OPTIMISATION OF THE QUALITY OF BREAD FROM WHEAT, BANANA AND CASSAVA COMPOSITE FLOUR

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DECLARATION

I declare that this dissertation presented for the award of a Master's degree in Food Technology has not been wholly or partially submitted in for any other degree or professional qualification.

Signed...

Date........29/11/2013..........................

APPROVAL

This is to certify that Henriettah Nakisozi carried out this research work titled "Optimization of the quality of bread developed from wheat, cassava and banana composite flour" under my supervision. I approve the submission of the work for the award of Master of Science in Food Technology.

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DEDICATION

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ABSTRACT

Bananas and cassava are important staple foods in terms of caloric intake in Uganda. These traditional crops are highly perishable with little value addition. This study examined the prospects of application of banana, cassava and wheat composite flour in the development of bread. Sixteen composite flour blends were prepared using D-optimal constrained mixture design within a range of 40 to 80% for wheat, 10 to 30% for cassava and10 to 30% for banana. Nutrient quality and sensory acceptability of the breads were optimized using design expert software. Baseline assessment on bread consumption was conducted from Kampala city. Information on bread consumption and purchasing behaviour was collected using a questionnaire. Banana (EAHB) cultivar cassava (NASE 19) and wheat were used for bread development. Moisture was analyzed using hot air oven (BTI-29, India), dry ashing for ash using a muffle furnace (FSL 340-0100, Gallenkamp, UK), Kjeldahl method for crude protein, Soxhlet method for crude fat and Gravimetric method for crude fiber. Total carbohydrate content of bread was determined by difference method. Loaf weight and loaf volume were evaluated using a digital laboratory scale (CE-4101, China) and rapeseed displacement method, respectively. Bread firmness was determined using a TA XTplus texture analyzer (Stable Micro Systems, Gudaiming, Surrey, UK). Sensory attributes (taste, odor, color, texture and overall acceptability) were evaluated on a five-point hedonic scale.

Bread consumption was high (66.4%) amongst young people. A large (37.1%) proportion of the population consumed bread daily and sweet bread was mostly (88.2%) liked. Over 70% of respondents preferred white bread to whole meal bread. Large companies (56.1%) were the main purchasing points because bread was considered to be fresh. Freshness (36.4%) was the most important criteria for preference of bread. Blending had a significant effect on carbohydrate, protein, ash, loaf volume, taste and odor ($p<0.05$). Banana addition increased mineral amounts in bread whereas cassava increased total carbohydrate. The optimum blending ratio for both nutritional and sensory acceptability was in the range of 71.2% wheat, 10.6% cassava and 18.2% banana flours. Loaf volume ranged between 803.3 and 1310cm^3. Loaf volume of bread processed from less than 70% wheat flour was inferior. Crumb firmness values ranged from 6.4 to 29.8 N. Bread developed from 50% wheat, 40% banana and 10% cassava had the highest (29.8 N) firmness whereas that made from 80% wheat, 10% banana and 10% cassava had the lowest (6.4N) firmness. It was concluded that controlled processing of banana and cassava flours, maintaining wheat flour around 70% and high proportion of banana flour (about 17%) in the blend, would lead to production of high quality bread with high content of minerals and bioactive compounds such as polyphenols, flavonoids etc. Bread from banana, cassava and wheat was of good quality to consumers.

CHAPTER ONE

INTRODUCTION

1.1 Background

Bread is a fermented confectionery product comprising mainly wheat, yeast, water, sugar, and salt (Ndife *et al.*, 2011). It is a good source of carbohydrates and dietary fibre (Ndife *et al.*, 2011). The dietary fibre content of bread depends on the type of flour and varies between 0.3 and 1.5 g/100 g fresh bread ((Romankiewicz *et al.*, 2017). Dietary fibre positively affects peristalsis of intestines and enables expelling of harmful metabolites ((Romankiewicz *et al.*, 2017)). Cellulose and hemicellulose are main fractions of dietary fibre in bread (Romankiewicz *et al.*, 2017). Bread consumption is high in Uganda and is attributed to taste and convenience (Noorfarahzilah *et al.*, 2014). Teaching institutions, families and casual laborers in cities and towns are the major bread consumers. According to Wojciech *et al.* (2011), 64.7% of urban population in Uganda consume bread compared to 31% of rural population. Bread is served as a snack and used as an ingredient in other culinary preparations such as sandwiches and fried items coated in bread crumbs (Wojciech *et al.*, 2011). It is also eaten as a main course of the meal for example salt bread and French bread.

Wheat flour contains approximately 12% proteins (Wang *et al.*, 2017). These proteins hydrate to form gluten, a viscoelastic matrix holding the starch granules that constitute the bulk of flour. Gluten comprises of a mixture of water insoluble proteins mainly gliadins and glutenins that affect bread structure (Wang & Zhang 2015). Baked bread's structure is a desirable characteristic in baking (Wang & Zhang 2015). Gliadins and glutenins range from 80 to 85% of the total proteins of wheat flour and impart the unique properties especially extensibility and elasticity to the wheat dough (Pauly & Delcour, 2018). Gluten proteins are dominated by hydrophobic amino acids particularly glutamine (Shewry, 2018). This amide bearing amino acid has a strong tendency of forming hydrogen bonds between protein strands which is a major factor in the physical structure of bread (Shewry, 2018). In addition, the glutenin protein chains of the subunits contain thiol groups from the amino acid cysteine, which form disulphide bridges that hold the glutenin macro-polymer and the gluten complex together (Ortolan & Steel, 2017). Aroma of bread is equally important to consumers (Wojciech *et al.*, 2011). Flour naturally contains α- and β-amylases, which breakdown starch in the dough to fermentable sugars, maltose and glucose (Rosell *et al.*, 2006). By artificially increasing the level of amylases in the dough, the quantities of these sugars available for yeast fermentation can be enhanced (Rosell *et al.*, 2006).

Partial substitution of wheat flour with non-gluten rich flour has been widely experimented in bakery (Shittu *et al.*, 2014). Maize, soya, banana, and cassava flour have demonstrated to be feasible in supplementing wheat flour (Shittu *et al.*, 2014). Uganda produces approximately 10.6 million metric tons of plantains (Kawongolo *et al.*, 2016). The cooking type bananas locally known as *Matooke*, a *Musa* spp. triploid acuminate genome group (AAA-EAHB) is the most commonly produced (Kawongolo *et al.*, 2016). Similarly, approximately 2.9 million metric tons of cassava are produced in Uganda (UBOS, 2010). It is estimated that cassava and banana wasted range from 10 to 25% and 22 to 45%, respectively, due to poor handling (Kawongolo *et al.*, 2016). This study focused on the prospects of using banana and cassava for bread making. Cassava is a good source of starch (about 80%) but is limited in proteins, fats and minerals (Montagnac *et al.*, 2009). Starch contributes to water absorption during dough development; gelatinization and pasting behaviour during baking (Calvin, 2016). Banana is rich in minerals especially potassium and bioactive compounds such as phenolics, carotenoids, biogenic amines and phytosterols (Ayele *et al.*, 2017). These compounds have antioxidant activities and are effective in protecting the body against various oxidative stresses (Ayele *et al.*, 2017).

1.2 Problem statement

Bananas are a major staple food in Uganda constituting 70% of the family food basket (Ariho *et al.*, 2015). They are grown by approximately 75% of the country's farmers on 40% of the total arable land (Ariho *et al.*, 2015). Uganda is currently the second largest banana producing country in the world, accounting for 10% of total global production (Karamura *et al.*, 2000). In spite of its high production and large acreage, the crop remains largely underexploited in terms of research and development as well as value addition (Karamura *et al.*, 2000). Bananas are also highly perishable accounting for 22 to 45% postharvest losses (Muranga *et al.*, 2010). Similarly, cassava production is the highest in Uganda estimated at 2.9 million metric tons (UBOS, 2010). Most of cassava production is for local consumption (Lyimo *et al.*, 2007). Its root is the most important part of the crop with a high starch content (25%) (Lyimo *et al.*, 2007). About 10 to 25% of cassava is wasted in Uganda(Lyimo *et al.*, 2007). The products derived from banana and cassava include fried chips, pancakes, crisps, wine, beer, juice and cakes which are high-energy foods. These local crops are also relatively neglected in Uganda and are mainly processed in traditional food preparations such as boiled and roasted forms (Westby, 2013). Use of bananas and cassava as composite flour in the development of bread would reduce postharvest losses and also add value to the two crops. Bread was chosen in this study because of its increasing demand, convenience as well as its commercial value and alternative way to utilise the lowly exploited crops like cassava and banana.

1.3 Justification

Wheat flour has been substituted with soya, maize, plantain, cassava, and millet flour in the bakery industry (Lyimo *et al.*, 2007). However, the prospects of using banana and cassava for bread making in Uganda is still limited. Cassava is rich in starch (about 80%) but is limited in proteins, fats and minerals (Montagnac *et al.*, 2009). Starch increases water absorption capacity during dough development and on interaction with gluten in dough, it creates a stable network that can retain carbondioxide in the dough structure and prevent collapse of bread during baking and cooling. On the other hand, banana is rich in minerals especially potassium, starch and bioactive compounds which have antioxidant activity and are effective in protecting the body against various oxidative stresses (Ayele *et al.*, 2017). Wheat flour for bread has functional proteins (gluten) that favour the processing of leavened aerated bread (Goesaert *et al.*, 2005). Banana serves as an ideal and low cost food source for developing countries where most of the populations rely mostly on bananas for food (Ayele *et al.*, 2017). Conversely, cassava is also a cheap, readily available and reliable source of carbohydrates. Therefore, partial substitution of wheat flour with banana and cassava flours has potential to enhance the nutrients and bioactive compounds and would help produce high quality bread. In Uganda, wheat is grown on a very small scale. The country's demand is met through importation which makes it and its products very expensive. Therefore, substituting wheat flour with cassava and banana in baking would reduce the bread production cost. In addition, this will reduce on the heavy reliance on wheat as a raw material and will provide income to farmers who grow bananas and cassava leading to increased employment and product diversification.

1.4 Objectives

1.4.1 General objective

To optimize the quality of bread developed using wheat, banana and cassava composite flour.

1.4.2 Specific objectives

1. To conduct a baseline assessment on bread consumption in Kampala city.
2. To determine the physical properties (loaf weight, loaf volume, crumb firmness) of bread developed from wheat, banana and cassava composite flour.
3. To determine the proximate composition of the bread developed using wheat, banana and cassava composite flour.
4. To determine the sensory properties (aroma, crumb colour, texture, taste and overall acceptability) of bread developed using wheat, banana and cassava composite flour.

1.5 Hypothesis

Substitution of more than 30% of wheat flour with banana and cassava flour produces bread which is heavy and of reduced loaf volume.

CHAPTER TWO

LITERATURE REVIEW

2.1 Banana production

Bananas and plantains *(Musa* spp.) are the world's fourth most important food crop after rice, wheat, and maize(Tripathi *et al.*, 2007). Over 120 countries grow the crop with an annual world production of around 104 million tons (Tripathi *et al.*, 2007). India is the largest producer of bananas, contributing up to 27% of world's banana production (Mohapatra *et al.*, 2010). Uganda ranks second after India in total banana production with an annual output of 9.84 million tonnes(UBOS, 2010). The Western and Central regions are the main banana producing regions in Uganda contributing approximately 61% and 30% of the national banana crop, respectively (Komarek, 2010). Production is mainly by smallholder farmers owning an average of < 0.5 ha, with medium-sized farms gaining prominence in Southern Uganda (Ouma *et al.*, 2014). Isingiro, Mbarara and Bushenyi districts in Southwestern Uganda are the major banana producing districts (UBOS, 2010). There are several types of bananas produced in Uganda classified as cooking bananas, roasting bananas, dessert bananas and brewing bananas (Byarugaba *et al.*, 2014). The cooking bananas, locally known as *Matooke* are the most widely produced in Uganda (Table 1).

Table 1: Estimated annual banana production in major banana growing countries

	Total production (tonnes)	Dessert banana production (tonnes)	Cooking banana production (tonnes)
World total	133,691,965	78,860,773	54,831,192
India	27,575,000	17,075,000	10,500,000
China	8,926,308	500,000	8,426,308
Uganda	9,840,000	569,000	9,783,100
Philippines	8,645,749	5,790,091	2,855,658
Brazil	6,892,622	6,402,622	490,000
Ecuador	6,739,739	6,145,527	594,212
Colombia	5,405,365	2,587,625	2,817,740
Indonesia	5,359,115	3,289,115	2,070,000
Rwanda	3,263,462	250,000	3,013,462
Nigeria	3,222,000	315,000	2,907,000
Others	47,822,605	35,936,793	11,373,712

Source: Lescot, 2015

2.2 Bananas and their origin

Bananas belong to the family *Musaceae*, within the order *Zingiberales*, subclass Liopsida division *Magnoliophyta* with two genera; *Musa* and *Ensente* (Stover and Simmonds, 1987). The genus *Musa* is comprised of all the edible bananas and plantains with over 50 species in five sections namely *Eumusa, Australimusa, Rhodachlamys, Callimusa* and *Ingentimusa*. Amongst these, *Eumusa* is the biggest and geographically most widely distributed section of the genus giving rise to the great majority of the edible bananas worldwide (Stover and Simmonds, 1987). Almost all of the 300 or more cultivars arose from two seeded diploid species, *Musa acuminata* Colla and *M. balbisiana* Colla. These are diploid, triploid and tetraploid hybrids of these genomes (Ploetz *et al.*, 2003) and the edible bananas are classified into different genomic groups: AA, AB, BB, AAA, AAB, AAAA, AAAB, and ABBB (Bakry *et al.*, 2009). The botanical classification based on agro-morphological variations helps in the differentiation of land races of dessert bananas (AA, AAA, AAB), cooking bananas (AAA, AAB, ABB), and plantain cooking bananas (AAB). However, more complete morpho-descriptors or molecular markers have led to classification of genomic subgroups for triploid bananas (Bakry *et al.*, 2009). Bananas have developed various economic uses based on their different morphological characteristics.

2.3 Banana consumption and utilization in Uganda

Uganda has the highest capita consumption of banana in the world estimated at 172 kg/person/year(Haggblade & Dewina, 2010). The cooking type (East African Highland Banana (EAHB) AAA genome group) locally referred to as *Matooke*is the preferred staple food in most regions of Uganda prepared by steaming wrapped in banana leaves. '*Pisang awak*' (local name '*Kayinja*', from the ABB genome group) is mainly used for brewing. The dessert banana '*Kisubi*' (AB genome group) and some EAHB AAA cultivars (e.g. '*Mbidde*') are also used for juice extraction and brewing. Some of the popular food uses of banana are chips, raw ripened fruit, cooked green banana, fermented and unfermented beverages, juice, puree, dried flour for cakes and infant formula food (Mohapatra *et al.*, 2010). Although bananas are widely consumed fresh in most parts of Uganda, their conversion into processed secondary products is still limited (Sole, 2005). Bananas undergo less industrial processing largely because investigations on their use, especially at unripe stage and suitability for various types of processing are still limited (Aurore *et* *al*., 2009). Brewing is the most common form of value addition among the value chain actors, being practiced by 25% of the farmers (Ariho *et al.*, 2015). The proportion of people consuming ripened bananas is 9%, banana juice (5%), banana crisps (2%), pancakes (1%) and roasted bananas (5%) (Ariho *et al*., 2015). All these products are commonly made from dessert bananas; *Sukali ndizi* and *Gros Michel*.

Banana flour has been used in production of a number of bakery products including cakes, cookies, crackers and bread. According to Langi *et al.* (2014), Goroho banana (*Musa acuminate,* sp), a plantain, locally grown in North Sulawesi was processed into flour to be used as a substitution material in the making of traditional cakes. In addition, White and Hotchkiss (2015), developed bread from cowpeas and plantain flour. It can be concluded that banana flour employed in this study could provide an opportunity for using local raw materials for use in high mineral breads in Uganda.

2.3.1 Banana-based products

2.3.1.1 Pancakes

This is a cake processed from ingredients consisting of flesh of dessert banana cultivars and cassava flour. Banana flesh is crushed manually and mixed with cassava flour to make a paste. The paste is molded into a disc shape and deep-fried with vegetable oil on strong fire until the surface turns golden brown for finishing. *Sukali ndiizi* (apple bananas) is normally used. Alternatively, the AAA cultivar of dessert banana, *Gros Michel*, can also be used but the pancakes made from *Sukali ndiizi* taste better.Pancakes are packaged in cling film and preserved with baking soda.

2.3.1.2 Banana cake

Ripe bananas mainly *Sukali ndizi* and *Gros Michel* are cut into pieces or mashed, followed by pulping using a blender. A mixture of wheat flour and sugar is added to the banana pulp and stirred. Then, lightly beaten eggs are added to the mixture. Once the mixture is combined, vanilla essence is added to improve flavour. The mixture is poured into a greased cake tin or bread tin and baked in a preheated moderate oven for about 30 to 45 min at 180°C depending on oven type.

2.3.1.3 Banana chips

Banana chips or crisps are hard, brittle fried products abruptly releasing energy that gives rise to characteristic sound effects when bitten. They are the most popular post-harvest processed products of cooking bananas in East Africa (Adeniji *et al.*, 2001). They are one of the most important foods usually fried in the form of crisps or sometimes in form of French fries. They are prepared by deep-frying round slices of unripe or slightly ripened plantain pulp in vegetable oil, and can be preserved for a long time given adequate packaging and storage facilities.

2.3.1.4 Banana beverages

Beverages made from bananas include juice, brewed beer, and distilled spirits. Juice is more often made for home consumption but brewed beer and distilled spirits are often processed by farmers for commercial purposes.

Banana wine: this is made from overripe bananas from *Mbidde* clone set particularly *Kisubi* cultivar. This shows the need for prospective studies to improve value of *Matooke*. Overripe bananas are used because of presence of high level of sugars in the fruit. The peels complement the pulp in providing the nutrients required for yeast and impart natural colour to the resultant wine. Water, sugar and yeast nutrients are also required in varying quantities.

Banana beer; Banana beer is made from ripe (but not over-ripe) East African Highland Bananas (Rietveld and Mpiira, 2013). To accelerate the ripening of bananas, a pit is dug in the ground, lined with dried banana leaves which are then set on fire to create warmth in the pit. Fresh banana leaves are laid on top of bananas wrapping very well to maintain warmth, and then the unripe bananas. These are then covered by more layers of fresh banana leaves and pseudostems. After 4-6 days, the bananas are ripe enough (Mohapatra*et al.*, 2010). This method only works in the dry season. During the rainy season, bananas are ripened by placing them on a hurdle near a cooking fire(Rietveld and Mpiira, 2013).This is known as rack method of ripening (Kyamuhangire *et al.*, 1996). *Kayinja* is the most common banana cultivar used for banana beer in Uganda. Once ripened, the bananas are peeled. After peeling, the bananas are kneaded using spear grass until soft. The juice is then strained and filtered to obtain clear banana juice, which is then diluted with water. Sorghum lightly roasted, milled into course flour and then added to the juice. This mixture is left to ferment for 24 hr and then filtered(Mohapatra *et al.*, 2010). After filtering, the beer is packaged in glass or plastic bottles. In commercial production, the beer may first be pasteurized before packaging to halt fermentation and extend shelf life.

2.4 Post-harvest losses of bananas in Uganda

Bananas are highly perishable with shelf life of 6 to 10 days at ambient temperature (Tock *et al.*, 2010; Nakasone and Paull, 1998; Robinson, 1996). About 35% of harvested bananas in Uganda are wasted as a result of little or no processing and poor post-harvest handling (Tribess *et al.*, 2009;

Rodriguez-Ambriz *et al.*, 2008).Post-harvest losses are caused by high respiration rates, biochemical changes associated with respiratory metabolism, ethylene biosynthesis, compositional changes, physical injuries, water loss and pathological breakdown (Adewumi *et al.*, 2009).Diversification of banana use is therefore required to reduce wastage and add value to the crop.

2.5 Nutrient composition of bananas

Bananas are important sources of calories providing 67 - 137 cal/100g fruit (Chadha, 2007). The fruit is abundant in indigestible compounds; resistant starch and non-starch polysaccharides which make up its dietary fiber (Ovando-Martinez *et al.*, 2009). Resistant starch and non-starch polysaccharides have low glycemic index or low digestibility making them desirable for diabetic patients. This property is desirable in foods such as bread (Cheirsilp & Umsakul, 2008; Aparicio-Saguilán, *et al.*, 2007; Agunbiade, *et al.*, 2006). Bananas also contain vitamins A, B (thiamine, riboflavin, niacin, and B_6), and C. Protein content of banana fruits varies depending on variety, altitude, and climate, and ranges between 3.8 and 4.2% (Akaninwor & Sodje, 2005; Lustre *et al.*, 1976; Loeseck,1950). The fat levels (1%) in pulp remains almost constant. Potassium is the most abundant mineral present in edible portion of bananas, followed by magnesium, calcium, and phosphorus (Leterme *et al.*, 2006). Potassium can help to decrease blood pressure in individuals who are potassium deficient and also reduces the risk of stroke (Debjit *et al.*, 2012). Bananas also contain bioactive compounds including flavonoids, alkaloids, phenols, steroids, glycosides, terpenoids, saponins, vitamin C, A, B_6, and B_{12}, gallic acid, and volatile components (Ayodele & Erema 2010). These bioactive compounds help reduce the blood sugar levels (Ayodele & Erema 2010; Zakpaa *et al.* 2010).

2.6 Cassava production

Cassava (*Manihot esculenta*) is a major food crop in the humid and sub-humid parts of Africa and a major source of dietary energy for millions of people in these regions (Asiedu *et al.*, 1992). World production of cassava is 160 million tonnes of fresh roots, with 80 million tonnes produced in Africa, out of which 2.9 million tonnes come from Uganda (UBOS, 2010). Cassava is essentially a carbohydrate food with about 75 to 83% and less than 2% protein (Ukpabi & Dafe, 1999; Cock, 1985). It emerges as a dominant staple of primary or secondary importance in many developing countries of the humid and sub-humid tropics in Africa and elsewhere. In Uganda, it is the most important root crop in terms of food security, employment creation, and income generation for crop producing households (Ugwu &Ukpabi, 2002).

2.7 Cassava deterioration

Cassava tubers are highly perishable and begin to deteriorate two to three days after harvesting (Asiedu *et al.*, 1992). After harvesting, the roots are susceptible to spoilage and begin to deteriorate 48 hr if no preservation measures are taken (Asiedu *et al.*, 1992). According to Cock (1985), post-harvest deterioration of cassava is related to two separate processes: physiological changes and microbial changes. Physiological deterioration often starts within 24 hr after harvest due to enzymatic effects, while microbial deterioration usually beings within a week of microbial growth. Therefore, cassava root must be processed as soon as possible after harvest to stop physiological processes and subsequent deterioration.

2.8 Uses of cassava in Uganda

Cassava is important, not only as a food crop, but even more as a major source of income for rural households (UBOS, 2010). As a food crop, cassava has some inherent characteristics, which make it attractive, especially to the small holder farmers. It is rich in carbohydrates that give it many end uses (Westby, 2013). Cassava provides a basic daily source of dietary energy (Falade & Akingbala, 2010). Approximately 30% of cassava produced is consumed by the producers, whilst the rest is sold on markets and processed into various indigenous products (Westby, 2013). Roots are processed into pastes, flours, fried cassava chips, starch or consumed freshly boiled or raw (UBOS, 2010). The leaves are also consumed as a green vegetable, which provides protein and vitamins A and B (Falade & Akingbala, 2010). Fresh roots and leaves are usually consumed or marketed close to their centres of production because of their perishability (UBOS, 2010).

2.9 Composition of cassava roots

Cassava roots contain about 55 to 65% moisture, 30 to 35% carbohydrates on fresh weight basis and 80 to 90 % on dry matter basis (Table 2). The starch content represents 80% of the carbohydrates produced (Montagnac *et al.*, 2009). Cassava is a poor source of protein as it contains only 1 to 3% protein on dry matter basis (Montagnac *et al.*, 2009) and is low in essential amino acids such as methionine, lysine, tryptophan, phenylalanine and tyrosine (Falade & Akingbala, 2010). Cassava contains two cyanogenic glucosides, namely linamarin and lotaustralin, present in all parts of the plant with the highest concentration in the root peel (Montagnac *et al.*, 2009). Normal levels of cyanoglucosides range from 31 to 630 ppm calculated as mg HCN/kg of fresh cassava root, although the content varies considerably depending on variety, climate and environmental conditions (Montagnac *et al.*, 2009). Sweet cassava varieties have often lower levels of cyanide than bitter varieties but there is no established correlation between the taste and the toxicity (Falade & Akingbala, 2010). In this study, sweet cassava varieties were used to develop composite bread to minimize effect on sensory properties.

Table 2. Proximate composition of fresh cassava roots

Component	Value (g/100 g)
Moisture	59.4
Carbohydrates	38.1
Protein	0.7
Fat	0.2
Crude fiber	0.6
Ash	1.0
Calcium(mg/100 g)	50.0
Vitamin C (mg/100 g)	25.2
Energy (kcal/100 g)	157

Source: Balagopalan (1988)

2.9 Flours used in bread making

2.9.1 Cassava flour

Cassava flour is one of the derivatives from cassava roots whose processing technology is cheap and easy (Abass *et al.*, 1998). Cassava flour is traditionally obtained from receiving roots which later are washed and/or peeled and then grated or chipped into slices or chips (UBOS, 2010). These can be dried on concrete floors by the sun, on trays or in artificial dryers (rotatory, trays, fixed bed or flash). The commonest technique applied is drying cassava chips by the sun, which depends on sunny seasons and large spaces. The dried chips are milled and the flour is sieved to obtain refined flour which finally is packaged and stored (Ospina, 2017).

A number of studies on the use of cassava flour in bread making have been conducted (Ospina, 2017). Most of the studies revealed that wheat flour can be replaced by 5 to 10% cassava flour without significant effects on processing and bread quality (Ospina, 2017). Substitutions of up to 30% have been made to obtain organoleptically acceptable breads (Jensen *et al.*, 2015; Defloor *et al.*, 1993). Some factors that influence the quality of bread made with wheat-cassava composite have been studied. These include; variety of cassava (Eriksson *et al.*, 2014; Eduardo *et al.*, 2013; Henao & Aristizábal, 2009; Shittu *et al.*, 2007), maturity of cassava (Defloor *et al.*, 1994; IITA, 1986), and the time and temperature of baking (Shittu *et al.*, 2007). Studies have shown that cassava flour absorbs a larger amount of water than wheat flour which could be explained by the higher starch content in cassava (Eduardo *et al.*, 2013; Defloor *et al.*, 1993). Henao &Aristizábal (2009) found that using 10% of cassava flour, the dough yield increases by 1.4%.

2.9.2 Wheat flour

Wheat is considered an important source of energy: it provides between 1220 and 1450 kJ per 100 g of cereal. Starch is the major component of wheat (61 to 65%) (Defloor *et al.*, 1993). Wheat is also an excellent source of dietary fibre (9 to 12%), composed mainly of cellulose, complex xylans, lignin and ß-glucans. The protein content of wheat is one of the highest in cereals, ranging between 10 and 15%, although deficient in lysine, one of the essential amino acids (Nwanekezi, 2013). The predominant amino acids are glutamic acid, proline, leucine and aspartic acid and are mainly concentrated in the germ (Defloor *et al.*, 1993). The fat content is low (1.7 to 2.0%), and is mainly polyunsaturated with absence of cholesterol (Jiang and Wang, 2005).. With respect to micronutrient composition, wheat is also a source of B vitamins, namely thiamine (B_1), riboflavin (B_2), niacin (B_6), and minerals. Calcium, phosphorus, iron, sodium, magnesium and potassium are also present (Nwanekezi, 2013). Other health promoting compounds in wheat include phytosterols, which have the ability to decrease serum cholesterol (Jiang and Wang, 2005). Wheat flour for bread production contains functional proteins, gliadin and glutenin which when mixed with water, forms a gluten network, giving the dough its strength and elasticity (Goesaert *et al.*, 2005).

2.9.3 Banana flour

Banana flour is essentially starchy and contains long outer amylopectin chains, which form an excellent source of resistant starch (RS) (Zuwariah and Aziah 2009). Resistant starch is defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals (Zuwariah and Aziah 2009). Resistant starch in colon appears to play an important role in protection from colon cancer, diverticulitis and hemorrhoids through production of short chain fatty acids (Annison & Topping, 1994). The other beneficial physiological effects of RS include decreased serum cholesterol and triglycerides level, increased fecal bulk and pre-biotic effects (Annison & Topping, 1994; Bjorck *et al.*, 1989; Shetty & Kurpad, 1986). Polyphenols are receiving increasing interest from consumers and food manufacturers for several reasons (Lolayekar & Shanbhag, 2012). Epidemiological studies have suggested associations between the consumption of polyphenol-rich foods such as bananas and the prevention of diseases(Debjit*et al.*, 2012). Polyphenols prevent various diseases associated with oxidative stress, such as cancers, cardiovascular diseases, inflammation and others (De Espana, 1977). Organolyptically acceptable breads have previously been formulated from wheat/plantain composite flours using up to 80:20

(w/w) % and 60:40 (w/w) % ratios of wheat: plantain flour as maximum acceptable levels of substitution (Mepba *et al.*, 2007). No studies have been conducted on bread made from wheat, cassava and banana composite flour to date.

2.10. Influence of baking temperatures on nutrients

Bread is a one of the most important sources of carbohydrates, in the form of starch, in the human diet. During baking, starch undergoes a series of changes known as gelatinization (Bloksma, 1986). The gelatinization of starch during baking is important in relation to its susceptibility to enzymatic breakdown. Processing conditions and ingredients may also influence the formation of RS in bread (Kale *et al.*, 2002). According to Djurle *et al.*, (2018), increase in baking temperature and time were found to increase RS in pumpernickel, whole meal barley (70%) and white wheat bread.

2.11 Role of baking ingredients

2.11.1 Water

Water is of great importance in the production of bread, since it hydrates flour particles and helps flour components to interact, producing a homogeneous mass of dough. During mixing, water is necessary for formation of dough and becomes distributed between the flour components for its fluidity. The rest of the added water remains as "free" water and forms the water phase. In this phase, the flour proteins are hydrated, partially absorbed by the flour starch, particularly the damaged starch fraction of the flour; soluble solids such as sugars, salt, soluble proteins are also dissolved, and the yeast cells are dispersed (Brown, 1993). The amount of water absorbed into the dough is mainly controlled by the quality of the flour. During fermentation, water acts as a solvent in the dough, and many of the reactions that take place at this unit operation cannot occur if there is no solvent. For example, water acts as a solvent for some of the released carbon dioxide gas to form carbonic acid. Carbonic acid contributes to the acid pH of the dough, providing a feasible atmosphere for the action of enzymes and yeast in the dough system. Water governs the major changes that take place during baking including starch gelatinization, protein denaturation, yeast and enzyme inactivation, and flavor and colour development. At the baking stage, the degree of starch swelling and gelatinization depends on the total amount of water present in the dough. For this, the temperature in the crumb does not exceed 100°C while the final temperature to completely gelatinize the starch must be below 100°C (Cauvain & Young, 1998). Based on the dependence between the level of added water and the quality of flours, it can be concluded that if too little water is added to the flour, the resultant dough will be firm and become difficult to mould. As a consequence, the bread will have a small volume and poor external appearance while, if there is too much water, the dough will be soft and difficult to mould, hence producing bread of poor quality (Cauvain, 2003). However, water also has plasticizer effects that increase softness and decrease bread firmness (Mohammadi *et al.*, 2014). Based on all these arguments, the standard ratio of water to flour weight (hydration) is 60 to 65% with an average of 63% which was applied in this study.

2.11.2 Bakers' yeast

Bakers' yeast (*Saccharomyces cerevisiae*) is used in the manufacture of bread, due to its ability to metabolize fermentable sugars in the dough and thus produce carbon dioxide and alcohol towards fermentation. Carbondioxide enables the dough to expand to the required volume through its action on internal pressure of the gluten network while the alcohol formed and other compounds released from secondary fermentation such as organic acids, aldehydes, ketones and other carbonyl compounds act as precursors in the development of taste and flavor (Poitrenaud, 2004; Rose & Vijayalakshmi, 1993).

2.11.3 Salt

Salt (sodium chloride) forms part of the dough ingredients. The amount of salt used varies between1.0 and 2.0 % depending on flour weight (Brown, 1993; Eliasson & Larsson, 1993). Salt stiffens the dough and makes it less sticky. In addition, salt increases the mixing tolerance of wheat dough, extends the dough development time and increases the dough resistance, elasticity and extensibility all of which are required attributes in bread making (Uthayakumaran *et al*., 2011; He *et al*., 1992). Salt is also partly required in bread making for the strengthening effect of the dough (Preston, 1989).

2.11.4 Sugar

Sugar (sucrose) is a disaccharide composed of two units, one of glucose and another of fructose. In bread production, 2 - 3% sugar is adequate to sustain yeast activity. Later, more sugar is released for gas production by the action of enzymes in the flour. Due to the affinity for water, sugar exerts a limiting effect on gluten formation during the dough preparation. This limitation of water availability is partly responsible for the effect on starch gelatinization (Cauvain & Young, 1998). Sugar is used as a substrate for the yeast during the early stages of fermentation. When added to dough, sugar is hydrolyzed almost instantly into glucose and fructose by the yeast enzyme invertase. Sugar acts as an antistaling ingredient inhibiting starch recrystallization (Levine & Slade, 1990). In the case of microbial growth, this is restricted through increasing levels of sugar in the dough formulation (Cauvain & Young, 1998). Sugar increases product volume and crust colour (Brown, 1993).

2.11.5 Shortenings

Shortenings are lipids in the form of fat or oil, but mostly consist of 25% solid fat at room temperature. Shortenings are used in bread production to impart tenderness, to give moister mouthfeel, confer structure, lubricate during chewing and contribute to flavor (Seibel 1970). Due to the liquid phase of shortenings, their tenderizing effect slows the staling process compared to the dough formulation without shortenings. Typical usage levels are in the range of 3 to 4% all-purpose shortening or 2 to 3% vegetable oil, although up to 5% (flour weight basis) can also be used (Stauffer, 1993). With the addition of shortenings in the dough formulation, a larger bread volume has been reported due to improved oven spring, softer crumb, less crisp crust and better keeping quality of the bread (Stauffer, 1993). Studies by Campbell (1972) showed a greater increase in loaf volume and improved crumb grain with additions up to 1.5 g (flour basis) of shortenings in the dough formulation. This effect is attributed to the lubrication on the gluten matrix during mixing that improves uniformity of the dough by lowering its resistance to diffusion while increasing the oven spring (dough expansion) (Eliasson & Larsson, 1993; Stauffer, 1993).

2.12 Bread making process

Bread can be produced using four major methods: (1) Straight dough fermentation, which is the traditional method, where all the ingredients are mixed together to form a dough and are left to ferment for 1 hr before baking; (2) Sponge dough method, which includes a two-stage process in which part of the total quantity of flour, water and other ingredients from the formulation are mixed to form a homogeneous soft dough. The homogeneous soft dough is left to rest in bulk for a prescribed time, after which the sponge is mixed with the remainder of the ingredients to form a homogenous dough; (3) Rapid processing method, which uses the different combinations of active ingredients and processing methods. A common element in this procedure is the inclusion of improvers to assist in dough development; and (4) Mechanical dough development method, a baking process with no need of a fermentation period. Dough development is achieved during high-speed mixing by intense mechanical working of the dough (Sedláček & Horčička, 2011; Cauvain, 2007; Cauvain, 2003).

Onuegbu *et al.* (2013) studied the effect of different bread making methods (rapid process, straight dough, sponge and dough, sourdough) on baking and the sensory properties of composite wheat-maize bread using varied levels of an addition of maize flour (0 to 20%). The sponge and dough method gave the best crumb texture, while crust colour did not show a significant difference in the rapid processing and sourdough methods. In addition, the authors highlighted that crumb texture did not show a significant difference in composite breads baked using the rapid processing dough, straight dough and sour dough methods.

On the basis of the above studies, the current study employed the straight dough bulk fermentation method. According to Eliasson & Larsson (1993), the bread making process using composite flour is considered to be the same as that described for wheat flour, which mainly includes three major stages-mixing or dough formation, fermentation and baking. The same approach was adopted in this study as each stage in the bread making process is critical in influencing the final product quality.

2.12.1 Mixing

Mixing is a critical step that blends ingredients together into homogeneous dough mass, retains air into the dough, and develops the gluten proteins into a continuous phase (Cauvain, 2007). Water is added to the dry ingredients and hydrates the flour through diffusion; the hydrated flour particles rub against each other and, in the process, the outer, hydrated layer is removed. As this process continues, the flour particles mainly the protein and starch become hydrated, bringing about dough development (Eliasson & Larsson, 1993). Belton (1999) proposed a model called "loops and trains" to explain the behaviour of dough with respect to the hydration of gluten proteins. With a low level of hydration, the proteins are disordered and have close interactions via hydrogen bonds but no regular structure. In the intermediate hydration, plasticization of the system facilitates the formation of hydrogen bonded structures, which is described as a low loop-to-train ratio. In high hydration, there is formation of hydrogen bonds that results in the formation of regions in which interchain interactions are broken. This is observed as a high loop-to-train ratio. That is, the more hydrated the flour is, the more viscoelastic properties the dough has. Therefore, high hydration is desirable because gluten is able to form a viscoelastic mass that is responsible for the gas retaining properties of wheat dough and the springy texture of bread.

2.12.2 Fermentation

The developed dough formed while mixing expands as the carbondioxide is retained in its structure during proofing. During proofing, yeast metabolism results in carbon dioxide release and growth of air bubbles previously incorporated during mixing, leading to expansion of the dough, which inflates to larger volumes and thinner cell walls before collapsing. The growth of gas bubbles during proofing and baking determines the characteristics of the bread structure and thus the ultimate volume and texture of the baked product (Olapade & Oluwole, 2013). There are two phases in the fermentation stage, bulk fermentation (first proof) and the main fermentation (known as the final proof). Dough has a resting period in bulk fermentation after mixing and before dividing, which varies from 0.5 to 3 hr (Ćurić *et al.*, 2014). The objective of this stage is to transform a piece of dough in a more elastic and more resistant form to being stretched without tearing in the moulding stage (Gould, 2007). After dough resting period, the bulk dough may be divided into individual pieces by weighting and then shaped. The moulded dough is placed either in tins or on a baking tray and kept in a proofing cabinet to continue fermentation (final proof). This step is necessary to work the fermented dough, divide the alveolus, and provide a uniform redistribution of gas. During the final proof, which lasts about 30 to 60 min, starch from the flour is progressively converted into sugars and dextrin by enzyme action (Ćurić *et al.*, 2014). The sugars feed the yeast, and the by-products are carbon dioxide and ethanol. As carbon dioxide is produced, the dough expands and retains it, and it is important that the skin remains flexible.

2.12.3 Baking process

Baking is the last operation in bread production, whereby due to action of heat, the dough is transformed into bread by firming (stabilization of the structure) and by the formation of the characteristic aromatic substances. Baking is achieved at temperatures ranging from 220 to 250°C (Ćurić *et al.*, 2014; Cauvain, 2003). During this process, the dough undergoes a series of changes due to rise in temperature. Initially, yeast activity ceases when a temperature of 55°C is reached. Subsequently, stability of the structure is sustained due to expansion of entrapped gas. As the temperature nears 60°C, starch starts to gelatinize. The starch granule first absorbs any free water from the dough and later from the protein membranes until it is fully gelatinized (Wiggins and

Cauvain, 2007; Cauvain, 2003). A final internal temperature ranging from 92 to 96°C is achieved for an adequately baked loaf (Ćurić *et al.*, 2014; Cauvain, 2003). The different temperatures reached inside and outside the dough cause formation of the bread crust and crumb. The different phases during baking are described as oven spring (enzyme active zone), gelatinization of starch, and browning and aroma formation. Crust formation is of great importance to the strength of the bread loaf and flavor development (Wiggins & Cauvain, 2007).

2.13 Importance of gluten in bread making

Gluten is a cohesive, viscoelastic proteinaceous material prepared as a by-product of the isolation of starch from wheat flour (Day *et al.*, 2006). Gluten confers high water absorption to the flour and makes the dough cohesive and viscous with high capacity for gas retention (Scherf & Köhler, 2016). This gives bread of high volume and with the typical crumb structure. Due to lack of gluten, flours from all other cereal species gives bread of lower volume, smaller pores and less elastic crumbs (Day *et al.*, 2006). During kneading, the flour first takes up water and mechanical energy is transmitted to the developing gluten. A continuous, viscoelastic gluten network with embedded starch granules is formed (Singh & MacRitchie, 2001). Both the quantity and the composition of the gluten proteins influence the behaviour of wheat flour during kneading and its mixing tolerance. Gluten is also responsible for the rheologilocal properties of the optimally developed dough. In particular, resistance to extension of a dough determines its gas retention capacity, and thus the bread volume and crumb structure. Low or extremely high resistance to extension leads to baked products with low volume, as the gas bubbles are either unstable and collapse, or cannot expand enough.

Gluten is a protein made up of two functionally distinct groups of storage proteins: the monomeric gliadins and the polymeric glutenins. Gliadins are present at higher levels (gliadin/glutenin ratio: 1.5 to 2.7:1), soluble in aqueous alcohols and their molecular weights are between 28,000 and 55,000 (Wieser, 2007). Glutenins consist of aggregated proteins, linked by disulphide bonds. Their molecular weights range from 500,000 to more than 10 million (Wieser, 2007). In contrast to the gliadins, only a small fraction with the lowest molecular weight is soluble in aqueous alcohols. The molecular weight distribution of the glutenins influences dough and baking properties. Glutenin macropolymer (GMP) in wheat flour ranges from 20 to 40 mg/g and is highly correlated with dough consistency and baking volume (Wieser, 2007). Glutenins are made up of protein subunits linked by disulphide bonds. The technological function of gluten is essentially determined by two parameters: the gliadin/glutenin ratio and the quantity of GMP. Glutenins form a three-dimensional network, which is responsible for resistance to extension and elasticity (Belton, 1999). Gliadins are regarded as "softeners" in dough, responsible for viscosity and extensibility (Khatkar*et al*., 1995). Therefore, optimal baking quality is based on a balanced ratio between gliadins and glutenins.

2.14 Bread quality

Bread is a staple food processed by baking dough of flour and water. It is popular around the world and is one of the world's oldest foods. Bread quality is of importance because bread is commonly consumed. However, it is difficult to define this quality since it is affected by many factors such as the nutritive value, taste, freshness, shelf-life and attractiveness for consumers. Loaf volume, specific loaf volume and crumb firmness are the main quality characteristic of bread (Katina *et al*., 2006). The most common method of assessing whole product volume is by the rapeseed displacement method (AACCI, 2011). In addition, image analysis methods have been applied for product volume measuring. Bread texture is a critical factor for consumer acceptance (Belz *et al.*, 2012). Crumb firmness is important in bread texture assessment because it is associated with the perception of bread freshness (Cauvain, 2003). This important parameter can be evaluated by texture profile analysis (TPA) as well as the sensory evolution test. In this study, texture profile analysis was used. The external appearance of the bread product is a major factor in attracting consumers using appearance by sight sensory attribute. Thus, other external bread quality parameters assessed include color, weight, height, shape, and flavor. Bread made from wholegrain wheat flour often has lower loaf volume, firmer dense crumb, and darker crumb and crust compared with bread made from refined wheat flour (Cai *et al.*, 2014).

The reduction in wholegrain or fiber-rich bread loaf volume is due to several factors including: (1) dilution of wheat gluten by the added fiber (2) mechanical interference of insoluble fiber with the formation of gluten network causing rupture of gas cells and (3) the higher water binding ability of fiber (both soluble and insoluble) causing less water available for the development of gluten network and less steam production (Courtin & Delcour, 2002). Bread improvers (Gomez *et al.*, 2003) or commercial enzyme mixtures (Katina *et al.*, 2006) can be used to improve the quality of wholegrain or high-fiber bread. However, pre-fermentation of wheat bran with lactic acid bacteria and yeast improve loaf volume and crumb softness (Katina *et al.*, 2006).

2.15 Chemical composition of bread

Bakery products are rich in carbohydrates because of the high content of starch and simple saccharides. Levels of the latter carbohydrates depend not only on chemical composition of flour but also on amounts of sugars added during the manufacturing process. Bakery products are an important source of dietary fiber. Its content depends on the type of flour and varies between 0.3 and 1.5 g/100 g fresh bread (Almeida *et al.*, 2013). Therefore, whole meal breads contain elevated levels of dietary fiber. The latter is not digested in human gastrointestinal tract but positively affects peristalsis of intestines and enables expelling of harmful metabolites. Cellulose and hemicelluloses are main fractions of dietary fiber in wheat bakery products (Almeida *et al.*, 2013).

Flour type and dietary fiber content also influence the concentration of other nutrients. Whole meal wheat and rye breads contain 2 to 5-fold more salts of iron, magnesium, manganese, copper and zinc than white breads (Diowksz*et al.*, 2000). Protein content varies between 4.5 and 8.0 g/100 g fresh bread. Fat content in bakery products is low and varies between 0.7% and 2.5%. Therefore, bread can be kept for a relatively long time. Bread is rich in B complex vitamins, in particular thiamine (vitamin B1), niacin, riboflavin, and folic acid as well as vitamin E. Higher levels of these valuable nutrients are contained in whole meal breads, particularly yeast-fermented wheat breads. Energy value of bread varies between 874 and 1924 kJ, it means 208-459 kcal/100 g fresh product with exception of pumpernickel and crisp breads, which are characterized by the higher energy value and content of nutrients (Diowksz *et al.*, 2000). It is estimated that bread satisfies around 50% of nutritional needs of human and around 30% of energy needs.

2.16 Factors affecting bread purchases

Human behaviour towards food choice is multifaceted, involving actions as well as physiological and psychosocial processes (Sobal *et al.*, 2014). Beside intrinsic and extrinsic product's characteristics, social context as well as consumers' attitudes, beliefs and opinions affect food choice and purchase (Barrios & Costell, 2004; Biloukha & Utermohlen, 2000). Intrinsic features include appearance, colour, shape, and presentation of the product, while the extrinsic ones include price, geographic origin, and additional information, such as nutritional facts, production chain, and environmental sustainability. In addition, consumers draw on product's experience attributes, e.g. convenience, freshness and sensory characteristics perceived while eating, and credence attributes such as healthiness and naturalness (Issanchou, 1996). Moving to bread, sensory attributes are major determinants of consumers' acceptability (Jensen *et al.*, 2011; Heenan *et al.*, 2009; Heiniö, 2006) and ingredients, processing, social context and demographic features contribute to the purchase decision (Heenan *et al.*, 2008; Hersleth *et al.*, 2005; Kihberg *et al.*, 2004; Shogren *et al.*, 2003).

2.17 Sensory quality

Sensory evaluation is defined as a science that is used not only to measure and evaluate the sensory properties of food but also to analyze and interpret the responses to those characteristics of foods as they are perceived by the human senses (sight, smell, touch, taste) (Stone & Sidel, 2004). The sensory quality of food products plays a key role in the selection of food. Hedonic testing is often used to determine consumers' attitude towards the food by measuring a degree of acceptability, overall liking, preference or sensory attributes (appearance, flavor, texture, aroma etc.) of a new product or improving the existent food product (Meilgaard *et al.*, 1999). The data obtained from this test can help to identify potential buyers of the product and the way in which the product can be introduced into the food market (Gámbaro, 2012; Kemp *et al.*, 2009; Cordonnier & Delwiche, 2008). Overall appearance includes all visible sensory attributes such as colour, size and shape as well as surface texture (Meilgaard *et al.*, 1999). Flavor involves sensory attributes such as taste, specific flavor, aroma and sweetness. Aroma is the odor of a food product, resulting from the process that involves the course of volatiles through the nasal passages when a person inhales them (voluntarily or otherwise) (Meilgaard *et al.*, 1999).

Texture is referred to as the tactile feel properties, measured as geometrical particles, and mechanical and moisture properties, by the tactile nerves in the surface of the skin of the hand, lips or tongue (Meilgaard *et al.*, 1999). This can be described as aerated, crispy, crumbly, gummy, soft, hard and moist (Setser, 1993). Overall liking can be defined as a complex expression of liking the product as a whole. The willingness to buy represents the extent to which a consumer has a positive attitude towards purchasing a product. Roininen *et al.* (1999) concluded in a quantitative study of important predictors of food that food choice is mainly predicted by taste, although appearance and smell are also important hedonic aspects for consumers' quality perception.

2.18 Advantages and limitations of RSM in modeling food quality

Response surface methods (RSM) are information based techniques and perform well when there is a rich tapestry of accurate information provided. They have gained immense popularity due to their ease of use as well as dexterity of being applied across the board of industrial applications. RSM offers techniques for mapping multidimensional patterns of responses to varying levels of control factors that are identified to govern physical processes(Madhuresh *et al.*, 2013). RSM is dependent on the use of regression analysis on data from experiments carried out at multiple levels and can be used to find approximate minima or maxima in response patterns provided such optima are within the design space of regressed points. RSM is a mathematical tool based on statistics (Myers & Montgomery, 1995; Box *et al.*, 1978). Several commercial tools have currently come to the market (e.g. MINITAB, Design Expert, E-Chip, Statistica, SAS etc.) that are prevalently used. In this study, MINITAB was employed. RSM techniques provide an efficient method of transferring information. The strength of the method lies in capturing accurate efficient smooth approximations for accurate data garnered from numerical or practical experiments at discrete data points in the design space (Venter *et al.*, 1997).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Baseline assessment on bread consumption in Kampala

In May 2018, a baseline study on bread consumption habits of respondents in Kampala city was carried out in the five divisions of Kampala, Nakawa, Central, Kawempe, Rubaga and Makindye. Using simple random sampling, close ended questionnaires were administered to 293 participants in Kampala. Kampala city lies at latitude, 0.3476°N and longitude 32.5825°E, and altitude 1,190 m. The sample size was computed using the method of Yamane (1967).

$$n = \frac{N}{1 + N(e)^2}$$

Where n is the sample size, N is the population size, and e is the level of precision. The population of Kampala is estimated to be 1,936,080 inhabitants (UBOS, 2010). With a precision level e of 0.05, the sample size was estimated at 400. Using the proportion (64.7%) of bread consumers, 259 was obtained for the study. To account for possible attrition, the number of respondents was increased to 293.

3.2 Experimental materials

Mature banana (EAHB) and cassava roots (NASE 19) were procured from Nakasero market, Kampala, Uganda. Wheat flour was purchased from Bakhresa Grain Milling (Uganda) Limited. Other ingredients included granulated sugar (produced from Kakira Sugar Works Limited, Lugazi, Uganda), Fermipan baking yeast (DSM bakery ingredient, Dordr-echt-Holland), baking fat (from

Mukwano Group of Companies, Kampala, Uganda) and salt (from Kensalt Limited, Mombasa, Kenya).

3.3 Experimental design

A constrained D-optimal mixture experiment was generated using Design-Expert (Version 10.0, Stat-Ease) software. Constraints used were; $50 \leq$ wheat ≤ 80, $10 \leq$ banana ≤ 40 and $10 \leq$ cassava ≤ 40; wheat + banana + cassava = 100. The control was 100% wheat flour. The proportions for each ingredient were expressed as a fraction of the mixture and for each treatment combination, the sum of the component proportions were equal to 100%. Sixteen runs were experimented to assess the optimal proportions of wheat, banana and cassava (Table 3).

Table 3: Design matrix for formulation of the composite flour

Run	Wheat proportion (%)	Banana proportion (%)	Cassava proportion (%)
1	64.86	10.00	25.14
2	70.19	15.26	14.56
3	64.99	25.01	10.00
4	64.86	10.00	25.14
5	60.24	19.82	19.94
6	50.00	32.60	17.41
7	50.30	24.72	24.98
8	64.86	10.00	25.14
9	50.30	24.72	24.98
10	64.99	25.01	10.00
11	50.00	10.00	40.00
12	80.00	10.00	10.00
13	50.00	40.00	10.00
14	57.58	32.15	10.00
15	50.30	24.72	24.98
16	54.97	14.66	30.37

3.4 Flour preparation

3.4.1 Banana flour

The bananas were peeled using a stainless steel knife and soaked in water for 30 min according to method by Osman (2001). They were then sliced using a knife into pieces of between 5 to 10 mm. Banana slices were placed on a tray and dried in a hot air drier (CK 80520, England) at 60 °C for 12 hr. After drying, the slices were milled into flour using a Molinex Super Blender Mill (720, China), sifted through 60-size mesh and stored in an airtight plastic container at ambient temperature until use.

3.4.2 Cassava flour

About 20 kg of mature cassava roots were purchased from Kireka market, Wakiso district. The roots were peeled, washed and sliced into 3 cm cubes. The slices were dried on a perforated tray in a conventional hot-air oven (PP 22, Genlab, England) at 55°C for 72 h. The dried chips were milled into flour using the laboratory rotor mill (D-6072, Germany) and sieved through a 0.5 mm aperture sieve (AS200, Germany). The flour was packaged in airtight containers and stored at 4°C until use.

3.5 Bread production

Bread from sixteen flour blends and a control flour were formulated following the straight dough method (American Association of Cereal Chemists, 2000). Wheat, cassava, and banana flour in proportions as shown in table 5, as well as yeast (15 g), sugar (120 g), salt (10 g), water (600 ml) and fat (20 g) were weighed using a digital laboratory scale (CE- 410I, China) and placed in a Kenwood dough mixer (A 907 D, England). The mixture was firstly blended at 90 rpm for 2 min for uniformity followed at 180 rpm for 6 min to allow for dough development. The dough was then divided into 585 g portions, manually rounded and molded into a loaf shape and placed in lightly greased tins. The tins were placed in a proofer (PW 120, Italy) for 40 min at 35°C and 85% relative humidity. The proofed dough was baked in a preheated oven (PW 120, Italy), with top and bottom heat, at 220°C, for 45 min. A control wheat bread was prepared simultaneously in the same oven under identical conditions. The bread was cooled for 2 hr, prior to loaf volume and loaf weight measurement. Baked breads were packaged in a low density polyethylene plastic bags and stored (at 24±2°C) for subsequent analysis.

3.6 Chemical analyses

3.6.1 Determination of moisture content

Moisture content of the bread samples was determined according to AACC, (2000), method No. 44-15A. A porcelain crucible, (<50 mm diameter and <40 mm deep), was cleaned with water and rinsed with distilled water. It was dried in a conventional hot-air oven for 30 min, cooled in a desiccator and weighed (W_o). A 2g sample was weighed using an analytical balance (224, Practum, Germany) and placed in a porcelain crucible. Using tongs, the porcelain crucible containing the sample was placed in an air drying oven at 105 ± 1°C for 4 hr. The sample was removed and placed in a desiccator, allowed to cool for 30 min at room temperature and reweighed (W_1). Percent moisture content of the bread was measured by weight difference before and after drying and calculated according to the following formula:

$$\text{Moisture content (\%)} = \frac{\text{weight loss}}{\text{weight of sample}} x\ 100$$

3.6.2Determination of total ash

Ash content was determined by AOAC (2000), method No. 923.03. A porcelain crucible was washed and dried at 120°C in an oven for 15 min and cooled for about 20 min in a desiccator at room temperature. The bread sample (2 g) was weighed into the dried porcelain crucible and placed in a muffle furnace (FSL 340-0100, Gallenkamp, UK) at 550°C for 1 hr until ashing was completed. The sample was cooled in the desiccator and weighed to obtain percent ash content.

$$\text{Ash content (\%)} = \frac{\text{weight of ash}}{\text{weight of sample}} x100$$

3.6.3 Determination of protein

Protein was determined by AOAC (2000), method No. 920.87. A 2.0 g bread sample was weighed into a digestion flask and 0.5 g of selenium catalyst added. Concentrated sulphuric acid (25ml) was added and the flask shaken for approx. 2 min to mix the contents. The flask was then placed on a digestion burner at 370°C and heated for 8 hr until the solution turned green and clear. The solution was transferred into a 100ml volumetric flask and made up to the mark with distilled water. Boric acid of 2% (25ml) was transferred into a 250ml conical flask and 2 drops of mixed indicator (bromocresol green and methyl red) solution added. Sodium hydroxide solution of 40% (15ml) was introduced into the decomposition chamber of the distillation apparatus. The digested solution (10ml) was introduced into a Kjedahl flask. Ammonia was distilled into the boric acid until it changed to bluish green. The distillate was titrated with standardized 0.1N sulphuric acid to a reddish colour. Protein content was calculated as a product of percent total nitrogen and a factor of 6.25.

$$\text{Nitrogen content (\%)} = \frac{(\text{Titre} - \text{Blank}) x\ 14.008\ x\ \text{Normality}}{\text{weight of sample}} x\ 100$$

Protein content = % Nitrogen x 6.25

3.6.4 Determination of fat

Sohxlet method of AOAC (2000), method no. 920.39 was used to determine fat. A 250 ml quick fit round bottom flask was washed and dried in an oven (OV 880, Gallenkamp, England) at 105°C for 25 min and allowed to cool to room temperature before it was weighed. A clean and dried muslin thimble containing about 5 g of dried sample covered with fat free cotton at the bottom and top was placed in the extraction column with the condenser connected. Petroleum ether (200ml, boiling point 40 to 60°C) was transferred into the round bottom flask and fitted into the extraction unit. The flask was heated with the aid of electro-thermal heater at 60°C for 8 h. After extraction, the thimble was removed and the solvent salvaged by distillation. The flask containing the fat and residual solvent was placed on a water bath (W-22, Chemyx, USA) to evaporate the solvent followed by further drying in the oven at 105°C for 30 min to completely evaporate the solvent. It was then cooled in a desiccator and weighed. The flask containing the extracted fat was dried on a steam bath (F-65G, Steamers, India) at 98°C to a constant weight. The flask was reweighed and percentage fat calculated as follows:

$$\text{Fat content(\%)} = \frac{\text{weight loss}}{\text{weight of sample}} x\ 100$$

3.6.5 Determination of crude fiber

Fiber content was determined according to AOAC (2000), method no. 962.09. Sulphuric acid of 1.25% (200ml) was added to a 3.0 g defatted sample in Erlenmeyer flask with a condenser connected to it and immediately set on a hot plate electric oven (900XP, China) heated at 130°C. The content was brought to boil within 1 min and the sample digested for 30 min. After digestion, the content was filtered through a linen cloth and subsequently washed with boiling water. The residue was washed back into the flask with 200ml boiling 1.25% NaOH solution and boiled for 30 min. The residue was transferred to a clean crucible with a spatula and the remaining particles washed off with 15 ml ethanol into the crucible. The crucible with its contents was dried in an oven at 105°C overnight and cooled in a desiccator and weighed. The crucible with its content was then ignited in a Gallenkamp furnace (FSL 340-0100, UK) at 550°C for 2 h, cooled and re-weighed. The loss in weight was considered as the fiber content and was expressed as a percentage of the initial weight of the sample as shown in the following formula:

$$\text{Fiber content (\%)} = \frac{\text{weight loss}}{\text{weight of sample}}\ x\ 100$$

3.6.6Determination of total carbohydrates

Carbohydrate amount was determined by the difference method as reported by Osborne and Voogt (1978). Total carbohydrate content of the samples was determined by subtraction of protein, fat, fiber, moisture and ash constituents from 100%.

3.6.7Determination of physical characteristics of bread

Loaf weight, loaf volume, crumb colour were evaluated. Loaf weight (g) was measured using a digital laboratory scale (CE- 410I, China) 60 min after the loaf was removed from the oven. Loaf volume was determined by rapeseed displacement method 60 min after baking with slight modification (millet was used in place of rape seed) (Wang *et al*., 2002; Iwe*et al*., 2017). A container was filled to the 5th volume of the loaf sample with seeds, until the seeds dropping from the height of ½ foot above the container were overflowing. The edge of a meter rule was used to cut off all seeds above the container rim such that the seeds form a plateau with the rim of the container. The millet seeds were then withdrawn from the container into the measuring cylinder and the volume recorded. The bread to be measured was placed in the same container. The millet grains were gently poured into the container until the bread was covered and leveled again. The volume of the remaining millet grains displaced by the bread was measured using a measuring cylinder and was considered as the volume of the bread expressed in cubic millimeters.

3.6.8 Bread firmness

Bread firmness was determined using a TA-XTplus texture analyzer (Stable Micro Systems, Gudaiming, Surrey, UK) according to AACC method 74-09 (AACC, 2001) on slices obtained from the center of each loaf. Each slice was cut with a commercial electric knife (Rohnson, Ak-8, P.R.C.) in order to have a slice thickness of 25 mm. The test was performed in compression mode using the 36mm diameter aluminium probe. Five crumb compression tests were performed for each slice making 15 readings per loaf and 30 readings per treatment. The aluminium probe was applied at five different points of the bread crumb. A total of 90 readings was taken per treatment and per replicate. The test modes were; pre-test speed with a compression of 1.0 mm/sec, test speed of 1.7 mm/sec, post-test speed of 10 mm/sec and a trigger force of 0.05 N. The peak force of compression was reported as firmness (AACC, 2000).

3.7 Sensory evaluation

Sensory evaluation of bread samples was performed after 12 h of storage at ambient temperature. Sensory attributes (taste, odor, crumb color, texture and overall acceptability) were evaluated on a five-point hedonic scale. Sensory evaluation was conducted by 48 randomly recruited untrained consumer panelists. Three digit-coded samples were presented to panelists in random order along with the cup of water to cleanse their mouth between sample tasting.

3.8 Statistical analysis

A D-optimal mixture design with 16 formulations of three ingredients was analyzed using Minitab Version 17 software to determine the optimum proportion of the ingredients to maximize proximate compositions, loaf volume and sensory acceptability response variables; and to minimize loaf weight response variable. Complete analyses of these response variables were conducted using the method described by Montgomery (2013). The analyses included verifying that the model did not have significant lack-of fit, and the normal distribution and constant variance assumptions on the error terms were valid. Independence assumption was valid through the random order of the runs. This was followed by testing the significance of each model term, and performing response optimization to identify the optimum proportions that jointly maximized the desired response variables within each of proximate composition and sensory evaluation. This was followed by constructing an overlaid contour plot to determine the "sweet spot" that jointly optimizes all response variables for each of these groups of the response variables. The mean comparison was done by least significant difference. A $p<0.05$ was considered as significant.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Determinants of bread choice and consumption in Kampala city

4.1.1Characteristics of population survey participants

Distribution of interviewees in Kampala comprised of 42.7% male and 57.3% female (Table 4). The majority (66.4%) of the respondents were youths with age ranging from 16 to 34 years. This was followed by the age category of 35 to 54 years at 29.7% and few (3.9%) were above 55 years. Of the 293 respondents, 49.5% had completed University degree, 22.8% secondary education, 12.1% tertiary education, and 8.3% primary education. Only 7.3% had completed postgraduate courses. Almost half (44.1%) of the respondents had a monthly income of less than UGX500,000/= (USD135) while 40.6% earned a monthly income within a range of UGX500,000/= to 1,000,000/= (USD270). A reasonable proportion (15%) of bread consumers earned more than UGX 1,000,000/= (USD270) per month.

These results demonstrated that bread is consumed by different age groups in Kampala city. The high bread consumption among young people could be related to convenience and changing life styles. Younger people are more driven towards sensory attributes of bread than health benefits whereas older people are normally inclined to health attributes in addition to being sensory averse (Quiroga *et al.*, 2016). However, being sensory averse may not necessarily be a dependent factor in this study. Since bread was purchased by mostly people earning below 500,000/=, it indicates that bread is relatively affordable.

Table 4: Characteristics of the population survey participants in number and percentage (%)

Characteristics		N	%
Size of the sample		293	100
Gender	Female	161	57.3
	Male	120	42.7
Age group	16 – 34 years	188	66.4
	35 – 54 years	84	29.7
	> 55 years	11	3.9
Education level	Primary school	24	8.3
	Secondary school	66	22.8
	Tertiary institutions	35	12.1
	University degree	143	49.5
	Postgraduate	21	7.3
Income status	< 500,000/=	126	44.1
	500,000 – 1,000,000/=	116	40.6
	> 1,000,000/=	43	15.0

4.1.2 Bread consumption

Bread was consumed on a daily basis by a relatively large proportion (37.1%) of the population. About 6.1% of the respondents consumed bread more than once a day. Those who ate bread twice a week accounted for 38.6% while 13.9% consumed it once a week. Sweet bread was the most liked bread type in Kampala and was consumed by 88.2% of the respondents. A small proportion (11.4%) of the consumers liked savory or salty bread. White bread was preferred by 72.2% of the respondents and whole meal bread by 24.9%. Only 2.5% of consumers did not know what type of bread they liked. A considerable proportion (46.2%) reported margarine to be their preferred topping while 26.9% preferred plain bread. Peanut butter and jam were preferred by 20.4% and 5.4% respondents, respectively. A large proportion (61.2%) of respondents preferred no filling, 20.3% preferred raisins while 18.5%, grains. Majority (60.5%) of the interviewees consumed 2 slices while 24.7% consumed 3 or more slices daily (Table 5).

Bread was consumed every day by most participants, which confirms a high consumption rate among people living in Kampala. An earlier study reported regular consumption of bread in Kampala with more than 66% of the population served three times a day (Moneera, 2017). High bread consumption has been reported in many developing countries with preference being white bread (Pomeranz, 1988). In this study, white bread was consumed more than whole meal bread attributable to soft texture and sweet taste. The difference in consumption for bread types may be related to price differences or a knowledge gap regarding the health benefits, sensory attributes and traditions (Sandvik *et al.*, 2014). Bread consumers in their thirties or forties and below are negative towards health attributes but positive towards sensory attributes (Xavier *et al.*, 2009). Consumption of white bread by most respondents in Kampala could be based on perception that whole meal bread is not healthier than other bread types (Ratinger *et al.*, 2015). Younger consumers who were the majority preferred bread's taste than any other attribute. This could explain the observed consumption distribution in favour of white bread and yet whole meal bread is associated with reduction of bowel transit time, diabetes, reduction in colorectal cancer and hypertension (Keogh *et al.*, 2003).

Table 5: Bread consumption habits of participants

Parameters		N	%
Frequency of consumption per week	Once a week	39	13.9
	Twice a week	108	38.6
	Once a day	104	37.1
	Twice a day	17	6.1
Quantity consumed per day	1 slice	3	1.1
	2 slices	170	60.5
	3 or more slices	69	24.6
	1 bun	20	7.1
	2 buns	10	3.6
	3 or more buns	9	3.2
Bread type preferred	White bread	200	72.2
	Whole meal bread	69	24.9
	Don't know	7	2.5
Type of topping used	No topping	75	26.9
	Margarine	129	46.2
	Jam	15	5.4
	Peanut butter	57	20.4
Type of filling preferred	No filling	169	61.2
	Raisins	56	20.3
	Grains	51	18.5

4.1.3 Purchasing behaviour and decisions of respondents

Large baking companies (56.1%) were the main purchase points preferred by consumers. Super markets were preferred by 35% of bread consumers. A small proportion (2.9%) of consumers bought bread from the streets while 5.4% did not have a definite purchase point. This is supported by the high preference of bread from large companies such as Hot loaf, Ntake and Tip top (Table 6). Freshness was the most important criteria for the preference of bread with 36.4% of consumers accepting it. Brand of bread, softness and springiness, and price were also important accounting for 22.5%, 11.4%, and 7.1%, respectively. The purchasing behaviour of 51.4% of respondents was not determined by the claims on packages (Table 8). Few consumers considered good packaging (2.5%), price (7.1%), health benefits (6.8%), appearance (3.2%), size of the loaf (2.9) and ingredients (7.1%) as key factors affecting their buying decision.

Bread was purchased from mostly large baking companies and supermarkets guided by freshness and brand of the product. Freshness and quality are important factors affecting preference for the bread-purchasing location (Gul *et al.*, 2003). Other factors for purchase were brand of bread and softness. Price was surprisingly less considered compared to other developing countries perhaps because many respondents could afford bread. Appearance of bread and packaging did not contribute much to bread purchase. Therefore, it can be concluded that consumers' bread choices do not depend on advertisement. Studies have found little or no evidence to support the view that advertising can affect bread demand (Duffy, 1999). Differences in the choice of bread brands are probably attributed to consumer beliefs and attitudes. Consumers go for products that satisfy their expectations and requirements. Few consumers purchased bread from streets on the assumption that street bread is not fresh. The size of loaf also had little impact on bread purchase.

Table 6: Bread purchase behaviour and decisions of respondents

Parameters		N	%
Frequency of bread purchase a week	7 times	33	12.0
	6 times	413	4.7
	5 times	26	9.5
	3 times	52	18.9
	2 times	104	37.8
	Once	47	17.1
Purchase points of bread	Large baking companies	157	56.1
	Supermarkets	98	35.0
	Street retail bakers	8	2.9
	It doesn't matter	15	5.4
Bread brand usually bought by clients	Ntake	37	13.2
	Hot loaf	45	16.0
	Capital shoppers	27	9.6
	Quality supermarket	3	1.1
	Tiptop	37	13.2
	Kiddawalime	28	10.0
	Milk bread	23	8.2
	Supa loaf	36	12.8
Factors considered when choosing bread	Brand of bread	63	22.5
	Appearance of the bread	9	3.2
	Freshness of the bread	102	36.4
	Softness and springiness of bread	32	11.4
	Ingredients	20	7.1
	Size of loaf	8	2.9
	Packaging	7	2.5
	Price	20	7.1
	Health benefit claims on the packaging	19	6.8
Claims considered to be important when buying bread	Gluten free bread	4	1.4
	Low on calories	13	4.6
	Whole grain/whole meal	14	5.0
	Contains seeds/fruit/grains	28	10.0
	Low salt content	20	7.1
	Enriched with nutrients	34	12.1
	Organic	2	7.5
	It doesn't matter	144	51.4

4.2 Physical properties of bread from wheat, banana and cassava composite flour

4.2.1 Physical characteristics

4.2.1.1 Loaf weight and loaf volume

Blending ratio affected ($p<0.05$) loaf weight and loaf volume as predicted by linear and/ or quadratic models (Table 9). The addition of banana and cassava flours to wheat flour increased the loaf weight. A composition of 50% wheat, 33% banana and 17% cassava flour composite had the highest loaf weight (643.0 g). The lowest (468.3 g) loaf weight was obtained from 80% wheat, 10% banana and 10% cassava flour blend (Table 8). The loaf volume of the bread decreased from 1310.0 to 803.3 cm^3 as the proportion of banana and cassava flours increased (Table 7). Bread with higher banana and cassava substitution levels showed lower loaf volume compared to the control. The lowest loaf volume (803.3 cm^3) was observed in bread with 25% banana and 25% cassava (Table 7).

Reduction in loaf volume with increase in banana and cassava flour quantity could be a result of reduced flour strength and a lower ability of the gluten network to enclose the carbon dioxide produced during fermentation (Montagnac *et al*., 2009). Similar findings on loaf volume of composite flours were reported by Bakare *et al*.(2016). Low protein network in the dough and weak interaction between starch and the gluten negatively affect loaf volume (Rosell *et al*., 2006). Bread loaf volume decrease has been demonstrated in breads produced from wheat and plantain flour blends (Ndife *et al*., 2011; Mashayekh *et al*., 2008; Dhingra & Jood, 2004). A study on cassava flour blended with wheat flour has shown similar behaviour in loaf volume (Jensen *et al*., 2015).

4.2.1.2 Firmness

Blending ratio did not affect ($p>0.05$) loaf firmness as predicted by linear/quadratic models (Table 8). Firmness increased from 6.4N to 29.8N as the amount of banana and cassava increased in the blends (Table 7). Bread developed from 50% wheat, 40% banana and 10% cassava had the highest (29.8 N) firmness whereas that made from 80% wheat, 10% banana and 10% cassava had the lowest (6.4 N). Firmness is attributed to mainly amylose and amylopectin matrix which contribute to overall bread texture (Schiraldi &Fessas, 2000). Gomez *et al.* (2013) reported that bread firmness is due to interactions between gluten and fibrous materials.

Table 7: Mean loaf volume, loaf weight and firmness mean scores for bread produced from wheat, banana and cassava composite flour

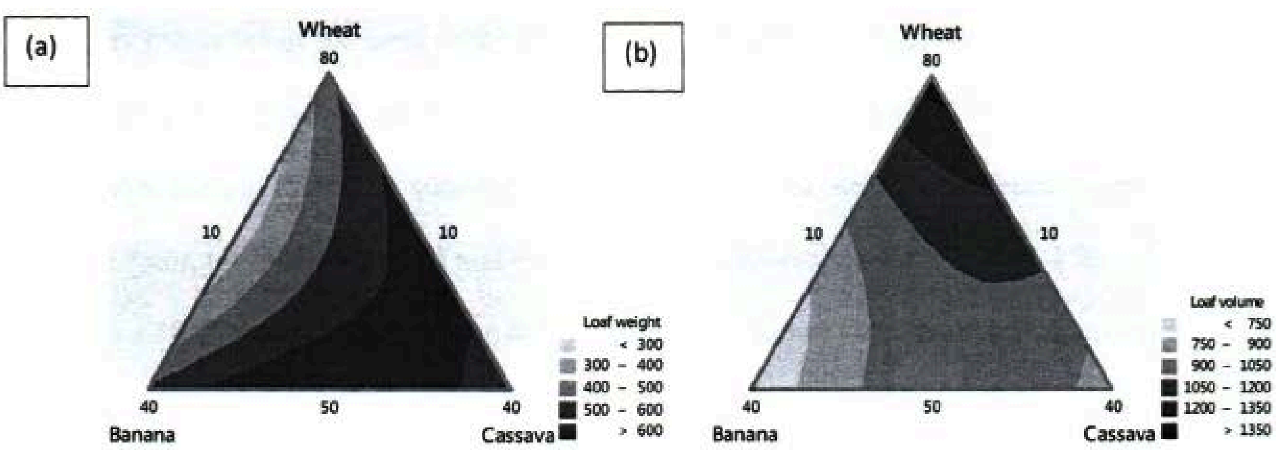
Treatment	Blends (WF:BF:CF) %	Loaf weight (g)	Loaf volume (cm^3)	Firmness (N)
1	65:10:25	522.0±0.6	1093.3±2.1	17.1±3.0
2	70:15:15	519.0±0.3	1126.7±2.2	8.0±1.2
3	65:25:10	509.3±1.0	1026.7±0.6	14.7±1.5
4	65:25:10	514.3±0.2	1073.3±0.3	6.9±1.7
5	60:20:20	527.3±0.5	1076.7±0.8	7.9±2.0
6	50:33:17	643.0±0.6	830.0±0.0	18.4±0.6
7	50:25:25	581.3±0.5	803.3±1.6	28.1±0.4
8	65:10:25	510.3±0.1	1050.7±0.0	9.8±0.1
9	50:25:25	591.3±0.8	804.1±0.0	11.1±2.1
10	65:25:10	514.0±0.6	1080.0±1.3	10.5±1.0
11	50:10:40	579.0±0.5	876.7±5.8	7.9±2.0
12	80:10:10	468.3±0.6	1310.0±5.7	6.4±2.9
13	50:40:10	572.0±1.0	903.3±1.3	29.8±0.6
14	58:32:10	508.0±2.0	1003.3±0.9	6.9±1.2
15	50:25:25	577.7±1.4	806.7±0.9	11.4±1.3
16	55:15:30	579.3±0.6	830.0±0.0	18.4±0.6
Control	100:0:0	412.3±5.0	1426.6±0.4	5.1±1.4

Values are reported as mean ± standard deviation WF: wheat flour; BF: banana flour; CF: cassava flour

Table 8: Lack of fit p-values and adjusted R^2 for physical and sensory properties of bread

Sources	Bread weight	Bread volume	Firmness	Odor	Taste	Texture	Crumb Color	OA
Linear	0.039	0.042	0.083	0.008	0.019	0.103	0.002	0.005
Quadratic	0.031	0.001	0.271	0.025	0.015	0.064	0.001	0.004
A*B	0.001	0.250	0.029	0.006	0.051	0.167	0.057	0.017
A*C	0.412	0.043	0.071	0.332	0.146	0.141	0.001	0.003
B*C	0.353	0.129	0.098	0.480	0.145	0.063	0.269	0.087
Lack of fit	0.052	0.054	0.236	0.320	0.282	0.069	0.121	0.142
R^2_{adj}	98.2%	90.7%	88.9%	96.0%	93.8%	96.7%	98.6%	98.0%

A = Wheat, B = Banana, C = Cassava, OA= Overall acceptance



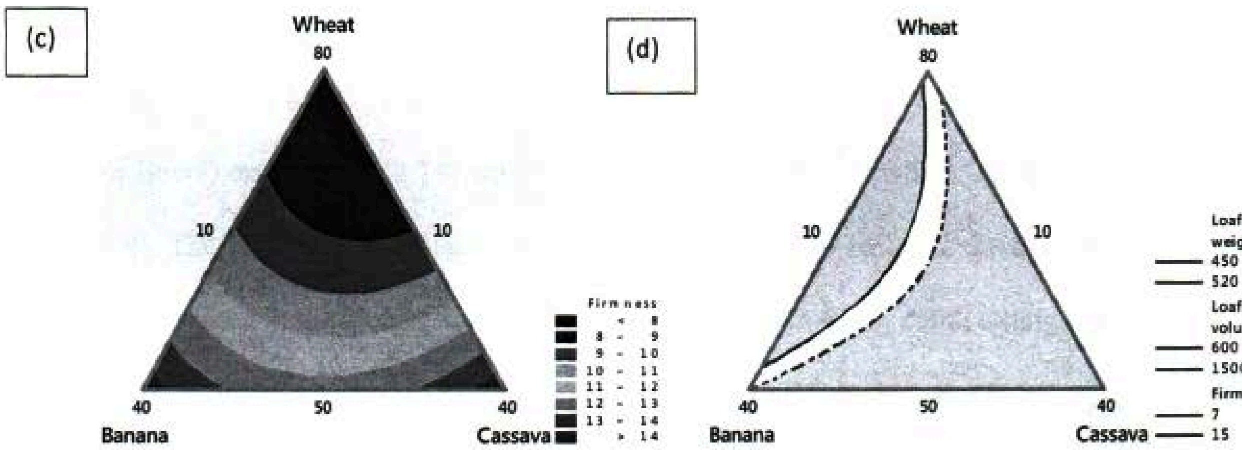


Figure 1: Contour plots and surface plots displaying physical characteristics of composite bread. (a) Loaf weight, (b) Loaf volume, (c) Firmness and (d) Overlaid contour plot of the three physical characteristics that shows the sweet spot (white area).

4.2.2Sensory properties of the bread

Sensory properties of the composite bread were affected ($p < 0.05$) by blending ratio as predicted by linear and/ or quadratic models. The absence of significant lack of fit in both models (Table 7) show that the linear and/ or quadratic models fit well to the sensory attributes. Mean scores of crumb color, taste, odor, texture and overall acceptability ranged from 2.17 to 4.13, 1.96 to 4.08, 3.04 to 3.79, 2.92 to 4.29 and 1.75 to 4.21, respectively. Sensory acceptance attributes of the bread increased as the proportion of wheat increased in the blends. The observation indicates that high supplementation of non-wheat flour (cassava: banana) at 30:30 contributes to low scores (3.5) on texture (Figure 2(d). High supplementation levels of other flours to wheat flour are known to reduce elastic properties of wheat flour dough leading to low gas retaining properties during fermentation (Jensen *et al.*, 2015; Noorfarahzilah *et al.*, 2014; Nwanekezi, 2013), which in turn reduces the texture quality of the final bread.

Bread aroma plays a primary role in creating consumer appeal. The aroma of banana and cassava flour supplemented bread samples scored maximum (3.79) in a ratio of 65:25:10 (wheat: banana: cassava flours) and lowest (2.25) was for the blend of 50:40:10 (wheat: banana: cassava flours) (Table 9). The less odor acceptance is, probably attributed to residual flavor of the bananas as bananas increased in the blend (Ndife *et al.*, 2011; Mashayekh *et al.*, 2008; Dhingra & Jood, 2004). The inclusion of bananas (10 to 40%) and cassava (10 to 40%) had shown an effect on the overall acceptability of the final bread scoring from 1.75 to 4.21 with the highest score being recorded in 80:10:10 and the lowest in 50:40:10 (wheat: banana: cassava) (Table 9). As in many parts of the world, consumers are familiar with the taste of the products they regularly consume. Bread with higher ratio of wheat (80%) and lower ratio of banana and cassava flour (10%) scored the highest, which is similar to those reported in previous studies (Udofia *et al.*, 2013; Mashayekh *et al.*, 2008; Eddy *et al.*, 2007; Dhingra & Jood, 2004). However, changing the proportion of cassava flour from 10 to 30% did not show a difference ($p>0.05$) in the taste of the bread which reflects the blunt taste nature of cassava flour (Jensen *et al.*, 2015; Masamba & Jinazali, 2014). The sweet spot was obtained by placing a range of color 4.1 to 4.3, taste 3.6 to 4.2, odor 3.8 to 4.0, texture 3.6 to 3.9 and overall acceptance of 4.0 to 4.3. The optimum region in this overlaid plot was where the criteria for all five response variables (color, taste, odor, texture and overall acceptability) were satisfied lying in the range of 53.5 to 75.7% for wheat, 11.0 to 29.0% for banana and11.5 to 25.2% for cassava (Figure 2(f). However, the optimum proportion 74% wheat, 10% cassava and 16% banana gave the maximum values of sensory quality responses (taste = 4.10, color = 4.08, odor = 3.87, texture = 3.8 and overall acceptability = 4.18).

Table 9: Sensory properties of bread produced from wheat, banana and cassava flour

Treatment	Blends (WF:BF:CF) %	Crumb color	Aroma	Taste	Texture	Overall acceptability
1	65:10:25	2.88	3.04	2.25	3.75	2.13
2	70:15:15	4.08	3.87	4.10	3.8	4.18
3	65:25:10	3.75	3.42	3.46	3.79	3.46
4	65:25:10	3.50	3.54	3.08	3.46	2.92
5	60:20:20	3.29	3.08	2.67	3.29	3.58
6	50:33:17	4.13	3.63	3.46	4.29	3.46
7	50:25:25	3.48	3.17	3.04	3.42	2.96
8	65:10:25	3.79	3.46	3.46	3.71	3.63
9	50:25:25	3.38	3.42	3.29	3.38	2.83
10	65:25:10	4.08	3.79	4.08	4.04	4.0
11	50:10:40	3.29	3.25	3.38	3.50	3.08
12	80:10:10	3.58	3.42	3.46	3.46	4.21
13	50:40:10	2.17	2.25	1.96	3.54	1.75
14	58:32:10	3.75	3.50	2.29	3.38	3.38
15	50:25:25	3.75	3.71	3.88	3.96	3.71
16	55:15:30	4.13	3.13	3.00	2.92	3.00
Control	100:0:0	3.75	3.17	3.75	4.51	3.79

Values are reported as mean ± standard deviation WF: wheat flour; BF: banana flour; CF: cassava flour

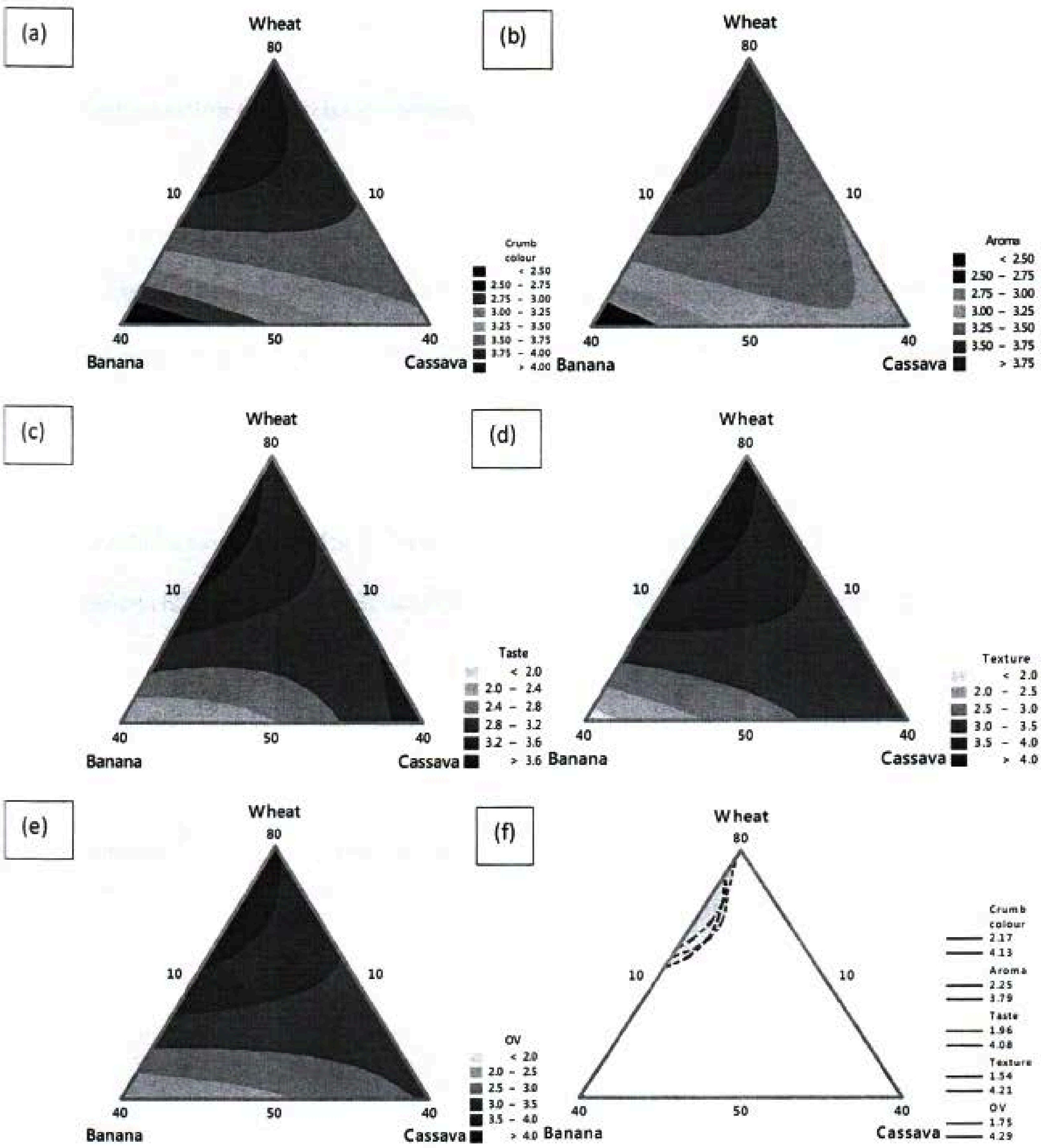


Figure 2. Contour plots and surface plots displaying sensory attributes of composite bread. (a) crumb color, (b) aroma, (c) taste, (d) texture, (e) Overall acceptance and (f) Overlaid contour plot of the five sensory analysis that shows the sweet spot (white area).

4.3 Nutrient composition of bread from wheat, banana and cassava composite flour

4.3.1 Optimization of proximate composition

The composite flours processed from wheat, banana and cassava had moisture, crude fiber, protein, fat, ash, and total carbohydrate in the range of 8.45 to 26.21%, 0.45 to 0.88%, 6.21 to 11.77%, 0.42 to 0.95%, 10.11 to 23.92%, and 41.37 to 72.65%, respectively (Table 10). Crude protein, fat, ash, and total carbohydrate of the bread were affected ($p<0.05$) by varying blending ratio as predicted by the linear and/ or quadratic models (Table 11). The highest crude protein content (11.76%) was from the formulation with 80% wheat, 10% banana and 10% cassava (Figure 3(a) which could be associated with the highest proportion of wheat flour, which contains high proteins. High banana blending proportion increased ($p>0.05$) ash whereas substitution of wheat with a high cassava blending ratio increased total carbohydrate content of the bread (Figure 3(d). A similar increase in ash content of the composite flour bread with banana flour addition was reported by Noorfarahzilah *et al.* (2014). However, as the level of cassava flour increased in the blend, the crude protein content of the bread decreased. The least crude protein content of 6.21% was obtained in the bread processed from the blend of 50% wheat, 10% banana and 40% cassava flour (Table 10). Proteins in roots and tuber crops are very low, which is a major impediment for their extensive utilization.

Fibre and carbohydrate levels could be attributed to cassava and banana flours which are high in both nutrients. This implies that the bread would be a source of high energy and nutrients dense food for consumers. According to Adeniji *et al.* (2008), fibre contributes to the health of the gastrointestinal system and metabolic system in man.

Table 10: Mean protein, moisture, fiber, fat, ash and carbohydrate scores for bread produced from wheat, banana and cassava flour

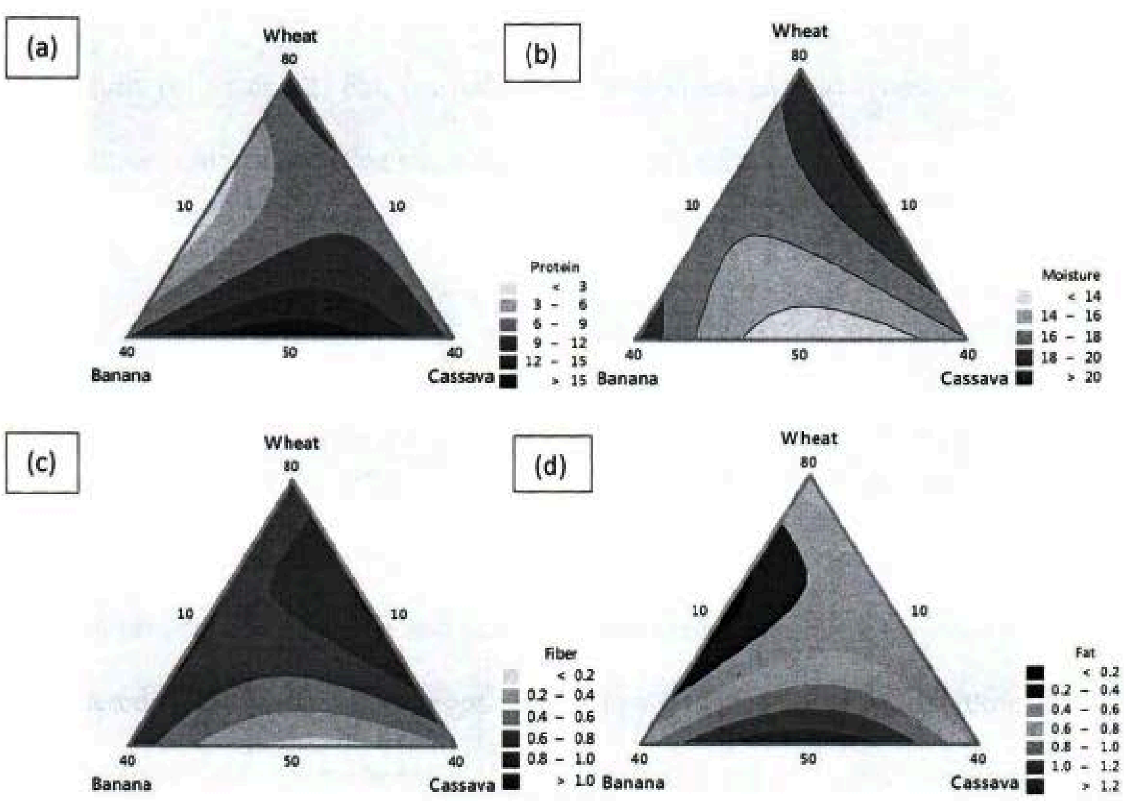
Treatment	Blends (WF:BF:CF) %	Moisture (%)	Fiber (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
1	50:40:10	17.76±0.90	0.77±0.37	8.87±0.02	0.72±0.00	23.92±2.14	55.26±2.69
2	80:10:10	22.48±0.03	0.61±0.10	11.77±0.06	0.81±0.09	12.40±1.57	52.98±1.73
3	65:10:25	22.41±0.11	0.88±0.04	10.63±0.01	0.80±0.06	16.62±4.26	41.37±4.26
4	50:25:25	9.08±2.19	0.55±0.02	7.13±0.07	0.54±0.09	10.06±0.45	49.66±2.66
5	50:10:40	12.56±0.62	0.46±0.00	6.21±0.06	0.42±0.00	10.42±2.39	72.65±1.72
6	55:15:30	26.02±1.02	0.51±0.02	7.87±0.09	0.79±0.03	11.25±1.39	67.81±0.28
7	55:30:15	22.36±2.23	0.54±0.19	9.80±0.18	0.75±0.05	10.16±6.05	56.39±3.88
8	60:20:20	9.56±0.62	0.80±0.00	8.21±0.06	0.54±0.00	20.06±2.39	61.17±1.72
9	70:15:15	8.45±2.00	0.78±0.21	10.79±0.09	0.95±0.06	20.89±1.73	61.91±3.67
10	65:25:10	19.46±2.08	0.81±0.18	10.11±0.06	0.75±0.04	19.71±2.84	45.09±0.60
11	50:33:17	10.09±0.65	0.68±0.05	7.50±0.07	0.66±0.16	23.73±1.02	64.48±0.51
12	65:10:25	20.25±1.32	0.46±0.11	9.93±0.06	0.66±0.02	11.65±2.73	57.05±4.08
13	50:25:25	10.19±1.24	0.54±0.02	8.37±0.05	0.55±0.06	12.28±2.19	68.21±1.08
14	65:10:25	20.62±1.32	0.66±0.11	9.59±0.06	0.76±0.02	17.88±2.73	48.66±4.08
15	50:25:25	18.99±2.19	0.45±0.02	9.18±0.07	0.47±0.09	11.31±0.45	59.32±2.66
16	65:10:25	26.21±0.11	0.48±0.04	10.84±0.01	0.76±0.06	10.47±4.26	51.06±4.26
Control	100:0:0	27.74±2.68	0.44±0.01	12.96±0.32	0.64±0.04	10.11±0.27	48.09±2.14

Values are reported as mean ± standard deviation WF: wheat flour; BF: banana flour; CF: cassava flour

Table 11: Lack of fit p-values, and adjusted R^2 for proximate compositions of bread.

Sources	MC	Fiber	Protein	Fat	Ash	Carb
Linear	0.097	0.588	0.024	0.434	0.014	0.024
Quadratic	0.045	0.527	0.025	0.032	0.016	0.015
A*B	0.391	0.807	0.310	0.246	0.005	0.253
A*C	0.032	0.440	0.017	0.504	0.132	0.023
B*C	0.340	0.477	0.297	0.217	0.376	0.295
Lack of fit	0.256	0.487	0.917	0.222	0.382	0.264
R^2_{adj}	87.8%	86.5%	99.6%	93.6%	94.4%	98.2%

A = Wheat, B = Banana, C = Cassava, MC = Moisture content, Carb = Carbohydrate



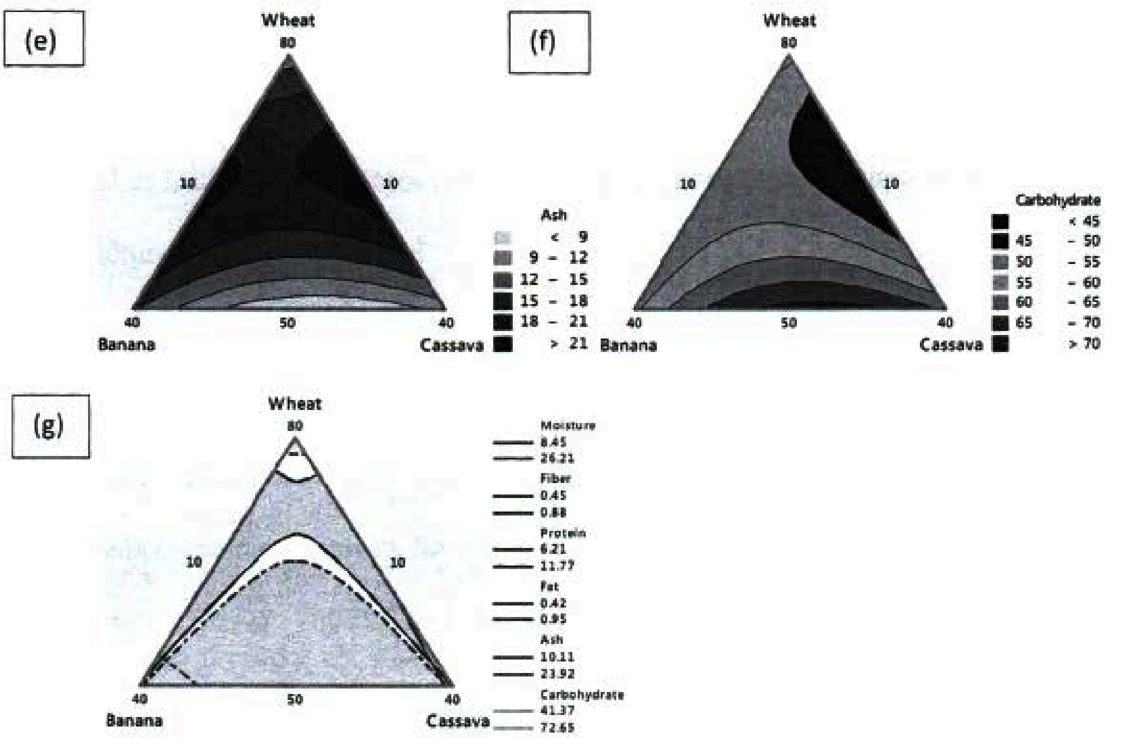


Figure 3. Contour plots displaying proximate composition of composite bread. (a) Protein, (b) Moisture, (c) Fiber, (d) Fat, (e) Ash, (f) Carbohydrate and (g) Overlaid contour plot of the six proximate compositions that shows the sweet spot (white area).

The minimum fat amount of 0.42% was in bread produced from 50% wheat, 10% banana and 40% cassava. Bread from composite flour of wheat at 70%, banana at 15% and cassava at 15% had the highest fat content of 0.95%. It was observed that as the amount of wheat increased in bread, fat increased proportionally (Garg *et al*., 2016; Noorfarahzilah *et al*., 2014; Dhingra & Jood, 2004). As the proportion of banana and cassava flour increased in the composite flour, the fat content decreased (Figure 3(d). These crops contain low fat (Montagnac *et al*., 2009; Eddy *et al*., 2007). Fat increases loaf volume because of its property that enhances incorporation and retention of air during mixing of the dough (Pareyt *et al*., 2011). In addition, fat imparts tenderness, moistness, lubricity, flavor, color and anti-bread staling qualities. Ash amount in bread increased from 10.11

to 23.92% with addition of banana flour from 10 to 40%. Similar increase in ash and inorganic nutrients in composite bread containing banana flour have been reported in previous studies (Sanful & Darko, 2010; Mashayekh *et al.*, 2008). Banana contributes to ash in the bread because of its abundant mineral content.

Carbohydrates in bread was influenced by the amount of cassava flour. The highest level (72.65%) was in a blend containing 50% wheat, 40% cassava and 10% banana (Figure 3(f). A similar trend in blends containing cassava flour has been reported influenced by the high amount of starch in cassava (Eddy *et al.*, 2007). The interactive effect of wheat and cassava was found to be significant ($p<0.05$) on moisture content of bread (Table 12). This is due to the high amount of starch in wheat and cassava that increases moisture absorption. There was no difference ($p>0.05$) in amount of fiber in the bread. Cassava and banana are low in fibre. The optimum response for proximate composition had fiber of 0.66%, protein of 10.00%, fat of 0.80%, ash of 19.20%, and carbohydrate of 53.77%. Desirability of 0.76 was the highest obtained from the blend comprising 64.42% wheat, 21.21% banana and 14.36% cassava (Figure 3(g).

4.4 Bread optimal mixture compositions

The regions of acceptability in the contour plots for protein, carbohydrate, fat, loaf volume, taste and overall sensory attributes were superimposed to determine the optimum formulation (Figure 4). The overall optimum values that would optimize high mineral and carbohydrate contents of acceptable sensory attributes of the bread were 71.2% wheat, 10.6% cassava, and 18.2% banana. Bread attributes like loaf volume and loaf weight of bread from less than 70% wheat flour in the mixture were inferior. Bread with high mineral and bioactive content can be processed from high proportion of banana flour (about 17%) than cassava flour and maintaining wheat flour at 70%. This is critical in diet since it favors incorporation of bioactive compounds such as phenolics, carotenoids, biogenic amines, phytosterols and resistant starch (Shao *et al.*, 2009). These organic compounds are desirable in diet since they exert positive effects on human health. Bioactive compounds have antioxidant activity and are effective in protecting the body against various oxidative stresses.

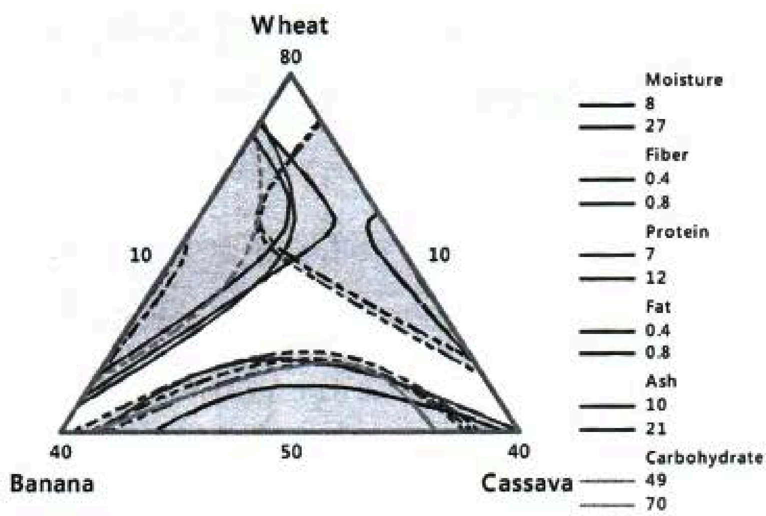


Figure 4. Overlaid contour plot that shows the sweet spot (white area).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Bread is a main dietary component of people in Kampala since majority consumed it daily. Freshness and brand of bread are the main factors driving bread purchases in Kampala. White bread is consumed more than the whole meal bread especially among the young people. It is considered to be cheap and so far the most popular in the communities. Banana and cassava have potential to be incorporated among bread ingredients. Partial substitution of wheat flour with banana and cassava flours in bread production decreases loaf volume and increases loaf weight. Increasing the proportion of banana increased the ash content of bread whereas increasing cassava proportion increased the carbohydrate content of bread. Addition of banana and cassava flours to wheat flour had little effect on the taste, color and texture of bread. Substitution of wheat with banana and cassava flour beyond 30% compromises the quality of bread. Optimum blending ratio was achieved at 71.2% wheat, 10.6% cassava and 18.2% banana. Utilization of cassava and banana flours in bread formulation has implication in improving nutrition and controlling price of bread from purely wheat flour.

5.2 Recommendations

The study generated data revealing the possibility of using banana and cassava flours in production of bread and how it affects the physical and sensory properties of bread. The study has raised several research gaps such as the effect of substituting wheat with cassava and banana in production of other products like cookies. In addition, the effect of baking temperatures on retention of nutrients in wheat, banana and cassava composite bread.

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