descriptors

Crusty texture

Not crusty **Very crust**

Chewy texture

Not chewy sample 109 Very chewy = sample 361

Rough texture of the crust

Not rough **Very rough**

Soft texture

Not soft **Verysoft**

Crumbly texture

Not crumbly) **Compact texture**

Not compact **very compact**

Slimy texture

Not slimy **very slimy**

Plastic texture

Kangumu (Not plastic) **Stiff porridge (Very plastic)**

Damp texture

Not damp **Very damp**

Damp texture

Kangumu (Not damp) **Stiff porridge (very damp)**

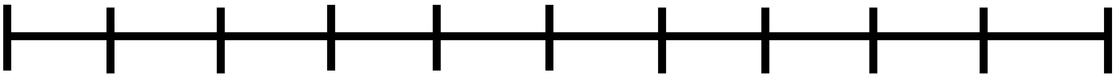
Question 5:

After swallowing the soybeans, rate the after taste of the sample ………………………….

After taste of the crumb

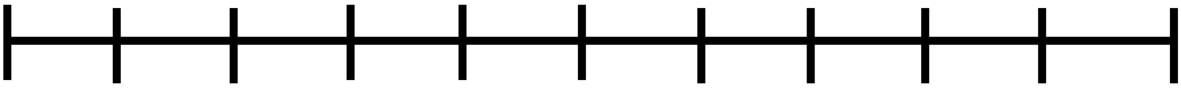
Fermented after taste

No fermented taste **Intense fermented taste**



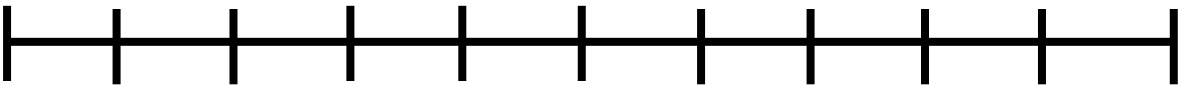
Gritty (grainy) residue in mouth

not gritty **very gritty**



Fibrous aftertaste

Pancake (Not fibrous) **Sample 361(very fibrous)**



THANK YOU

APPENDIX V: Consumer Acceptability Sheet

WELCOME TO THIS BREAD TASTING SESSION.

Age: …………………. Gender: ……………………….. Tray Number: …………………

PART A-INSTRUCTIONS

You are provided with eight (8) samples of bread .Please taste the samples in the order presented from left to right .Take a sip of water before you start tasting and in between tasting the different samples. Indicate your liking or disliking by placing a check mark on at the relevant bar on the scale provided for each attribute.

Sample No.																
Scale	**Appearance**	**Smell**	**Flavour**	**Texture**	**Appearance**	**Smell**	**Flavour**	**Texture**	**Appearance**	**Smell**	**Flavour**	**Texture**	**Appearance**	**Smell**	**Flavour**	**Texture**
Like extremely																
Like very much																
Like moderately																
Like slightly																
Neither like nor dislike																
Dislike slightly																
Dislike moderately																
Dislike very much																
Dislike extremely																
Sample No.																

Scale	Appea rance	Smell	Flavour	Textur e	Appea rance	Smel l	Flavour	Texture	Appea rance	Smell	Flavour	Texture	Appea rance	Smell	Flavour	Texture
Like extremely																
Like very much																
Like moderately																
Like slightly																
Neither like nor dislike																
Dislike slightly																
Dislike moderately																
Dislike very much																
Dislike extremely																

PART B

Rank the 8 samples from the most liked at 1 to the least liked at 8 by entering the sample code in the appropriate position.

1	2	3	4	5	6	7	8

PART C

INTENT TO PURCHASE EVALUATION

On a scale of 1 to 5 (1 being likely and 5 being most likely), indicate with an” X” the likelihood that you would purchase each product if it were available for purchase.

Sample code:_______

1 ____________

2____________

3____________

4____________

5____________

Sample code:_______

1 ____________

2____________

3____________

4____________

5____________

Sample code:_______

1 ____________

2____________

3____________

4____________

5____________

Sample code:_______

1 ____________

2____________

3____________

4____________

5____________

Sample code:_______

1 ____________

2____________

3____________

4____________

5____________

Sample code:_______

1 ____________

2____________

3____________

4____________

5____________

Sample code:_______

1 ____________

2____________

3____________

4____________

5____________

Sample code:_______

1 ____________

2____________

3____________

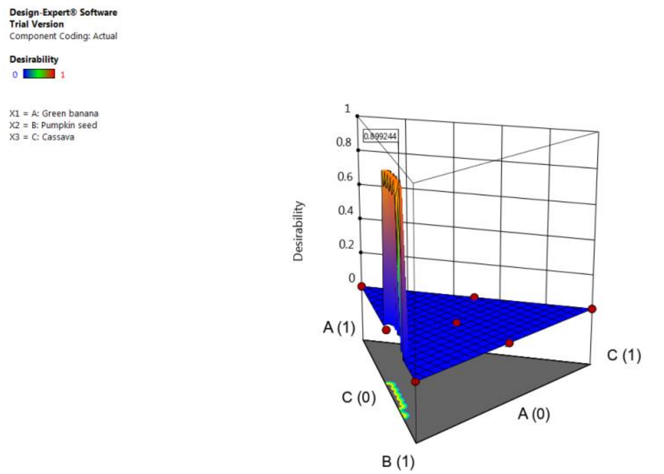
4____________

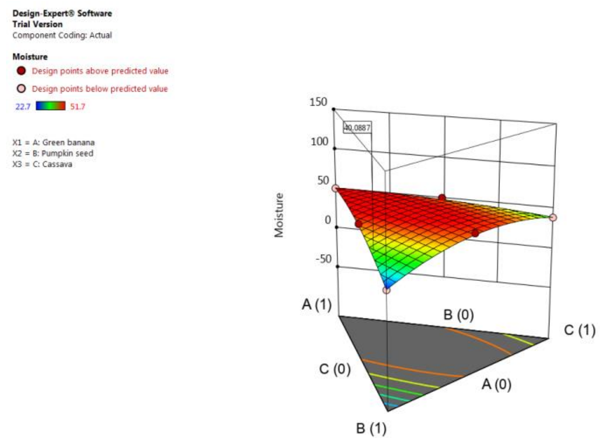
5____________

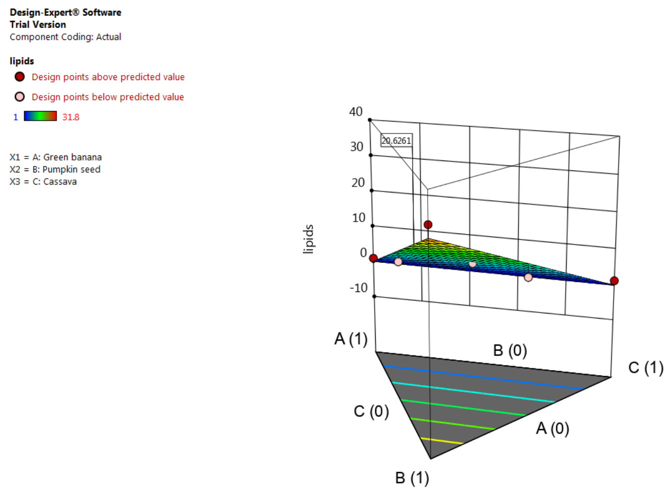
ANY OTHER COMMENTS

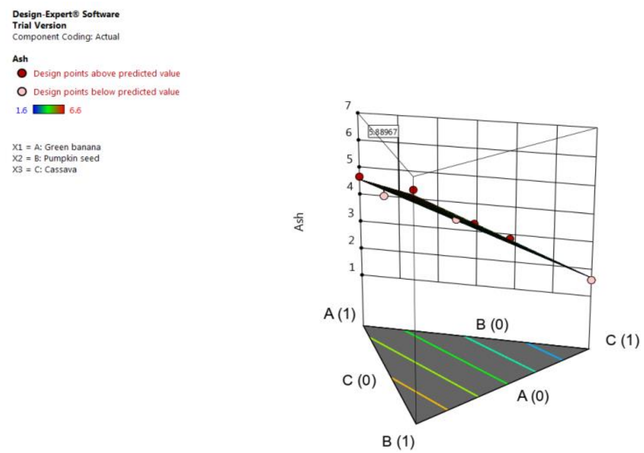
APPENDIX VI: 3D Optimum Surface Plot

Appendix VI. 1: 3D Optimum Surface Plot for desirability, moisture, lipid and Ash

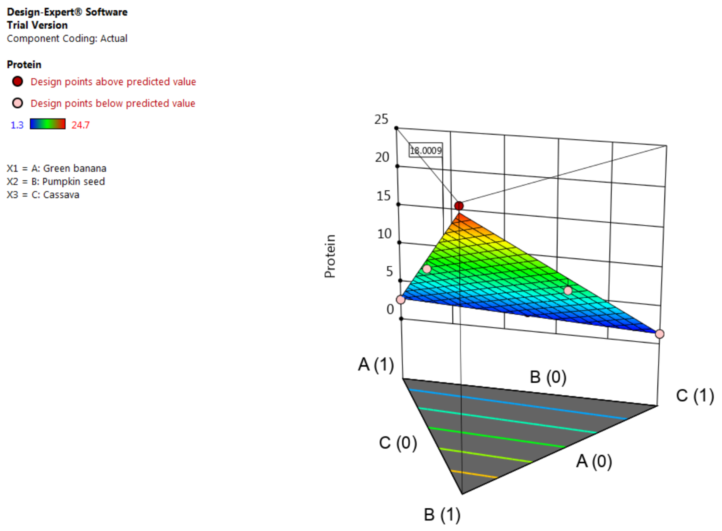


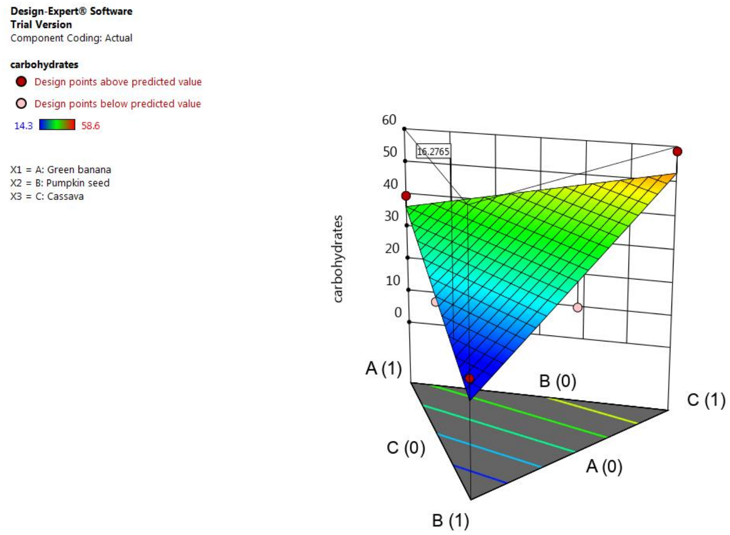


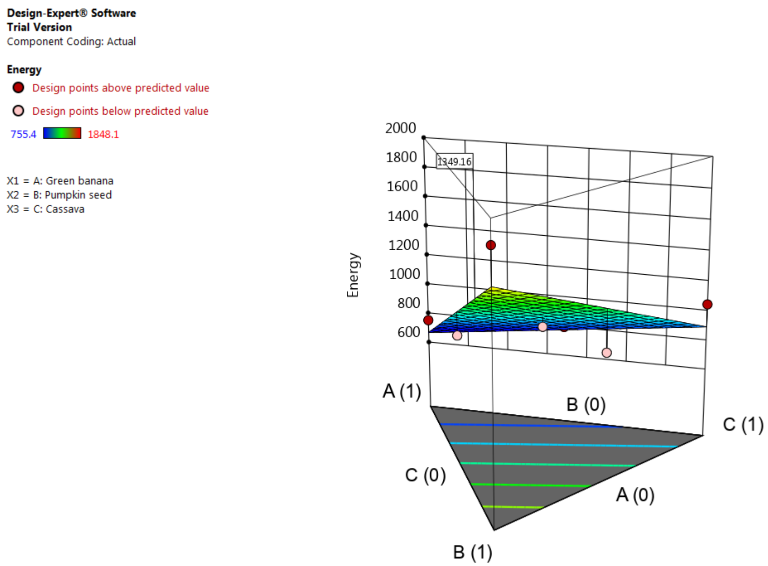


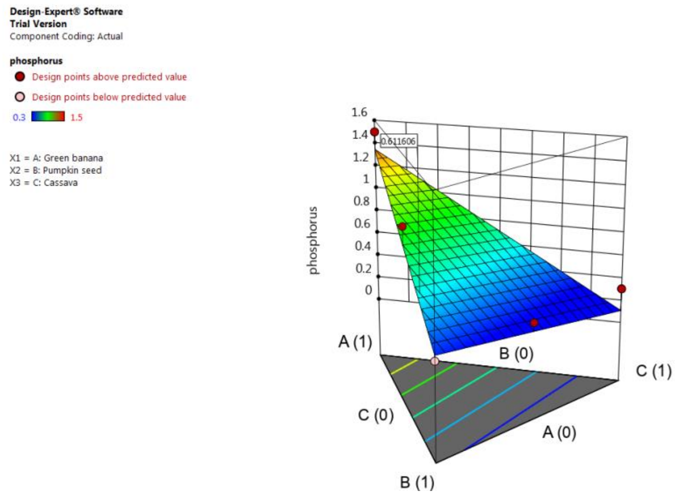


Appendix VI. 2: 3D Optimum Surface Plot for protein, carbohydrate, energy and phosphorus

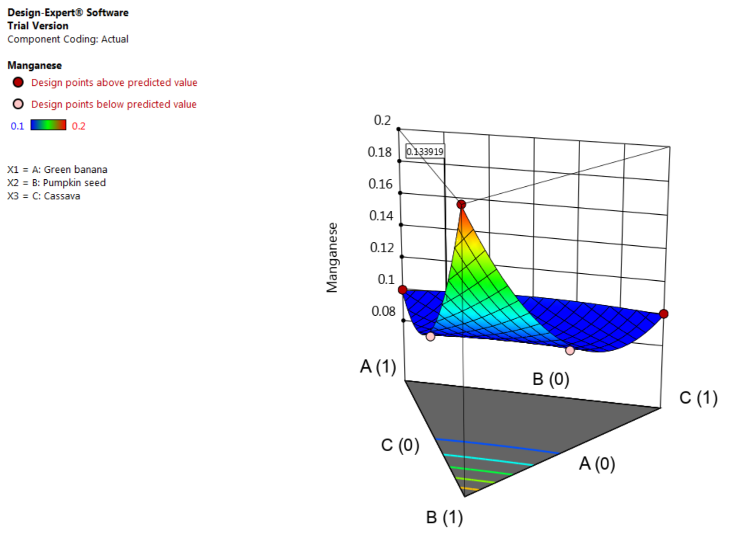


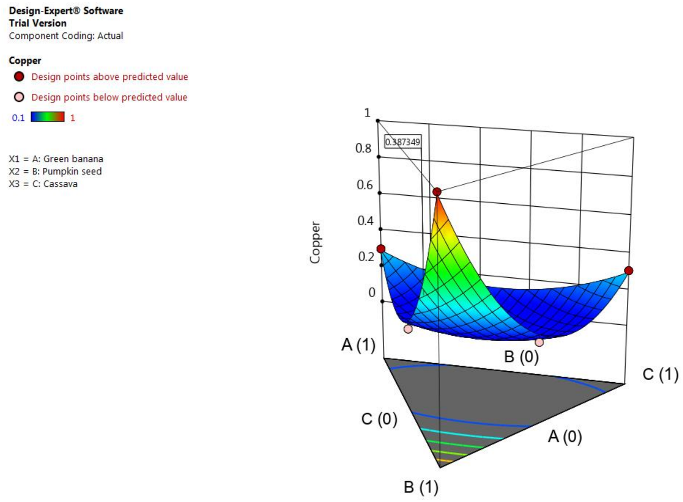


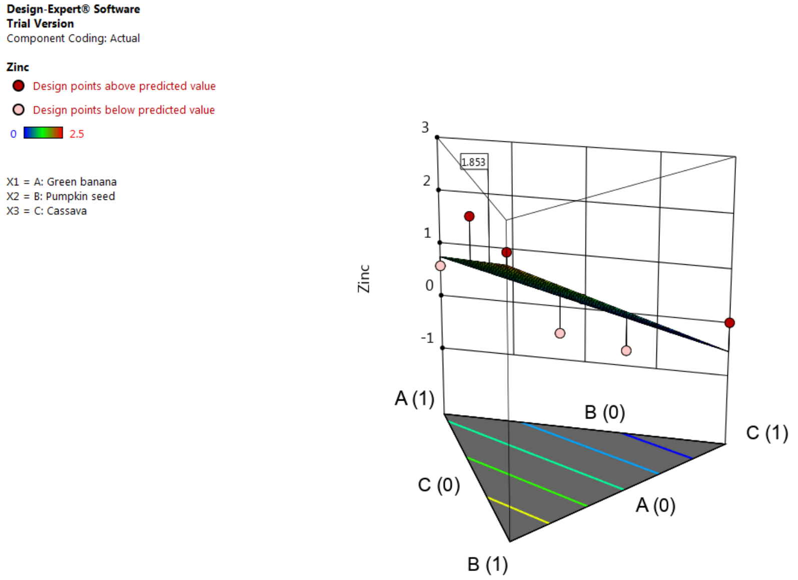


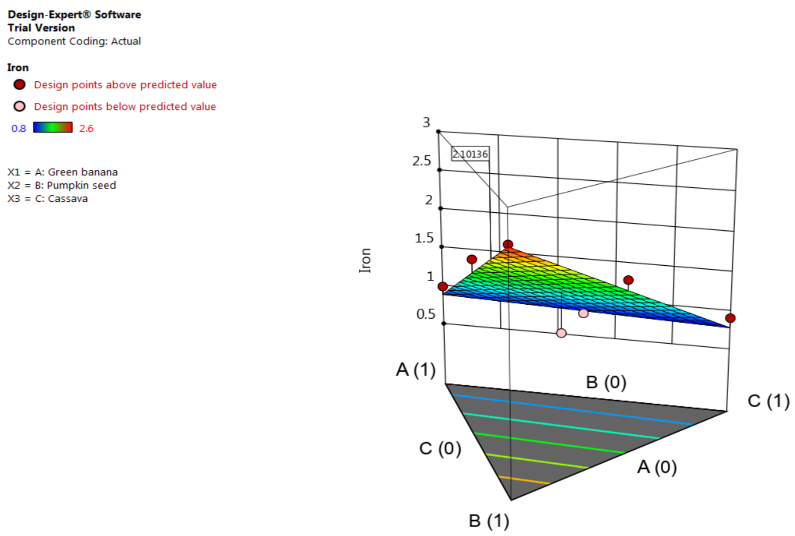


Appendix VI.3: 3D Optimum Surface Plot for manganese, copper, zinc and Iron.

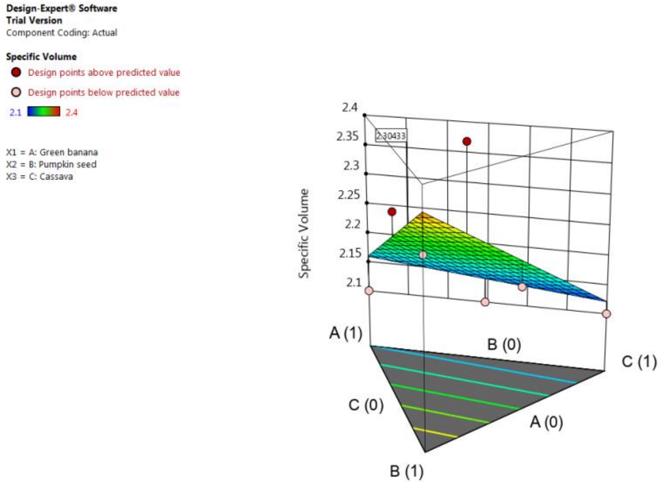


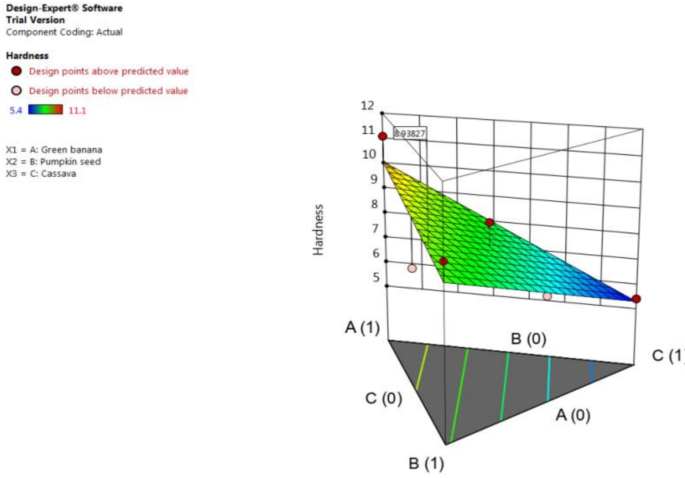


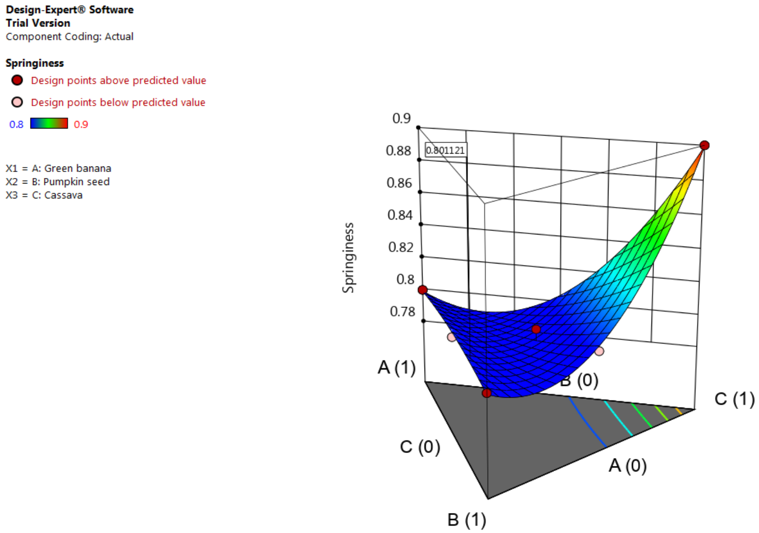


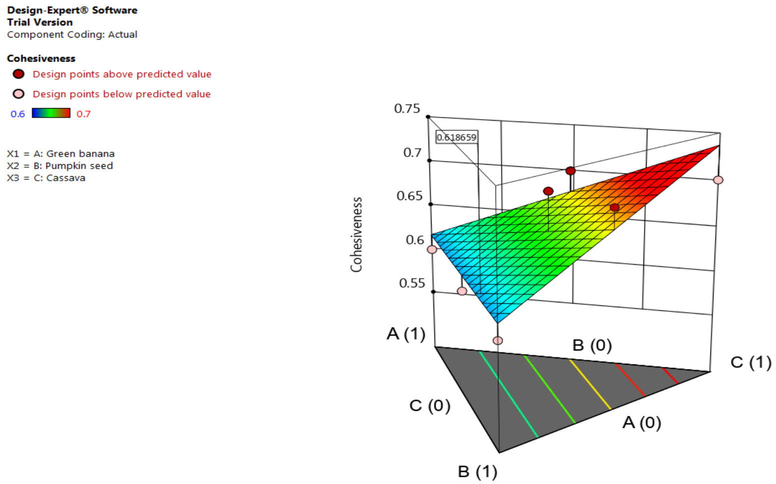


Appendix VI.4: 3D Optimum Surface Plot for specific volume, hardness, Springiness and Cohesiveness

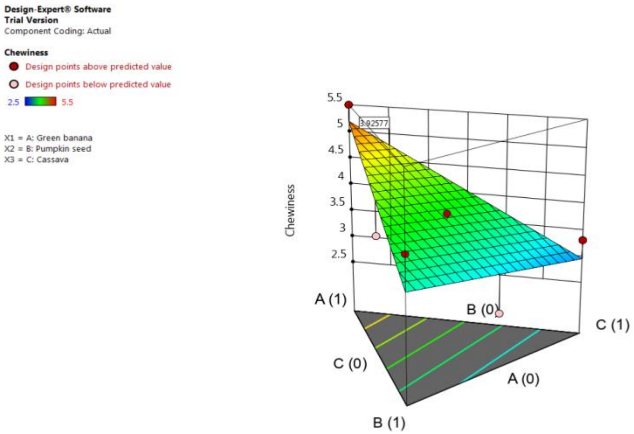


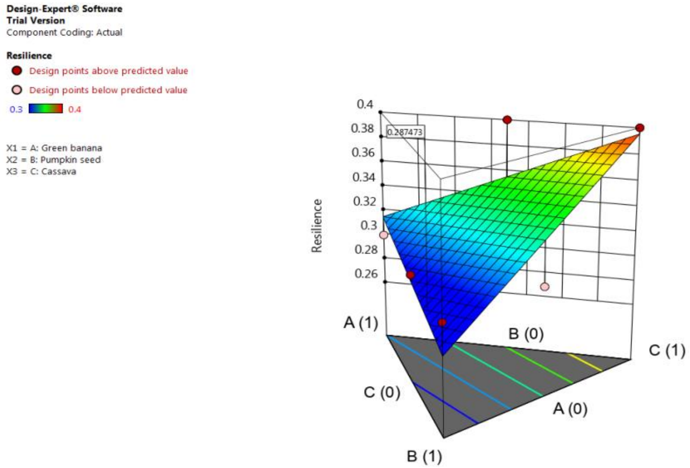


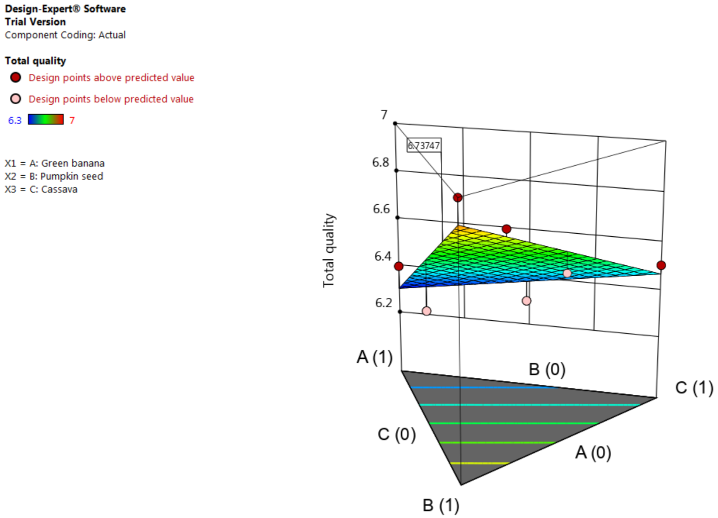


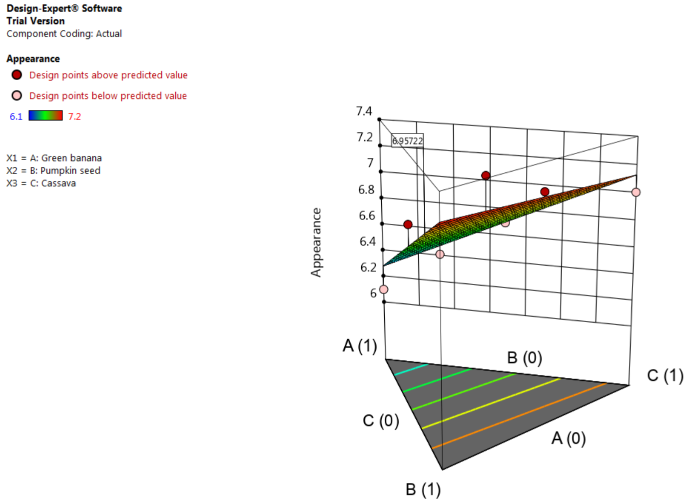


Appendix VI.5: 3D Optimum Surface Plot for Cohesiveness, Resilience, Total quality and Apperance

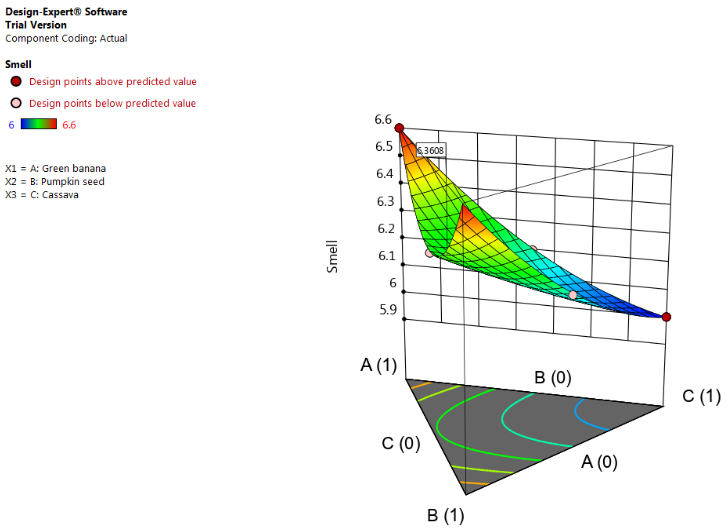


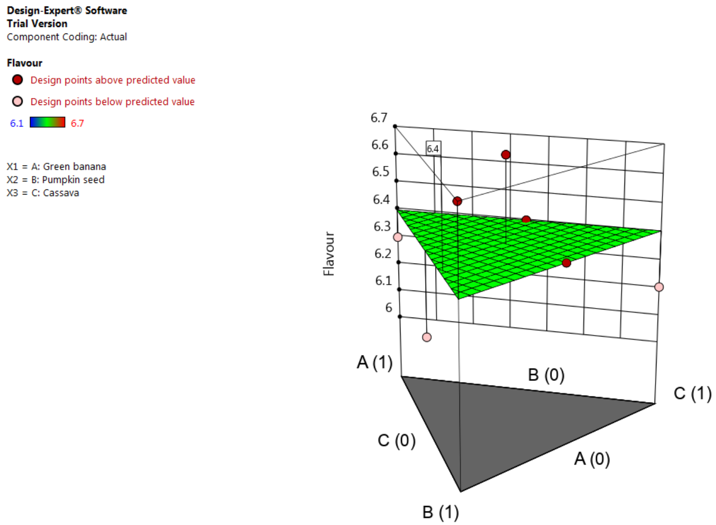


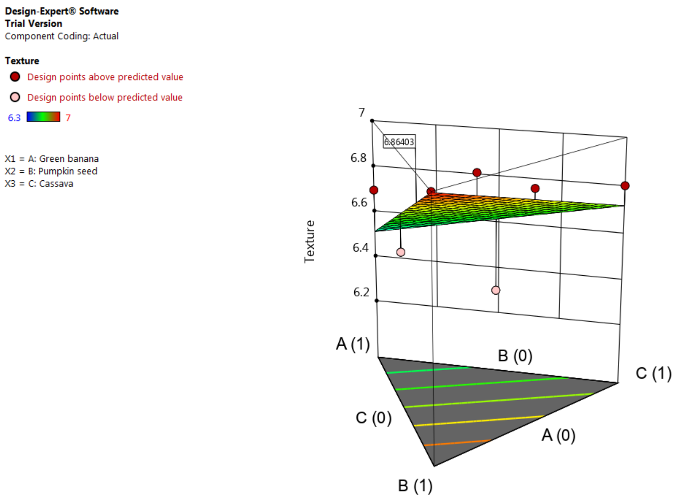




Appendix VI.6: 3D Optimum Surface Plot Smell, Flavor and Texture







APPENDIX VII: NACOSTI Research Permit

THIS IS TO CERTIFY THAT:
MS. LILIAN JEPKEMBOI SONGOK
of UNIVERSITY OF ELDORET, 0-30100
eldoret,has been permitted to conduct
research in *Nairobi, Uasin-Gishu*
Counties

on the topic: *OPTIMIZATION OF GLUTEN FREE BREAD PREPARED FROM GREEN BANANA, PUMPKIN SEED AND CASSAVA COMPOSITE FLOURS*

for the period ending:
13th June,2017

Permit No : NACOSTI/P/16/21631/11478
Date Of Issue : 17th June,2016
Fee Recieved :ksh 1000

....
Applicant's Signature

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

CONDITIONS

1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit
2. Government Officers will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2) hard copies and one(1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice

REPUBLIC OF KENYA

NACOSTI

National Commission for Science, Technology and Innovation

RESEARCH CLEARANCE PERMIT

Serial No. A 9629

CONDITIONS: see back page

APPENDIX VIII: Ministry of Education Research Authorization

REPUBLIC OF KENYA

HARAMBEE

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

STATE DEPARTMENT OF EDUCATION

Telegrams: **"EDUCATION",** Eldoret
Telephone: **053-2063342** or **2031421/2**
Mobile : **0719 12 72 12/0732 260 280**
Email: cdeuasingishucounty@yahoo.com
: cdeuasingishucounty@gmail.com
When replying please quote:

Ref: No. MOEST/UGC/TRN/9/Vol II/191

Office of The County Director of Education,
Uasin Gishu County,
P.O. Box 9843-30100,
ELDORET.

24th June, 2016

Lilian Jepkemboi Songok
University Of Eldoret
P.O Box 1125- 30100
ELDORET

RE: RESEARCH AUTHORIZATION

This office has received a letter requesting for an authority to allow you carry out research on ***"Optimization of gluten free bread prepared from green banana, pumpkin seed and cassava composite flours"*** Within Uasin Gishu County**".**

We wish to inform you that the request has been granted for a period ending **13th June, 2017.** The authorities concerned are therefore requested to give you maximum support.

We take this opportunity to wish you well during this research.

Okumu Stephen
For: County Director of Education
UASIN GISHU.

OK/LS

APPENDIX IX: NACOSTI Research 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

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/16/21631/11478**

Date: **17th June, 2016**

Lilian Jepkemboi Songok
University of Eldoret
P.O. Box 1125-30100
ELDORET.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Optimization of gluten free bread prepared from green banana, pumpkin seed and cassava composite flours,"*** I am pleased to inform you that you have been authorized to undertake research in **Nairobi and Uasin Gishu Counties** for the period ending **13th June, 2017.**

You are advised to report to **the County Commissioners and the County Directors of Education, Nairobi and Uasin Gishu Counties** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

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

Copy to:

The County Commissioner
Nairobi County.

The County Director of Education
Nairobi County.

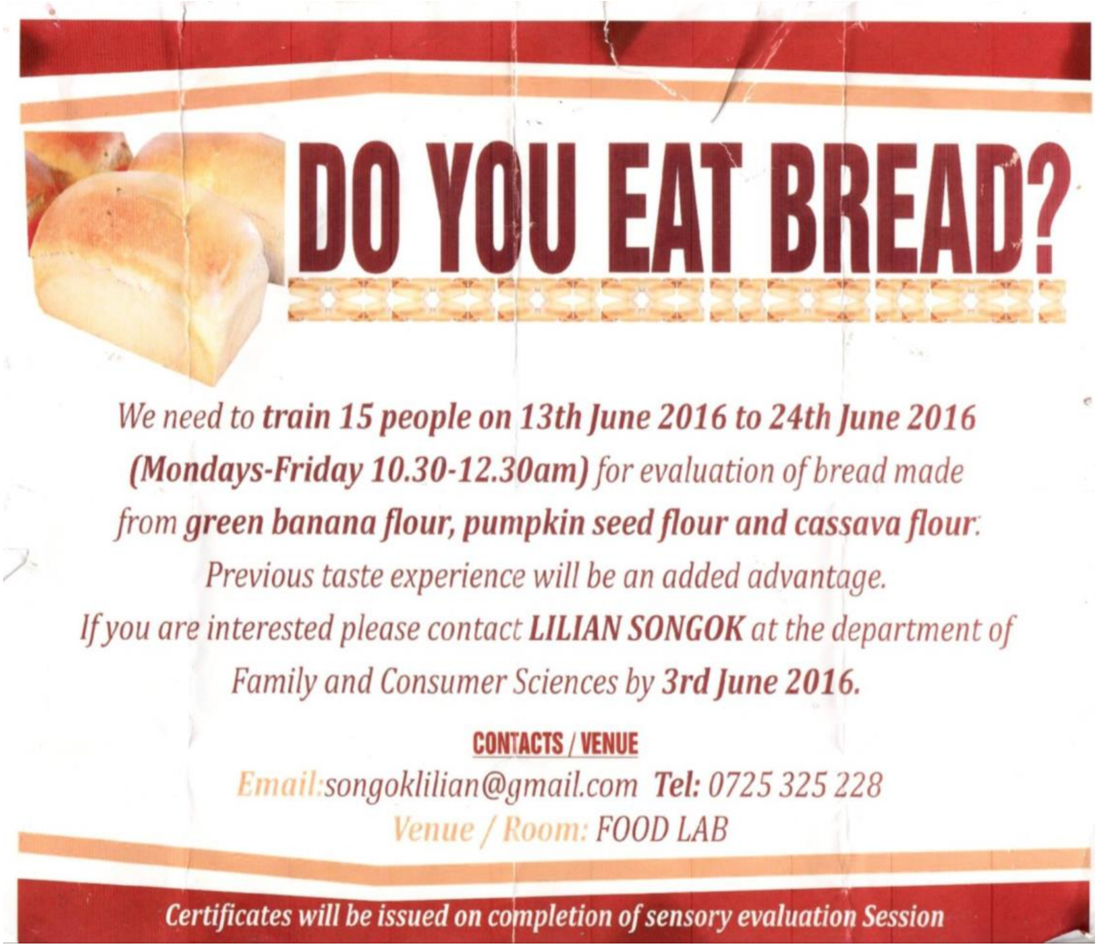
The County Commissioner
Uasin Gishu County.

COUNTY COMMISSIONER
NAIROBI COUNTY
P. O. Box 30124-00100, NBI
TEL: 341666

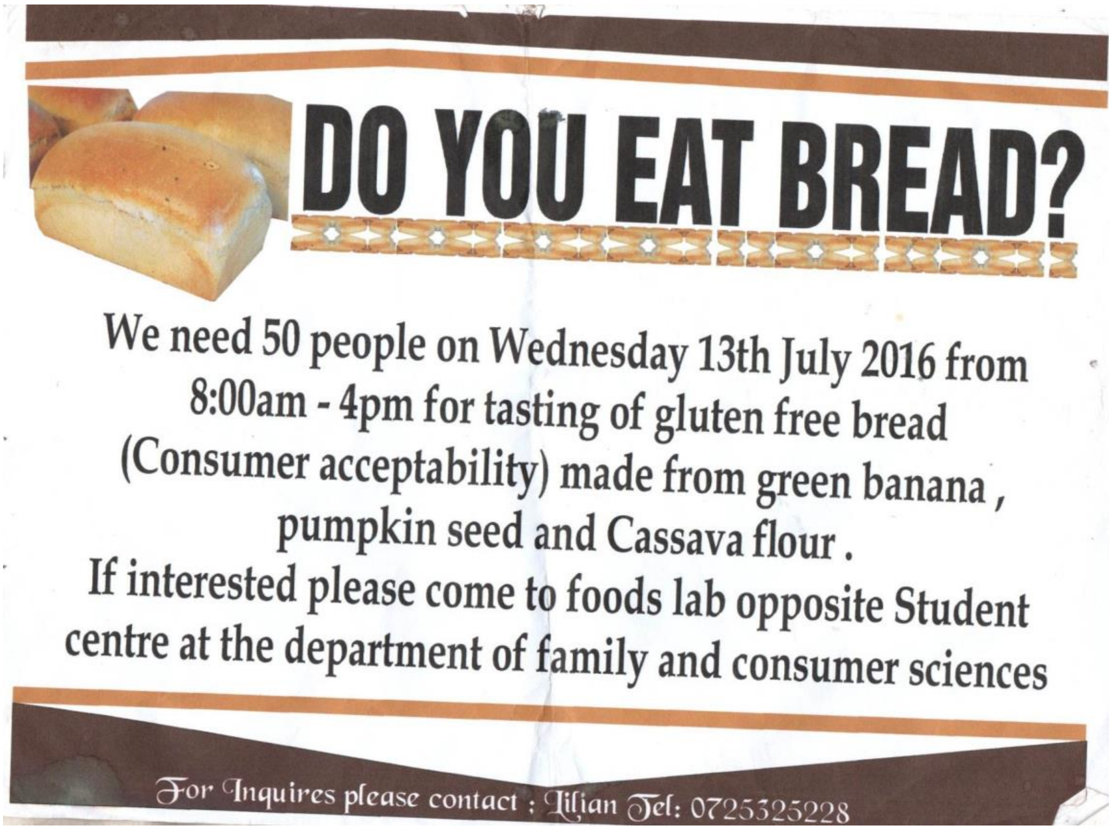
COUNTY COMMISSIONER
UASIN GISHU COUNTY
20/6/16

30 NOV 2016
MINISTRY OF EDUCATION
P. O. Box 74629, NAIROBI.

APPENDIX X: ADVERT ON DESCRIPTIVE SENSORY TRAINING



APPENDIX XI: ADVERT ON CONSUMER ACCEPTABILITY



APPENDIX XII: CONFERENCE PAPER AT UNIVERSITY OF NAIROBI

DETERMINATION OF PHYSICOCHEMICAL CHARACTERISTICS OF GLUTEN FREE BREAD PREPARED FROM GREEN BANANA PUMPKIN SEED AND CASSAVA COMPOSITE FLOURS

Lilian J. Songok [1], Charlotte. Serrem[2], Florence Wamunga[2], Calvince Onyango[2]

[1]Department of Family and Consumer Science, University of Eldoret

[2]Faculty of Agriculture and Biotechnology

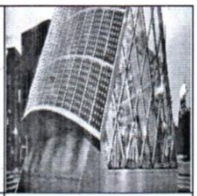
Corresponding author: songoklilian@gmail.com

Abstract

Awareness and increase in diagnosis of celiac disease and gluten intolerance in African countries has created a need for developing improved quality gluten free breads. Locally available food products: green banana, pumpkin seed and cassava flours, which are gluten free and have ideal baking qualities are underutilized in commercial bread production. A study was conducted to develop gluten free bread formulations using green banana, pumpkin and cassava flours and to determine their physicochemical properties. A 3 by 3 simplex centroid design experiment was used where all the components blended together to produce the final product, which constituted 100% of the flour. Eight variations of bread were produced. The first three were (single) composed of 100% each of banana, pumpkin seed, and cassava flours. The next three formulations (binary) contained 50:50 banana: pumpkin, pumpkin: cassava and banana: cassava. The seventh sample (centroid) had banana: cassava: pumpkin at ⅓: ⅓: ⅓ ratios, while the eighth sample served as the control with 100% wheat. The bread loaves were produced using straight-dough procedure. To establish the proximate composition of flours and bread, moisture, crude protein, crude fat, ash, carbohydrate and energy contents were determined using the AOAC internationally approved methods. Pumpkin seed flour and pure pumpkin seed bread recorded higher in ash, lipids, protein and energy with values of 5.37%, 36.22%, 20.07% and 2247.72kj respectively for flour and 6.62%, 38.40%, 29.50% and 2274.87kj for bread. Moisture content varied in both gluten free flours and bread with cassava flour recording higher moisture levels while control wheat flour had less in moisture with mean of 13.12% and 7.10% respectively. However, among gluten free breads pure green banana showed an increase in moisture level of 51.65% while pure pumpkin seed flour maintained a lower level of moisture of 5.46%. Lower levels of moisture

content are beneficial in keeping the shelf life of both flours and bread. Elemental analysis showed that green banana flour and pure green banana bread was most abundant in phosphorus, copper, zinc and iron with values of 0.63%, 073% and 0.95% for flour and 0.51%, 0.28%, 0.57% and 1.02% for bread respectively. Pumpkin seed flour and bread proved to be rich in manganese with a mean of 0.17% and 0.15%respectively. Cassava flour the showed least amounts of manganese and zinc with mean of 0.13% and 0.0% while overall centroid bread green banana pumpkin seed cassava showed least amounts of phosphorus, manganese and zinc with means of 0.21%, 0.05% and 0.0% respectively. Through compositing these raw materials can result in a nutritious final product which is rich in diverse mineral nutrients, affordable and consumed widely. The product will go along in dealing with food security issues, have health benefits both to patients with celiac disease and for those conscious about health.

APPENDIX XIII: CONFERENCE PROGRAM

	1ST ANNUAL CONFERENCE ON SCIENCE FOR DEVELOPMENT: SUPPORTING MANUFACTURING, AFFORDABLE HOUSING, UNIVERSAL HEALTHCARE AND FOOD SECURITY 24TH -25TH OCTOBER 2018	
	COLLEGE OF BIOLOGICAL AND PHYSICAL SCIENCES (CBPS),	
	P.O. BOX 30197 - 00100, NAIROBI, KENYA	
	DAY 1: WEDNESDAY, 24TH OCTBER 2018	
TIME	**ACTIVITY AND VENUE**	
8.00-9.00AM	**ARRIVAL AND REGISTRATION OF PARTICIPANTS. MILLENIUM HALL I**	
9.00-10.30AM	**OFFICIAL OPENING CEREMONY: MILLENIUM HALL I**	
	OPENING CEREMONY SHORT SPEECHES	**MC: PROF. PATRICK WEKE**
	1. CHAIR OF THE ORGANIZING COMMITTEE	
	2: STATUS OF RESEARCH IN SCIENCE: DEAN SPS, DIRECTOR SBS, DIRECTOR SCI, DIRECTOR ICCA	**RAPPT: SR.ADMIN. SPS**
	3. THE PRINCIPAL, COLLEGE OF BIOLOGICAL AND PHYSICAL SCIENCES (CBPS), UNIVERSITY OF NAIROBI	
	4. THE VICE CHANCELLOR, UNIVERSITY OF NAIROBI	
	GROUP PHOTO	
10.30 -11.00 AM	TEA BREAK	
		CHAIR & RAPPOTEUR
11.00-12.00PM	**PLENARY SESSION: MILLENIUM HALL I: KEYNOTE SPEAKER: PROF. CATHERINE NGILA, DEPUTY DIRECTOR , MORENDAT INSTITUTE OF OIL & GAS, KENYA PIPELINE COMPANY**	CHAIR: DR. B. KULOHOMA, RAPP: ....
12.00 - 1.00PM	PARALLEL SESSIONS WITH PARTICIPANT SPEAKERS (15 MINUTES PER SPEAKER)	
	PARALLEL SESSION 1: FOOD SECURITY: VENUE: ROOM 1:MILLENIUM HALL I	
12.00-12.15PM	CYRUS GITHUNGURI - ONFARM ASSESSMENT OF THE MAIZE YIELD GAP OBTAINED BY CURRENT FARMING PRACTICES AND IMPROVED CROP NUTRITION IN SEMI-ARID EASTERN KENYA DURING THE SHORT RAINS SEASON	CHAIR:DR. J. NYONGESA
12.15.12.30 PM	S.D. KIOGORA - WASTE TO WEALTH: BIOLOGICAL INNOVATIONS IN BIO-RESOURCE RECOVERY FOR FOOD SECURITY AND	RAPPT: ....

12.45PM		
12.45-1.00PM	QUESTIONS AND ANSWERS SESSION	
1.00-2.00PM	LUNCH BREAK	
2.00-3.00PM	**PLENARY SESSION: MILLENIUM HALL I: KEYNOTE SPEAKER: DR. NICHOLAS OZOR, EXECUTIVE DIRECTOR, AFRICAN TECHNOLOGY POLICY STUDIES NETWORK (ATPS), NAIROBI, KENYA**	CHAIR: DR. N. ONYANGO
3.00-4.00PM	2 MINS POSTER INTRODUCTIONS FOR EACH POSTER	RAPPT: ....
4.00-5.00PM	TEA BREAK AND POSTER SESSION	
	DAY 2: THURSDAY, 25TH OCTBER 2018	
TIME	**ACTIVITY AND VENUE**	**CHAIR & RAPPOTEUR**
8.45-9.30AM	**PLENARY SESSION: KEYNOTE SPEAKER: DR. WILLIS ODEK, CHIEF OF PARTY/SENIOR TECHNICAL ADVISOR, UNC/MEASURE EVALUATION TANZANIA**	CHAIR: DR. J. MUTEMI
9.30 - 10.15 AM	**PLENARY SESSION: MILLENIUM HALL I: KEYNOTE SPEAKER: PROF. RAPHAEL MUNAVU, PROFESSOR OF CHEMISTRY, UNIVERSITY OF NAIROBI**	RAPPT:.........
10.15-10.30AM	TEA BREAK	
10.30 - 1.00PM	PARALLEL SESSIONS WITH PARTICIPANT SPEAKERS (15 MINUTES PER SPEAKER)	
	PARALLEL SESSION 1: MANUFACTURING: VENUE: ROOM 1: MILLENIUM HALL I	
10.30-1045AM	JACOB OKETCH OKUNGU –ASYMPTOTIC NORMALITY BEHAVIOUR OF A NON-PARAMETRIC ESTIMATOR FOR A FINITE POPULATION TOTAL USING EDGEWORTH EXPANSION (SCIENCE AND TECHNOLOGY FOR SUSTAINABLE INDUSTRIAL TRANSFORMATION IN MANUFACTURING, VALUE ADDITION AND JOB CREATION)	CHAIR: DR. C. OLUDHE
10.45-11.00AM	MOSES NDUNDA - EXTENDING USABILITY OF OLD FIBRE OPTIC CABLES IN THIRD-WORLD ECONOMIC ZONES	
11.00-11.15AM	MMBAGA J - CELLULOSE-ZEOLITE NANOCOMPOSITES: SYNTHESIS AND CHARACTERIZATION USING POWDER X-RD, SEM, EDX & FTIR METHODS FOR LEAD & CADMIUM REMOVAL FROM WATER	RAPPT: ....
11.15-11.30AM	WILSON NGURU - SUPPLIER SELECTION PROCESS BASED ON FUZZY LOGIC	
11.30-11.45AM	LYDIA KITUNGULU - SCIENCE- INVESTMENT PARTNERSHIPS FOR TRAINING AND TECHNOLOGY TRANSFER	
11.45-12.00PM	ABEGA NGONO JEAN MARIE - FINANCING AND EMPLOYMENT OFFER OF THE ENTERPRISES IN CAMEROON	
12.00-12.15PM	NGUGI C.N. - MORPHOMETRICS BASED IDENTIFICATION OF INDIGENOUS ENTOMOPATHOGENIC NEMATODE ISOLATE TK-1	

12.15-1.00PM	QUESTIONS AND ANSWERS SESSION	
	PARALLEL SESSION 2: FOOD SECURITY: VENUE: ROOM 2: ICCA 101	
10.30-10.45AM	NDEDE ELIZAPHAN OTIENO -BIOCHAR EFFECTS ON PLANT AVAILABLE WATER OF SANDY SOILS AS A STRATEGY FOR CLIMATE-SMART AGRICULTURE	
10.45-11.00AM	PETER MWANGI MUCHIRI -AQUACROP SOFTWARE, THE KEY TO STUDYING THE EFFECTS OF CLIMATE CHANGE ON CROP YIELDS	CHAIR: DR J. KATENDE
11.00-11.15AM	TIMOTHY MAYABI WAMALWA - AN ARTIFICIAL NEURAL NETWORK MODEL FOR PREDICTING MAIZE PRICES IN KENYA	RAPPT: ....
11.15-11.30AM	LILIAN SONGOK - DETERMINATION OF PHYSICOCHEMICAL CHARACTERISTICS OF GLUTEN FREE BREAD PREPARED FROM GREEN BANANA PUMPKIN SEED AND CASSAVA COMPOSITE FLOURS.	
11.30-11.45AM	LUKORITO CROMWEL -INCREASING RESILIENCE OF AFRICAN SMALLHOLDER AGRICULTURE TO CLIMATE VARIABILITY AND CHANGE IMPACTS THROUGH VALUE ADDED CLIMATE INFORMATION SERVICES	
11.45-12.00PM	MAUREEN KERUBO - AQUACULTURE FOR FOOD SECURITY: ARE THERE VIABLE ALTERNATIVES FOR TILAPIA (OREOCHROMIS NILOTICUS) FINGERLINGS DIET?	
12.00-12.15PM	EMILY BOSIRE - MODELLING IMPACTS OF CLIMATE CHANGE ON SORGHUM PRODUCTION IN THE SEMI-ARID ENVIRONMENT OF MACHAKOS COUNTY	
12.15-12.30PM	AYIENDA KEMUNTO CAROLINE - DROUGHT EARLY WARNING INFORMATION SYSTEM IN A CHANGING CLIMATE: AGRICULTURE AND LIVESTOCK PRODUCTION IN KILIFI COUNTY, KENYA (SCIENTIFIC RESEARCH, TECHNOLOGY AND INNOVATIONS FOR SUSTAINABLE FOOD PRODUCTION)	
12.30 - 12.45PM	CECILIA NGUGI - MORPHOMETRICS BASED IDENTIFICATION OF INDIGENOUS ENTOMOPATHOGENIC NEMATODE ISOLATE TK-1	
12.45-1.00PM	QUESTIONS AND ANSWERS SESSION	
	PARALLEL SESSION 3: HEALTH: VENUE: ROOM 3: ICCA G1	
10.30-10.45AM	LEONIDA KERUBO OMOSA - CYTOTOXICITY OF PLUMBAGIN, RAPANONE AND 12 OTHER NATURALLY OCCURRING QUINONES FROM KENYAN FLORA TOWARDS HUMAN CARCINOMA CELLS	CHAIR: DR E. M. MWANGI
10.45-11.00AM	MOSES MUTAKI - APPLICATION OF RANDOM SURVIVAL FORESTS AND ACCELERATED FAILURE TIME SHARED FRAILTY MODELS IN UNDERSTANDING UNDER-FIVE CHILD MORTALITY IN KENYA	RAPPT: ....
11.00-11.15AM	IMMANUELLA KIBII - UTILITY OF EDXRF IN ELEMENTAL STUDY OF HERBAL EXTRACT SUSPECTED TO INDUCE PRODUCTION OF OXYTOCIN	

APPENDIX IX: SIMILARITY REPORT

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AGR/PGF/001/012 By Songok Lilian Jepkembol

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