EFFECT OF PECTIN AND EMULSIFIERS ON QUALITY AND STABILITY OF WHEAT COMPOSITE BREAD

By

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DECLARATION

I declare that the thesis herewith submitted to the Department of Biotechnology and Food Technology, Durban University of Technology, for the award of Masters in Applied Science Degree in Food Science and Technology is my research work and has not been previously submitted for a degree at any other University or Higher Institution of Education.

As the candidate's supervisor, I agree to the submission of this thesis.

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DEDICATION

I hereby dedicate this thesis to my Lord and Saviour, the giver of life, the Almighty God for given me the opportunity to complete this programme. I also like to dedicate this thesis to my lovely and supportive husband, Oluwatoyin Sunday Ajibade, who believe so much in me.

Our love is forever in Jesus name (Amen).

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ABSTRACT

Fortification and supplementation of wheat flour with other flour sources containing essential amino acids such as lysine, for bread production could help overcome the problem of protein-energy malnutrition. Indigenous and largely underutilised crops such as millet and bambara groundnut could be incorporated into staple foods such as bread. In this study, the rheological behaviour and quality characteristics of dough and bread made from wheat-millet-bambara flour (WMB) containing mixtures of emulsifiers and apple pectin were investigated for their suitability in breadmaking. WMB was prepared by substituting wheat flour (WF) with 25% each of millet flour (MF) and bambara flour. Pectin was added (1.0-2.0 g/100 g flour) and emulsifiers namely sodium stearoyl lactylate (SSL) (0.25-0.4 g/100 g flour), polysorbate 80 (PS80) (0.5-0.8 g/100 g flour), and diacetyl tartaric acid ester of monoglycerides (DATEM) (0.1-0.25 g/100 g flour) were mixed and added in different proportions. A Mixolab was used to measure the rheological behaviour of dough. The resulting bread was analysed for physical characteristics, nutritional composition, and organoleptic properties. Bread samples were stored at room temperature (±25℃), refrigeration (4℃) and freezing (-18℃) for 7 days. The bread samples were then investigated for firmness, compression energy, colour, visual observation of mould growth (VO), total aerobic plate count (APC) and fungal counts (FC). From the Mixolab analysis of composite dough, a significant increase ($p<0.05$) in the dough development time and dough stability were observed. The loaf volume, specific volume and proximate composition of the composite bread increased significantly ($p<0.05$) relative to the control. The protein content (33%), protein digestibility (85%) and some essential amino acids (lysine: 54.6%; threonine: 36.4%) increased significantly ($p<0.05$) compared to wheat bread (control) WF. Sensory evaluation revealed above-average acceptability for composite bread. Also, the pectin-treated bread (PTB) was significantly different ($p<0.05$) in firmness (8.47 N) compared to wheat flour bread (WF) (10.33 N) at -18℃ after 7 days of storage. The WF had the lowest firmness (8.32 N) at room temperature (±25℃) storage lower than the PTB (9.25 N) and emulsifier-treated bread (ETB) (12.37 N) after 3 days storage at room temperature (±25℃). Bread firmness decreased significantly ($p<0.05$) with an increase in storage time for all samples. The APC for all bread samples ranged from 3.02 log cfu/g to 6.19 log cfu/g and fungal count (FC) ranged from 3.48 log cfu/g to 4.86 log cfu/g. The PTB had the highest APC (6.19 log cfu/g) among bread samples stored at room temperature (±25℃) while it also had the lowest APC

(3.02 log cfu/g) at the same storage temperature (±25℃). It was found that all bread samples stored at -18℃ did not show no sign of mould growth.

The use of bakery products' acceptable limits of emulsifiers and pectin for this study significantly improved the dough rheology, physical characteristics, nutritional and sensory acceptability of WMB composite bread. The shelf life studies showed improved firmness, low microbial counts and a slower rate of degradation in cold storage conditions. This study revealed that there is potential for supplementation and fortification of wheat bread with flours from millet and bambara sources.

Keywords: Wheat-millet-bambara flour, composite flour, rheological behaviour, bambara groundnut, Mixolab, emulsifiers, pectin

ABBREVIATIONS

FAO – Food and Agriculture Organization

WHO – World Health Organisation

MT – Million tonnes

Nm – Newton meter

PREFACE

This work has the following conferences presentations.

Conference papers

1) **Ajibade O. B.** and Ijabadeniyi O. A., Effect of Pectin and Emulsifiers on Physical, Nutritional Quality and Consumer Acceptability of Wheat Composite Dough and Bread. 30th EFFoST International Conference, Vienna, Austria, November 27th to December 1st, 2016.
2) **Ajibade O. B.** and Ijabadeniyi O. A., Effect of Pectin and Emulsifiers on Physical, Nutritional Quality and Consumer Acceptability of Wheat Composite Dough and Bread. 1st Interdisciplinary Research, Innovation and Postgraduate Conference, Durban, June 13 – 15th 2017.
3) **Ajibade O. B.** and Ijabadeniyi O. A., Influence of Different Storage Conditions on Texture and Microbial Qualities of Wheat-Millet-Bambara Composite Bread. South African Association of Food Scientist and Technologist (SAAFoST), Durban, September 2017.

CHAPTER ONE: INTRODUCTION

Bread is a major staple food in different parts of the globe, including Southern Africa. The high cost associated with the importation of wheat flour into many developing countries has prompted researchers to seek alternative flours for bread production (Temba *et al.,* 2016). African countries are the world's biggest wheat importers with more than 45 million tonnes imported in 2013 at around 15 billion US dollar (FAO 2013). The total importation cost of wheat to South Africa in 2013 was reported to be R56, 535,574 and the figure is expected to rise as demand for bakery products increases (FAO 2016). Lysine is an important amino acid, and like all cereals, wheat flour used for bread production is deficient in lysine which is significant in solving protein-energy malnutrition (PEM) (Temba *et al.,* 2016). Many developing countries, including African countries, suffer from PEM (Wu *et al.,* 2014). South Africa has observed cases of PEM in children nine years and below (Labadarios *et al.,* 2005). The national food consumption survey (NFCS) reported a large majority of homes in South Africa were food-insecure, energy deficit and nutrient deficient which are the common causes of stunted growth in some adults and children 9 years under. This report has called for mandatory food fortification in South Africa (Labadarios *et al.,* 2005). The NFCS likewise found that more than one out of five children in South Africa are stunted because of malnutrition and that the trend would get worse as food prices increase (Labadarios *et al.,* 2005). The Global Nutrition Report (2015) stated that a diet of pap or bread alone retards the physical and mental growth of children. Baking with wheat (*Triticum aestivum*) alone does not provide adequate nutrients for staple food such as bread (Mal *et al.,* 2010) and a diet based on wheat bread alone may lead to malnutrition. The problem of PEM can be solved through fortification and supplementation of wheat flour with other sources of protein. When cereals and legumes are judiciously selected and combined, adequate essential amino acids are taken in (Wu *et al.,* 2014). Nutritional problems caused by poor protein intake in developing African countries can be solved through the incorporation of cheap, indigenous, protein-rich and readily available food crops, into staple food items (Temba *et al.,* 2016). Furthermore, traditional crops such as bambara groundnut (*Vigna subterranea*) and millet (Pearl millet; *Pennisetum glaucum*) have been advocated as possible crops to address food security challenges in African countries (Wu *et al.,* 2014). These crops are good sources of protein and essential amino acids.

Bambara groundnut is an indigenous and underutilised legume that originated from Africa (Temba *et al.,* 2016). It has great potential, high yield and is drought tolerant (Mazahib *et al.,* 2013). It has several advantages including the fact that it is highly nutritious with high protein (between 26-27%) (Arise *et al.,* 2015; Elemam 2015), carbohydrate (65%), fat (6.5%), phosphorus (380 mg/100g), calcium (73 mg/100g) and methionine (Stephens 1994; Saleh *et al.,* 2013). It is also rich in lysine (5-7%) which is limited in wheat grains (Stephens 1994; Omoikhoje 2008). In addition, moderate amounts of B vitamins and small amounts of minerals and vitamin A have been reported in bambara groundnuts (FAO 1988). A report by Amarteifio and Moholo (1998) shows that bambara groundnut contains 0.097% calcium, 0.007% iron, 1.2% potassium and 0.003% sodium, which makes a well-balanced food with a caloric rate equal to that of a high-quality cereal grain. Bambara has been widely investigated in the production of milk (Obizoba and Egbuna 1992), fermented condiments (Amadi *et al.,* 1999), composite flour, soup thickener (Echendu 2004) and various local delicacies such as beanballs, steamed bean gel (moimoi) (Obizoba 1983; Enwere 1996). Bambara has also been used in the production of composite bread (Alozie *et al.,* 2009; Muhammad 2014; Erukainure *et al.,* 2016).

Millet, a non-gluten cereal, is in the same class with bambara groundnut and it is understudied but has a lot of potentials to alleviate food shortages among Africa populace (Saleh *et al.,* 2013). Threonine and lysine are low in millet, but methionine is high (1.5-2.5%) (Saleh *et al.,* 2013; Obilana *et al.,* 2014). Micronutrients and phytochemicals occur in abundance in millet (Mal *et al.,* 2010; Singh *et al.,* 2012). Apart from good nutritional values, the glycemic index of millet products is on the low side (Kenijz and Sokol 2013). The increase in the percentage of millet will likely result in a decrease in the glycemic index of the composite flour which may be beneficial for people suffering from heart disease, diabetes, and hypertension (Saleh *et al.,* 2013). Millet has been used in making various health focus foods, functional foods and traditional food products such as 'loloh' (fermented Saudi Arabia bread), ready-to-eat cereal breakfast, biscuits/cookies, muffins, cake, biofilm, ethanol production and beer (Ferriola and Stone 1998; Bouis 2000; Keppler *et al.,* 2006; Wang *et al.,* 2006; Changmei and Dorothy 2014). Millet has been combined with wheat flour to make composite bread (Saleh *et al.,* 2013; Schoenlechner *et al.,* 2013).

Exploring the potential of bambara and millet grains in breadmaking could reduce the incident of PEM among African populace. The substitution of wheat with millet and bambara in breadmaking

could also reduce the over-reliance of African countries on wheat and could thus reduce the cost of wheat importation. Although, the use of flours other than wheat in bread production either singly or as a composite had limited success due to low or absent of gluten in these flours (Abdualrahman *et al.,* 2012; Angioloni and Collar 2013; Erukainure *et al.,* 2016). Also, the results of the addition or composite formation with these non-gluten flours for breadmaking are not desirable at a level beyond 20% (Shittu, 2007). As the supplementation of wheat flour with other various flours increase, the viscoelastic properties of dough decrease and this causes reduced handling properties and poor bread quality. The bread produced above the quoted level (20%) had no comparable sensory qualities with that of wheat bread (Abdualrahman *et al.,* 2012; Singh *et al.,* 2012; Erukainure *et al.,* 2016). Published work on the supplementation and fortification of wheat flour in breadmaking has been studied extensively, there is little information on large replacement of wheat flour with other flour sources at 50% and above using flours from tubers, pseudo-cereals and legumes. There is a need to study the effect of non-gluten flours when used above 20% level in composite flours in breadmaking especially when technological aid materials such as emulsifiers and hydrocolloids are used.

Emulsifiers are food improvers that belong to the general group of food enhancers called surfactants or surface-active agents (Stampfli and Nersten 1995). Surfactants like emulsifiers and hydrocolloids such as apple pectin and other improvers have been effectively added to increase the viscoelastic properties of dough and bread made from composite or non-gluten flours (Gómez *et al.,* 2013). Emulsifiers induced quality properties into bread qualities and no emulsifier has all the necessary properties needed to make a bread or baked product that has all the acceptable characteristics (Sharma *et al.,* 1990). They are often combined to induce desirable quality parameters needed for bread production (Gómez *et al.,* 2013). Emulsifiers such as Polysorbate 80 (PS80), sodium stearoyl lactylate (SSL) and diacetyl tartaric acid ester of monoglycerides (DATEM) have the effect of dough strengthening, crumb softening, increased volume and improved crumb structure on wheat bread (Xiujin *et al.,* 2007; Gómez *et al.,* 2013). They have desirable properties that improve bakery product appearance and are safe for consumption (GRAS) (Gómez *et al.,* 2004). Emulsifiers affect bakery product during the early time of baking and also during fermentation, mixing/mechanical handling, moulding, proofing, and transport (Gómez *et al.,* 2004). Such bread usually has good volume and improved crumb structure (Gómez *et al.,* 2004). Emulsifiers such as SSL and DATEM enhanced cassava, maize and wheat composite

bread's specific volume. The composite bread produced from wheat and millet (40%) flour produced bread with large volume and good crumb structure when emulsifiers were added (Eduardo *et al.,* 2014). Pectin has also been found to help in retaining gas in the dough, increased dough volume, improved crumb structure and retard staling process in bread (Kenijz *et al.,* 2013). Composite flour of wheat, cassava, and maize resulted in bread with increased volume up to 3% when pectin was added (Eduardo *et al.,* 2013).

Bread production, storage, consumption, and distribution face challenges such as fast staling rate, loss of moisture, short storage or shelf life, microbial degradation among many other economic problems (Saranraj 2012). Bread, like various processed foods, faces physical, chemical and microbiological spoilage. The shelf life is reduced by physical and chemical spoilage, loss of moisture, staling and microbial spoilage by bacteria and mould leading to economic loss (Saranraj 2012). The addition of improvers such as hydrocolloids and surfactants may also affect the water absorption of dough and final bread moisture content (Gómez *et al.,* 2013). There is reportedly a link between the starch content of the baked product, moisture content and the rate of bread degradation (Patel *et al.,* 2005). Different studies have reported ways in which structural changes in starch can be transformed to reduce staling in bread when surfactants and emulsifiers are used as improvers in bakery products (Armero and Collar 1998; Stauffer 2000; Patel *et al.,* 2005). High moisture content in bread encourages moisture migration, which causes interaction between starch and gluten, this causes degradation of bread (Patel *et al.,* 2005). Also, microbial spoilage, especially by moulds is common due to excess moisture within and around packaged and unpacked bread (Saranraj 2012). Mould spoilage is a serious and expensive problem in the making and consumption of bread as they grow under low water activity and promotes the growth of other microbes (Saranraj 2012). Bread spoilage caused by bacteria is increasing due to non-use of preservatives and the addition of bran and other flours (composite flours) for bread production (Saranraj 2012). Although the effects of hydrocolloids and emulsifiers on dough and bread have been studied, there is little research that has centred on the joint outcome of both emulsifiers and pectin on the quality of composite bread containing millet and bambara flours. Hence, the intention of this research work is to investigate the rheological properties of dough made from wheat, millet, and bambara (50%, 25%, 25%) composite flour using standard Chopin+ protocol by mixolab after addition of different concentrations of pectin and emulsifiers (SSL, DATEM, and PS80). The physical, nutritional and sensory characteristics of the composite bread will be determined. The

work also examined the consequence of the addition of these improvers (emulsifiers and apple pectin) on the texture of stored composite bread in different storage conditions; room (±25 ^{o}C), refrigeration (±4 ^{o}C) and freezing (±-18 ^{o}C) temperatures. The resulting composite bread was analysed for texture and microbial loads during storage periods (0 - 7 days).

Published work showed that bambara groundnut and millet flour addition when used to form composite flour beyond a certain level (>20%) gave bread with increased staling rate (Alozie *et al.,* 2009; Eduardo *et al.,* 2016). Sivam *et al.* (2012), tested the suitability of millet and wheat composite flour for breadmaking and the wheat-millet ratio of 60-40 w/w was found suitable but the staling rate was high. In his later work, he used hydrostatic pressure to treat millet batter and added it to the bread recipe. The result showed an improved bread with desirable characteristics but of low volume. When the fuzzy sensory analysis was used to score bread produced from a composite of millet and wheat by Singh *et al.* (2012), the final bread had a high acceptability level close to the whole wheat bread but that was due to the addition of raw gluten in the formulation. Bambara groundnut addition into the composite of wheat gave bread with improved protein content at 10% addition of bambara flour. The addition above this level (>10%) gave bread with low specific volume and poor crumb structure (Alozie *et al.,* 2009; Erukainure *et al.,* 2016). Eduardo *et al.* (2014) produced bread from composite mixtures of wheat, cassava, and maize with the addition of hydrocolloids and pectin. The results showed that bread without improver gave bread with low specific volume and poor crumb structure compared to the bread samples with addition of improvers.

In all these studies, the composite wheat flours resulted in the dough with poor viscoelastic properties and low-quality bread with a marked decrease in volume, specific volume and poor crumb structure (Gonzaga *et al.,* 2015). To improve the characteristics of both composite dough and bread, there is a need for the use of improvers such as emulsifiers and pectin to improve the viscoelastic properties of dough, dough handling, final breadcrumb and crust quality (Gonzaga *et al.,* 2015).

1.1 Problem statement

The replacement of wheat flour with non-gluten flours usually results in bread with poor quality. Also, when non-gluten flours' (tubers, coarse-cereals, and legumes) percentage level in the composite is increased (50% and above) for breadmaking, the effect further lead to fast degradation

of bread. The effects of these flours, when used for wheat supplementation in bread production, together with improvers such as emulsifiers and hydrocolloids, need to be studied. Also, most reported works either focused on the fortification of bread to address nutritional challenges or on bread supplementation majorly for economic reasons. The production of composite bread may simultaneously address the combination of nutritional and economic challenges. Thus, combining the nutritional suitability of bambara groundnut and supplementation ability of millet into composite bread formation for staple food such as bread is of high importance

Although the effects of hydrocolloids and emulsifiers on the quality of dough and bread have been studied, there is a dearth of information on the combined effect of both emulsifiers (SSL, DATEM and PS80) and pectin on the quality of composite dough and bread containing wheat, millet and bambara groundnut flours, especially when the level of wheat flour is reduced to 50% level in the composite flour and millet and bambara groundnut are incorporated at 25% each.

1.2 Aim

The aim of this research is to determine the effect of emulsifiers and pectin on the quality of composite flour dough and bread made from wheat, Bambara groundnut, and millet.

1.3 Hypotheses

- Composite dough with improved rheological characteristics and bread with desirable physical properties can be produced using emulsifiers and pectin.
  According to Kenijz *et al.* (2013) pectin positively influenced the rheological and physicochemical quality parameters of composite dough and bread.
- Bread with improved texture, storage stability, and slow degradation rate can be produced due to the action of emulsifiers to the composite flour.
  According to Eduardo *et al.* (2016) emulsifiers reduces the rate of firming of bread during storage at room temperature and rate of staling of composite wheat bread.

1.4 Objectives

The objectives of this study are:

- To determine the proximate compositions of composite flour blends formed.

- To determine the effect of emulsifiers and pectin on the rheological and textural properties of the dough and bread produced
- To determine the physical properties of bread after production.
- To determine the consumer acceptability of the composite bread produced with emulsifiers and pectin.
- To study the texture and microbial quality of the produced bread during storage at different temperatures for 7 days.

CHAPTER TWO: LITERATURE REVIEW

2.1 Protein-energy malnutrition

Protein-energy malnutrition can be defined as a variety of pathological conditions rising from a lack of adequate protein and calories intake (Ernest *et al.*, 2013). Malnutrition, in contrast, is defined as a state of an irregular physiological state triggered by inadequate, unstable or extreme consumption of micronutrients and macronutrients (Batool *et al.*, 2015). These two conditions are prevalent in poor and developing countries all over the world. African countries have a high incidence of malnutrition; within this period between 2012 to 2014, more than 214 million people suffered from undernourishment (Temba *et al.*, 2016) (Table 1). One of the major public health problems is protein-energy malnutrition (PEM) and is a huge burden as it is expensive to treat. It is related with poor health and physical malfunctioning of the human body (Temba *et al.*, 2016). PEM has been identified as the cause of kwashiorkor, marasmus, and marasmic-kwashiorkor and these have been associated with other deadly diseases such as malaria, diarrhoea, tuberculosis, anaemia and even death (Müller and Krawinkel 2005; Ernest *et al.*, 2013). In sub-Sahara Africa, there is slow progress in combating the menace of malnutrition among the populace. This is due to the consumption of cereals or starchy foods which are low in protein and some micronutrients and lack supplementation with proteins from animal sources or legumes (Temba *et al.*, 2016). The initiative by FAO to end hunger in Africa by 2025 with a target of no hunger in West African by this year (FAO 2015), can only be achieved if introduction of local foods that are high in protein is the centre tool or remedy to eradicate malnutrition and its health challenge in emerging countries (Becker 2007). The major staple food groups popular in African countries are the tubers, roots, and cereals with a small amount of protein originated from an animal, 60% of this represents the total energy supply for the Africa populace (Galati *et al.*, 2014). Maize, rice, sorghum, and millet are major cereals forming part of the main diet of African countries (Galati *et al.*, 2014). Fibre from these cereals are significant and they are a viable source of energy, minerals, and vitamins, though, considerably limited in lysine and tryptophan an essential amino acids (Galati *et al.*, 2014). Nutritional security cannot be guaranteed for any diet based on cereals alone due to this deficiency (Sarwar *et al.*, 2013). Legumes are a good source of lysine though they are deficient in amino acids that contain sulphur, they are a cheap source of good amino acids such as methionine and cysteine (Sarwar *et al.*, 2013; Galati *et al.*, 2014). Legumes, however, have some antinutritional factors that

reduce their use. They are relevant in cereal-based foods where they can increase the protein content (Temba *et al.,* 2016). Studies by Igbabul *et al.* (2015) affirms that a product made by combining cereals and legumes have better nutrients and energy value than those made from either alone. Forming composite with cereals and legumes increase the energy and protein content of a food product and this can solve malnutrition problems in Africa (Temba *et al.,* 2016). One of the best ways to reduce the problem of PEM is the preventative method which is compositing inexpensive and readily available indigenous crops such as cereals and legumes into the staple food item.

Table 1. Number of malnourished (millions) and percentages (%) of malnourished around the world, (1990-1992 & 2012-2014)

	1990 - 1992		2012 - 2014	
Region	Number	Percentage	Number	Percentage
World	1014.5	18.7	805.3	11.3
Developed region	20.4	<5	14.6	<5
Developing region	994.1	23.4	790.7	13.5
Africa	182.1	27.7	226.7	20.5
Sub-Saharan Africa	176.0	33.3	214.1	23.8
Asian	742.6	23.7	525.6	12.7
Eastern Asia	295.2	23.2	161.2	10.8
South-Eastern Asia	138.0	30.7	63.5	10.3
Southern Asia	291.7	24.0	276.4	15.8
Latin America and Caribbean	68.5	15.3	37.0	6.1
Oceania	1.0	15.7	1.4	14.0

Adapted from Temba *et al*. (2016)

2.1.1 Cereals

Cereals belong to the monocot family *Poaceae* and they are usually planted for the eatable part of their grain that contains the germ, endosperm, and bran (Sarwar *et al.,* 2013). The major cereals include millet, barley, rice, maize, sorghum, oats, wheat, and rye (Wrigley 2016). They ranked highest in terms of energy supply and food provision globally in comparison to others cereals (Temba *et al.,* 2016). Cereals are the basic food of man since prehistoric times and were consumed long before breadmaking was developed (Saranraj 2012). The global production of cereals was about 3 billion MT in 2014 (FAO 2016), this level of production shows cereals as an important dietary item. In terms of nutrition, cereals have a relatively small amount of protein but have a

high level of carbohydrate. The high amount of vitamins, dietary energy, insoluble dietary fibre, minerals (iron and zinc), phytochemicals and other antioxidant activities (Bouis 2000). Sorghum, maize, barley, pearl millet, oats and finger millet are known as coarse cereals and are considered excellent nutritious food due to their nutrient content (Aghamirzaei *et al.,* 2013). The nutrient components of cereals are depicted in Table 2. Cereals are limited in some essential amino acids needed for humans nutrition (Temba *et al.,* 2016). Supplementation and fortification of cereals (wheat flour) with low-cost crops, like non-gluten cereals and legumes, may, therefore, help render wheat-based products more nutritious (Noorfarahzilah *et al.,* 2014).

2.1.2 Wheat

Wheat (*Triticum aestivum)* has been a crop with a lengthy history of use by humans as food. It has been appreciated as a major source of energy through its carbohydrate, and in more recent times for its supply of valuable proteins (Amjid *et al.,* 2013). This combination of carbohydrates and proteins gives wheat its unique properties for making bread of different kind of taste (Singh *et al.,* 2012). The occurrence of gluten in wheat protein network and the capacity to form an elastic dough in the presence of gluten proteins are the major characteristics of wheat that made it suitable for breadmaking (Shewry 2009). The major proteins in wheat are glutenin and gliadin, these are responsible for gluten development during mixing. The exceptional functional properties of gluten appeared in leavened bread (Ahlborn *et al.,* 2005). Gluten present in the proteins of wheat is accountable for the protein-starch interaction, this provides specific viscoelastic properties in bread dough. These rheological properties are greatly in control of gas cell formation and stabilisation also, in retaining of gas during proofing (Gan *et al.,* 1989). The protein content and the quality of-of gluten present in the grain and flour of wheat is mainly related to breadmaking quality. They also have a high influence on properties of dough that include the ability to absorb water, stability in mixing, the capability to retain CO_2, and the volume of bread (Noorfarahzilah *et al.,* 2014).

Table 2. Nutrient Components of Common African Cereals Grains

Nutrients	Maize	Sorghum	Pearl Millet	Finger Millet	African Rice	Foxtail millet	Common millet	Little millet	Barnyard millet	Kodo millet	Wheat
Protein (%)	9.2	11.6	11.5	7.3	7.9	11.2	12.5	9.7	11.0	9.8	11.6
CHO (%)	73	77	70	74	7.1	63.2	63.8	60.9	55.0	66.6	71.0
Fat (%)	4.6	3.4	4.7	1.3	75	4.0	3.5	5.2	3.9	3.6	2.0
Dietary Fibre (%)	2.8	9.1 -11.5	9.7	11.7	1.8	na	na	na	na	na	na
Ash (%)	1.2	1.6	2.3	2.6	3.5	3.3	3.1	5.4	4.5	3.3	1.6
Calcium (mg/100g)	48.3	29	36	35.8	23	31	8	17	22	35	30
Iron (mg/100g)	4.8	4.5	9.6	9.9	1.9	2.8	2.9	9.3	18.6	1.7	3.5
Energy (kg/100g)	1471	1374	1443	1396	1392	351	364	329	300	353	248
Thiamin (mg/100g)	0.38	0.38	0.38	0.42	0.1	0.59	0.41	0.3	0.33	0.15	0.41
Riboflavin (mg/100g)	0.2	0.15	0.21	0.19	0.04	0.11	0.28	0.09	0.1	0.09	0.1
Niacin (mg/100g)	3.6	4.3	2.8	1.1	4.3	3.2	4.5	3.2	4.2	2.0	5.1

Adapted from Saleh *et al.* (2013) *CHO - Carbohydrate , na; not available

2.1.3 Gluten

Gluten is the protein fraction of wheat grain which gives wheat its unique ability to form a dough. It is the prolamin fraction of the storage proteins, attached to the gluten proteins (Shewry 2009). It forms elastic dough when there is an interaction between the proteins in the grains to form a protein-protein network (Seilmeier *et al.,* 1991). It has solid non-covalent and covalent forces of its protein and forms a constant matrix of protein deposits (fuse when water is added to form a dough) (Tosi *et al.,* 2009). Gluten proteins are the major element that guarantees the desirable outcome in wheat products and it influences the water absorption, cohesion, extensibility, viscosity, resistance to deformation, elasticity, tolerance to kneading, the ability to gas retention and dough strengthening properties (Noorfarahzilah *et al.,* 2014). Gluten protein is known for its importance in giving an easily handled and high-quality baked goods and in products that need extensible and elastic dough (Saleh *et al.,* 2013). Most cereal flours are devoid of gluten and their addition into bakery products impairs the breadmaking quality of wheat flour (Noorfarahzilah *et al.,* 2014). The wheat flour forms a dough which is a viscoelastic material with the help of gluten and joins the viscous liquid and elastic solid part to create rheological characteristics. The protein (viscoelastic) network plays a major part in dough processing also in the physical appearance of the final bread (Rosell *et al.,* 2007). When gluten is absent in flour instead of dough, a batter is gotten, and this usually gives the bread a texture that is crumbling, unfamiliar colour and other quality defects (Noorfarahzilah *et al.,* 2014). Bread production from low gluten dough cannot retain gas except hydrocolloids replace or strengthen the gluten in the flour mixtures or composites (Torbica *et al.,* 2010).

2.1.4 Legumes

Legumes are a member of the family of the plant kingdom; *Fabaceae* (or *Leguminosae*). Their different parts (fruit, seed, pod) are used as food and are ranked second based on human consumption rate (Sánchez-Chino *et al.,* 2015). They serve as a source of income, help in food security, soil fertility and increase the nutrition of world population with over 30 species grown all over the world. Chickpea (*Cicer arietinum*), cowpea (*Vigna unguiculate*), common bean (*Phaseolus vulgaris*), pigeon pea (*Cajanus cajan*), groundnut (*Arachis hypogaea*) and soybean (*Glycine max*) are some of the common legumes (Temba *et al.,* 2016) (Table 3).

Table 3. Chemical Composition of some selected legumes (g/100g)

Legume type	Species	Protein	Carbohydrate	Fat	Ash	Fibre
Bambara groundnut	*Voadnzeia subterranea*	32.40	51.79	7.35	5.78	2.68
African yam bean	*Sphenogtylis stenocarpa*	37.21	44.40	9.49	5.35	3.55
Bambara groundnut	*Vignal subterranea*	20.60	56.51	6.60	3.25	6.34
Jack bean	*Canavalis ensiformes*	26.20	57.83	1.95	6.51	1.07
Pigeon pea	*Cajanus cajan*	24.46	56.83	4.78	4.58	1.10
Cowpea	*Vigna unguiculata*	24.13	56.60	4.37	4.73	0.97
Soya bean	*Glycine max*	42.80	19.80	22.80	5.20	2.30
Bauhinia seed	*B. galipinii*	38.50	23.30	24.20	4.50	1.4
Chick pea	*Acararietinum*	22.83	57.19	5.43	3.04	3.50
Lentil	*Lensculinaris medicus*	31.12	52.63	0.81	2.62	3.68
Kidney bean	*Phaseolus vulgarius*	20.09	57.67	2.46	3.85	6.78
Mung bean	*Vigna radiataf*	22.70	58.99	1.36	3.35	4.70

Adapted from Arise *et al.* (2016)

These crops are estimated to be cultivated widely in Sub-Sahara Africa each year and about 19 million metric tonnes annually has been recorded (Abate *et al.,* 2012). Legumes are a good source of protein, good storage ability and cheap in comparison to animal products. They play a prominent role in nourishment, specifically when mixed with other food items (Broughton *et al.,* 2003). Legumes comprise a wide range of amino acids, carbohydrates, fats, vitamins and minerals in their grains (Table 3). Legumes are easily attacked by microbes that produce toxins and they also contain some anti-nutritional factors (Temba *et al.,* 2016). Processing methods have been employed in the reduction or total elimination of anti-nutrients in legumes (Saleh *et al.,* 2013). Developed processing methods (soaking, roasting, cooking, sprouting and grinding) are effective in the reduction of these anti-nutrients (Musa *et al.,* 2016). When cereals and legumes are judiciously selected and combined, a desirable pattern of essential amino acids comparable to or

greater than the reference protein is obtained (Noorfarahzilah *et al.,* 2014). Legume products, when effectively applied in baked goods can give a high protein product with enhanced amino acid level. Legume protein is high in lysine, but they are deficient in amino acids that contain sulphur. This made legumes and cereals a match because cereals are low in lysine but rich in sulphur amino acids (Noorfarahzilah *et al.,* 2014). The use of legumes as a vehicle for protein intake has been studied extensively (Abdualrahman *et al.,* 2012; Noorfarahzilah *et al.,* 2014; Erukainure *et al.,* 2016).

2.1.4.1 Legumes amino acids

Legumes storage proteins amino acids include glutamic and aspartic acid which may include glutamine and asparagine, respectively (Mune *et al.,* 2011). These amino acids are responsible for 25-40% of all the amino acids in leguminous seed as depicted in Table 4 (Arise *et al.,* 2016). The high content of arginine has been reported in faba bean and peanut but this can be compared with bambara groundnut, soya, and cowpea (Arise *et al.,* 2016). Higher arginine content of approximately 8.1% has also been reported for bambara groundnut. Arginine helps in preventing heart diseases, while glutamine helps support the immune system and improve athletic performance (Adebowale *et al.,* 2011). The lysine content of bambara (6.3 g/100 g protein) is like that of mung bean (Kudre *et al.,* 2013) and soybean (Adebowale *et al.,* 2011). Indigenous legumes such as bambara groundnut, mung bean, and faba bean have been shown to be adequate sources of lysine just like soybean according to FAO/WHO reference (Adebowale *et al.,* 2011; Kudre *et al.,* 2013). Bambara has methionine content that is higher than that of marama bean and faba bean but like black bean and soybean (Adebowale *et al.,* 2011; Amonsou *et al.,* 2012; Kudre *et al.,* 2013; Pastor-Cavada *et al.,* 2014). High methionine content (1.8%) was also reported for bambara (Ijarotimi and Esho 2009). Based on these reports, high contents of methionine and arginine in bambara coupled with high lysine content makes it a good candidate for supplementation and fortification of staple food products such as bread and thereby may reduce the incidence of protein-energy malnutrition among population groups in developing countries.

Table 4. Amino acid composition of some legume proteins (g/100g)

Legume types	*ASP	GLU	ARG	LYS	HIS	ALA	ILE	LEU	MET	PHE	PRO	VAL	TRP	CYS	SER	THR	TYR	GLY
Bambara groundnut	9.6	15.4	5.9	6.3	3.0	3.5	3.8	7.3	1.3	5.3	2.7	4.3	7.3	ND	3.2	2.8	3.3	3.1
Marama bean	9.4	15.2	8.0	5.7	2.7	3.5	4.3	7.9	1.0	3.7	7.2	4.8	ND	6.1	5.5	3.2	11.4	5.9
Kidney bean	10.9.	15.3	5.3	4.9	3.4	3.8	5.2	8.5	1.6	5.9	3.0	5.2	ND	0.9	4.6	3.7	3.2	3.6
Mung bean	8.5	12.5	6.4	6.2	2.8	3.7	3.9	7.4	1.3	5.8	3.0	4.6	6.4	0.5	3.9	2.8	3.2	3.2
Faba bean	11.9	16.4	11.8	7.1	2.8	4.4	3.9	7.9	0.9	5.8	4.1	4.8	0.7	1.1	4.6	4,8	2.5	4.1
Black bean	9.6	14.1	6.4	6.0	2.9	3.6	4.0	7.4	1.3	5.7	2.9	4.6	7.6	ND	3.6	2.5	3.3	3.2
Soya bean	11.6	19.1	7.7	6.4	2.8	4.3	4.0	7.8	1.4	5.2	ND	5.0	ND	ND	5.6	4.1	3.8	4.7
Peanut	11.2	18.7	11.4	3.4	2.1	4.0	3.1	6.2	1.1	3.6	4.3	3.8	0.9	1.2	4.9	2.7	5.1	4.6
Cowpea	12.2	18.9	6.8	6.9	2.5	4.4	4.6	7.7	1.2	5.7	3.9	5.4	3.8	1.0	5.5	3.8	3.2	4.1

* Asp – Asparagine, Glu – Glutamine, Arg – Arginine, Lys- Lysine, His – Histidine, Ala – Alanine, Ilu – Isoleucine, Leu – Leucine, Met – Methionine, Phe – Phenylalanine, Pro – Proline, Val – Valine, Trp – Tryptophan, Cys – Cysteine, Ser – Serine, Thr – Threonine, Tyr – Tyrosine, Gly – Glycine. ND: Not determined
Adapted from Arise *et al.* (2016)

2.1.5 Bambara groundnut

2.1.5.1 Background, history, and structure

Bambara groundnut (*Vigna subterranea)* has been classified as a legume species and it originates from Africa and belongs to the *Fabaceae* plant family and subfamily; *faboidea* (Mazahib *et al.,* 2013). It is herbaceous and an intermediate, annual legume that self-pollinates with a well-developed taproot (Arise *et al.,* 2016). Bambara has lateral stems which develop from the root and on this are the leaves. Bambara leaves are presented as trifoliate while petiole is grooved, stiff and long, with a green or purple colour base. The leaves and flower buds arise alternately at each node (Hillocks *et al.,* 2012). The pudding behaviour is like that of groundnut where flower stalk moves down after fertilisation, pushing the young developing pod into the soil where it will develop and mature (Hillocks *et al.,* 2012; Yao *et al.,* 2015). Bambara is usually grown at an average temperature of 2 ^{o}C – 28 ^{o}C and reaches maturity by 3 to 5 months. The bambara plant is very adaptable and tolerates drought conditions better than most crops. It can grow well on well-drained soil and on soil that is low in nutrients and requires pH of about 5.0 - 6.5 (Brough *et al.,* 1993; Bamshaiye *et al.,* 2011; Yao *et al.,* 2015). With so much food insecurity and malnutrition among developing countries and the continent of Africa, bambara can be a crop of hope to alleviate malnutrition and poverty because of its drought-tolerant characteristics especially in regions of the world where water scarcity is prevalent (Basu *et al.,* 2007). Bambara groundnut seeds leave with stem and colour varieties which comprises white to cream, yellow, red, brown, purple, and black as shown in figure 1. Bambara is majorly from Yola, Nigeria, and northern Cameroon. It has now been cultivated in many African countries and different continents (Azam-Ali 2001). The main producing areas of bambara in South Africa are Limpopo, Mpumalanga and KwaZulu-Natal provinces (Murevanhema and Jideani 2013). Bambara is cultivated by poor women farmers in some parts of Africa. Bambara has many uses and it is considered a complete food (Linnemann 1990; Bamshaiye *et al.,* 2011).

Figure 1. Bambara groundnut (seeds and plant). (Images from the University of Nottingham (www.nottingham.ac.uk). Adapted from Cleasby *et al.* (2016)

2.1.5.2 Uses of Bambara Groundnut

Bambara groundnuts have diverse ways of utilisation, although they are mainly used for human consumption. The seeds are consumed either when immature or fully ripe and dry (Elemam 2015). Salt and pepper are added to freshly boiled pods of bambara and eaten as snacks in many West African countries. Bambara groundnuts can be roasted and crushed to make soup with or without condiments (Goli *et al.,* 1997; Murevanhema *et al.,* 2013). Reports from recent research shows that bambara groundnut is applied in the preparation of various foods such as bread, biscuit and in cake production (Okafor *et al.,* 2015), vegetable milk and yoghurt (Murevanhema *et al.,* 2013; Falade *et al.,* 2015). Bambara groundnut was studied for the production of baby food, industrial products and animal feed (Elemam 2015). Milk and bread were produced in Zambia with Bambara groundnut. The milk produced by bambara have a preferred flavour in comparison to milk made from pigeon pea, cowpea and soybean (Brough *et al.,* 1993). Bambara groundnut can replace animal protein and it can also fight malnutrition and resolve the problem of food shortage in Africa due to its high essential amino acid content (Hillocks *et al.,* 2012). In Nigeria, bambara groundnut paste is used to make steamed products called Okpa. Okpa is a cooked, dough-like gel made from bambara paste. It is wrapped in banana leaves and boiled. Bambara has been widely investigated for processing into milk (Obizoba *et al.,* 1992), fermented condiments (Amadi *et al.,* 1999), composite flour and soup thickener (Echendu 2004) and various local delicacies such as beanballs, steamed bean gel (moimoi) (Obizoba 1983; Enwere 1996). It was also reported by Alozie *et al.* (2009) in the production of bread at different ratios with wheat flour. These qualities show the

ability of bambara groundnut as a rich protein source for the fortification of bread and various novel food products. Bambara groundnut has been investigated for medicinal uses and it has been reported that the Lio tribe in Kenya use water from boiled bambara seeds to cure diarrhoea (Adeola and Orban 1995). Bambara leaves and roots have been used as an anti-inflammatory, aphrodisiac and antibiotics material in some parts of Africa (Hillocks *et al.,* 2012). The black landraces have the popular belief of being used for treating impotence in Botswana (Bamshaiye *et al.,* 2011).

2.1.5.3 Nutritional profile of bambara groundnut

The seeds of bambara can be regarded as a nutritious and a complete food due to its high protein quantity (20.5-27.0%), carbohydrate content (54.5-69.3%), and fat (5.3-7.8%). The level of essential and sulphur containing amino acids found in bambara is higher than the amount found in most legumes (Ijarotimi *et al.,* 2009; Mune *et al.,* 2011; Murevanhema *et al.,* 2013; Arise *et al.,* 2016) (Table 5). Bambara is quite rich in fibre, iron, calcium, and potassium. Bambara is considered a complete food due to the presence of most nutrients in its seeds (Musa *et al.,* 2016). Marama bean, just like bambara groundnut (Amonsou *et al.,* 2012; Murevanhema *et al.,* 2013) and soya bean are good sources of protein (Adebowale *et al.,* 2011; Amonsou *et al.,* 2012) and like pulses such as cowpea, kidney bean, and mung bean. The protein content of bambara is sometimes varied within the landraces. The mineral contents and nutrients of the dark seeded landraces (black and red) are more than the cream colour seeds (Murevanhema *et al.,* 2013). The crude protein in the seeds (25%) is reportedly higher than in the pods (19%) and hulls (Murevanhema *et al.,* 2013). The composition of amino acids present in bambara groundnut was reportedly significant. The protein is high in lysine and methionine (Okpuzor *et al.,* 2010). However, lysine and leucine are the major essential amino acids in bambara seed (Mazahib *et al.,* 2013). For example, the lysine content was found to stand at 6.82 g/16 gN, methionine was 1.85 g/16 gN and cysteine; 1.24 g/16 gN and this is likened to soybean with lysine; 6.24 g/16 gN, methionine; 1.14 g/16 gN, and cysteine; 1.80 g/16 gN respectively. A high crude protein content was also reported (17.5-21.1%) and this makes bambara a good source of plant protein. The carbohydrate content of bambara ranged between 50-60% and their biological availability is high. The starch in the seeds accounts for between 24 to 56% in comparison with other oil legumes (peanuts 14% and soybean 32%) which has a lower carbohydrate content (Arise *et al.,* 2016). The total sugars consist of the oligosaccharides of the raffinose family (raffinose 0.35 g/mg, stachyose 1.57 g/mg) which is about

31-76% (Hardy 2016). These sugars are responsible for the effect of flatulence; a human gastric indigestion of legumes in some people. The problem of flatulence has been solved in soybean through germination of seeds before using it for food products. The seed germination method has also been used for bambara to reduce the incidence of flatulence after consumption (Murevanhema *et al.,* 2013). The chemical composition of legumes starch is characterised by high amylopectin content (Schuster-Gajzágó 2004). Oyeleke *et al.* (2012) reported starch to be the highest component (48.12 g/mg), which is an indicator of the high energy content of the bambara seed. The soluble fibre content of bambara has been reportedly high compared to other legumes (Murevanhema *et al.,* 2013). Bambara has a fat content (6-8% of dry matter) higher than found in cereals, but it is negligible for it to be used as an oil source (Brough *et al.,* 1993). However, it has been reported that oil from bambara is extracted in Congo through roasting and pounding (Goli *et al.,* 1997). The bambara hull has a fat content higher than found in the pods while the least is the seed; 4.3%, 3.5%, and 1.6% respectively. Except for the oilseeds (soybean; 20% and peanuts; 52%), legumes generally have 7% oil content and this provides essential fatty acids in the human diet (Murevanhema *et al.,* 2013). The oil is composed mainly of polyunsaturated fatty acids (Schuster-Gajzágó 2004). Bambara can be used to provide a balanced nutrition in zones with expensive animal protein and the farming of various legumes is economically unfavourable due to bad environmental conditions (Murevanhema *et al.,* 2013; Yao *et al.,* 2015). Bambara remains underutilised despite its good food potentials and is neglected even though it has the potential to perform a critical part in food security, revenue generation and food culture of the rural people. The nutrient composition of bambara is depicted in Table 5.

2.1.5.4 The amino acid composition of legumes

The main amino acids of legume storage proteins are glutamic and aspartic acid which may include glutamine and asparagine, respectively (Mune *et al.,* 2011). These amino acids make up about 25-40% of the total amino acids in leguminous seed as shown in Table 4. A higher amount of *arginine* has been reported in Faba bean (Pastor-Cavada *et al.,* 2014) and peanut (Latif *et al.,* 2013) but with similar comparison with bambara groundnut when compared with soya (Adebowale *et al.,* 2011) and cowpea (Elharadallou *et al.,* 2015).

Table 5. Nutrient composition of bambara groundnut

Component	Value
Proximate	g/100g
Ash	2.0 - 3.6
Carbohydrate	54.5 - 69.3
Crude fat	1.6 - 6.7
Crude fibre	1.8 - 12.9
Crude protein	17.0 - 27.0
Iron	5.9 - 7.1*
Potassium	1240 -1290*
Phosphorus	296 – 320*
Sodium	3.7 - 4.8*
Calcium	7.8 – 13.5*

Adapted from Arise *et al.* (2016) *mg/100g

Adebowale *et al.* (2011) had reported a higher arginine content of approximately 8.1% for bambara groundnut. Legumes have been recognised as protein sources (including Bambara) that are rich in arginine and glutamine. Arginine helps in the prevention of heart disease, while glutamine helps support the immune system and improves athletic performance (Adebowale *et al.,* 2011). The lysine content of bambara (6.3 g/100 g protein) is like that of mung bean (Kudre *et al.,* 2013) and soybean (Adebowale *et al.,* 2011). Based on recommended reference lysine intake from protein source by FAO/WHO (1989), indigenous legumes such as bambara groundnut, mung bean (Kudre *et al.,* 2013) and faba bean (Pastor-Cavada *et al.,* 2014) contained adequate sources of lysine just like soya bean (Adebowale *et al.,* 2011). Thus, these indigenous legumes offer some potentially significant nutritional attributes making legumes a good protein supplement to cereals that are known to be deficient in lysine. The methionine content of bambara (Kudre *et al.,* 2013) is higher than that of marama bean (Amonsou *et al.,* 2012) and faba bean (Pastor-Cavada *et al.,* 2014) but comparable to the black bean (Kudre *et al.,* 2013) and soya bean (Adebowale *et al.,* 2011).

However, higher methionine content (1.8%) was reported by Steve Ijarotimi *et al.* (2009) for bambara. This higher methionine and arginine content of bambara, if researched into, may lead to differences in its functionality when compared to other legumes. The amino acids found in bambara groundnut and other legumes are shown in Table 4.

2.1.6 Millet

2.1.6.1 History and cultivation of millet

Millets are grasses (cereals) with small kernels and are grouped because of similar cultivation technique. French word "mille" denotating thousand millets, meaning the small size of millet can have thousands of seeds in them (Taylor *et al.,* 2010). Millet is critical in food availability and economy of several under-developed countries in the world. They are commonly cultivated in India, Africa, and China. Millet is thought to be one of the first grains cultivated by man. The first recorded reports on the cultivation of millet dates to about 5,500 BC in China (Crawford 2006). They are extremely important crops in semiarid regions where other crops normally do not survive (Bora 2014). They are easy to cultivate, inherently bio-diverse and can be grown together with varied crops (Dendy 1995). Another good use of millets that make them a preferred choice in areas where they are cultivated is their short harvest period (45-65 days) (Bukhari *et al.,* 2011). Millet is an important drought-resistant crop and is non-gluten, it is assumed as a food for the poor (Kaur *et al.,* 2014). Another name for millet is *ragi* or *mandia* common to Indian or Asia region. Millet is popular in dryland areas of rural India and it is used for various purposes by the inhabitants (Pradhan *et al.,* 2010). The world production of millet grains was 28,384,668 metric tonnes and the highest producer was India (annual production of 11,420,000 tonnes) (59.8%) (FAO 2016). The commercialization of millet products is less advanced in southern Africa than in West Africa (Changmei *et al.,* 2014). Finger millet and pearl millet have been used industrially in the production of malt and unmalted adjunct for beer brewing in eastern, central and southern Africa regions (Saleh *et al.,* 2013). Millet is drought-resistant, ranked 6th in terms of world production record (Bora 2014). Short growing season, resistance to pests and diseases and high harvest during drought conditions compared to other cereals are some of the advantages of millet cultivation (Devi *et al.,* 2014). Recently, millet grain is getting positive attention from the developing countries through utilisation as food and in some developed countries for production of ethanol and biofilms (Dewettinck *et al.,* 2008). Millets are small-seeded with different types of species

such as finger millet (*Eleusine coracana*), pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italic*), kodo millet (*Paspalum setaceum*), barnyard millet (*Echinocloa utilis*), proso millet (*Penicum miliaceum*) and little millet (*Panicum sumatrense*) (Bouis 2000). Pearl millet (*Pennisetum glaucum*) is the most important millet, it represents about half of millet total production (Taylor *et al.,* 2010). Millets are generally known as coarse cereals also, sorghum (*Sorgum bicolor*), maize (*Zea mays*), barley (*Hordeum vulgare*) and oats (*Avena sativa*) (Bouis 2000; Kaur and Kapoor 2001). The most commonly consumed millet is the Pearl millet, cultivated in tropical parts of Asia, Africa, and Latin America. The highest producer of pearl millet is India and is mainly grown in northwestern parts (Dendy 1995; Obilana 2003). It is also the major millet grown in Nepal and Bhutan (Mal *et al.,* 2010), China, however, mainly produces foxtail millet. In Africa, pearl millet production is concentrated in the sub-Sahara and drier areas of northern and eastern Africa (Obilana 2003; Bora 2014). Proso millet is mainly grown in developed countries like Australia and America (FAO 1995).

2.1.6.2 Millet structure

Millet seed is like one-third of the size of wheat. Millet seed is small but it is packed with condensed nutritional value due to the large germ part of the seed in ratio to endosperm (Taylor *et al.,* 2010). Pearl millet seed is roundly shaped (Jain and Bal 1997) (Figure 2). They come in sizes and weight, ranges between 2 mg, 3 mg, and 15 mg. In contrast to other tropical cereal grains (maize and sorghum), millet seeds are minor grains (Dendy 1995). Most cereal grains have large air space, unlike pearl millet grains that have a closed packed grain with little air space. The density of pearl millet grain is about 1.6 g/cm^3 (Dendy 1995; Jain *et al.,* 1997), evidently bigger compared to wheat (1.39 g/cm^3), maize (1.39 g/cm^3), rice (1.24 g/cm^3) and sorghum (1.24 g/cm^3) grains (Dendy 1995). Sorghum has a similar structure as pearl millet, but the pearl millet kernel is smaller with a smaller endosperm and proportionately bigger germ than sorghum (Abdelrahman and Hoseney 1984).

The pericarp of pearl millet grain is about 8% with 17% germ (which is proportionally large) and 75% endosperm (Abdelrahman *et al.,* 1984). The pericarp, the germ (embryo) and the endosperm have values ranges between 7.2 to 10.6%, 15.5 to 21% and 71 to 76%, respectively (Abdelrahman *et al.,* 1984). The kernel is a third of the germ, and the endosperm is between 50 to 60% of the kernel (Zeleznak and Varriano-Marston 1982). The surface of the pericarp was covered with thin

waxy cutin, this helps to reduce the effects of weathering (Obilana 2014). The colour of the kernel is influenced by the presence of pigments in the epicarp (McDonough *et al.,* 1986). The tube and cross cells which formed the endocarp are often indistinguishable. The pearl millet resistance to mould attack is linked to the mesocarp layer which is different across genotypes (McDonough *et al.,* 1986). The endocarp exists inside the pericarp, under the pericarp, is found a layer of the thin seed coat, and then a single one cell thick aleurone layer (Obilana *et al.,* 2014). Pearl millet has a variety of colour from yellow, grey, brown and purple (Obilana *et al.,* 2014); at times grains may have diverse colours. This may be because of the presence of dense pericarp that usually coats the aleurone layer pigments of some grains varieties. The existent of tinny pericarp usually give light and even colour/pigments throughout the aleurone. Thin pericarp and absence of pigments give white kernel grain colour (Obilana *et al.,* 2014). Pigments can easily be removed during decortication where colour pigmentation is located within the pericarp to give acceptable flour colour (McDonough *et al.,* 1986). Under the seed coat lies a single layer of aleurone cells that form the first layer of the endosperm (Zeleznak *et al.,* 1982). These cells walls are thick and contain protein bodies and oval lipid bodies in their cytoplasm. The pearl millet starchy endosperm contains a corneous and floury component and is used to classify the cells. The floury component is soft and floury while the corneous component is stiff and vitreous-like. At the outer layer and the centre, the corneous and the floury portion are found in bigger sizes respectively (McDonough *et al.,* 1986). Pearl millet consists of three separate parts which are the corneous, peripheral and the floury endosperm. There existed three layers of cells within the peripheral endosperm and it comprises thick different protein mediums (McDonough *et al.,* 1986). There is the presence of minute polygonal starch granules rooted in the protein matrix and this characterises the starch granules. The hard texture of the endosperm is caused by the continuity of the protein matrix and the physical contact between the starch granules and storage proteins. The protein bodies of the peripheral endosperm are tightly packed and they leave separate indentations in the starch granules (McDonough *et al.,* 1986). The starch granules of the protein bodies have many air holes, this gives chalky presence to the floury endosperm. There exists a discontinuous matrix of protein in the floury endosperm of pearl millet (Obilana *et al.,* 2014). The ratio of the germ or embryo (a major structural part) of pearl millet to the endosperm is higher than that of other cereals (Dendy 1995). The germ comprises of two main parts; the embryonic axis and scutellum cells. The storage body; scutellum serves as storage for protein, lipids, minerals, and enzymes (Dendy 1995).

Scutellum cells are smoothly round in appearance and their sizes range from 25 to 35 µm in diameter (McDonough *et al.*, 1986).

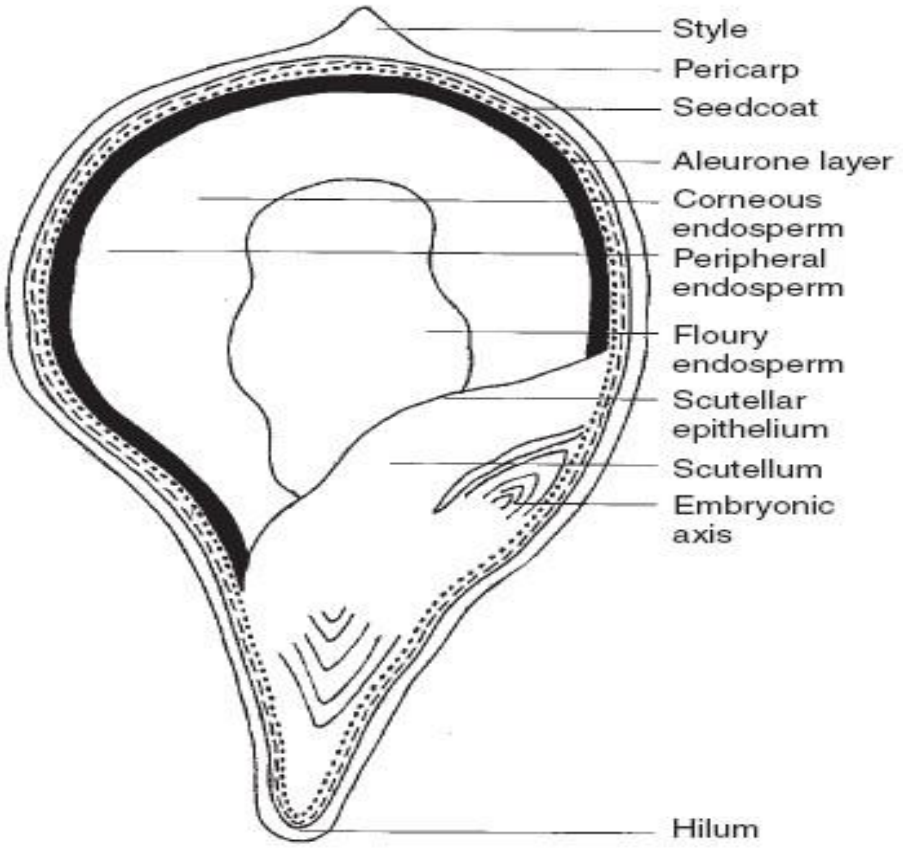

Figure 2. Pearl millet grain longitudinal section. (Barrion 2009)

2.1.6.3 Nutritional components of millet grain

Millet protein and fat contents ranged between 6-11.8% and 2.5 - 4.8% respectively. Most common cereals also have a close value, for instance, maize (9.2% protein, 4.6% fat) and sorghum (10.4% protein, 3.1% fat). Millet grain protein is known to be a good source of quality amino acids though deficient in threonine and lysine, they are moderately rich in methionine (Mal *et al.*, 2010). Millets contain many phytochemicals and micronutrients (Singh *et al.*, 2012). They are a rich source of soluble and insoluble dietary fibres, resistant starch, antioxidants and minerals (Ragaee *et al.*, 2006). There chemical composition is about 2.8% crude fibre, 92.5% dry matter, 2.1% ash, 13.6%

protein, 7.8% fat and 63.2% carbohydrate (Ratnavathi 2016). The protein content of foxtail millet showed that it can be used to supplement protein in protein deficient foods due to high lysine (Mohamed *et al.,* 2009). The health advantages of finger millet have been traced to high polyphenol contents (Chethan and Malleshi 2007). It contains 81.5% of carbohydrate, 9.8% of protein, 4.3% of crude fibre, and 2.7% of mineral which are similar to cereals and many other types of millet. It has crude fibre and mineral contents which are significantly better when compared to wheat (1.5% minerals and 1.2% fibre) and rice (0.2% fibre and 0.6% minerals); also a well-balanced protein with increased threonine, lysine, and valine (Ravindran 1991; Sripriya *et al.,* 1997). Black finger millet chemical composition is known to be closely comparable to ordinary finger millet (8.71 mg/g dry weight fatty acid and 8.47 g/g dry weight protein) (Glew *et al.,* 2008). Little millet and kodo millet ranked highest in fibre content (between 37% to 38%) among all cereals. The fat portion of kodo millet has a high polyunsaturated fatty acid compared to other cereals (Hegde and Chandra 2005). Proso millet has a protein content of which dry matter is 11.6%, this is comparable with that of wheat, and it was significantly better-off in amino acids level (leucine, isoleucine, and methionine) (Kalinova and Moudry 2006). Although pearl millet consumption is vast, it has some certain anti-nutritional factors (polyphenols and phytic acid) which hinder its digestibility (Simwemba *et al.,* 1984). Abdalla *et al.* (1998) reported pearl millet to have 88-91% dry matter, l.6-2.4% ash, 2.6-4.0% crude fibre, 2.7-7.1% oil, 8.5-15.1% crude protein, 58-70% starch and 354-796 mg g^{-1} phytic acid. Mineral contents were l0-80, 180-270 and 450-990 mg g^{-1} for Ca, Mg and P, respectively and 70-110, 4-13, 53-70, 18-23, l0-18 and 70-180 µg g^{-1} for K, Na, Zn, Mn, Cu, and Fe, respectively (Abdalla *et al.,* 1998). Pearl millet nutrient composition when compared to other main cereal grains in terms of gross energy content (363 Kcal/100g) is high (Table 2). The high-fat content of the grain allows high energy, and this is linked to the large germ size (Obilana *et al.,* 2014). Pearl millet contains several minerals, including the substantial level of phosphorus, calcium, iron, magnesium, and it is a fair source of thiamine and B vitamins (Obilana *et al.,* 2014). The pearl millet grain contains some level of thiocyanate and flavones, this is goitrogenic (Osman and Fatah 1981; Akingbala *et al.,* 2002). The presence of these bioactive compounds is reduced significantly by various processing methods. The roasting method used in this work will reduce the anti-nutritional bioactive substances present in pearl millet which may favour the digestibility of the composite bread. Apart from good nutritional values, pearl millet foods are popular for the low glycemic index of their food products

(Kenijz *et al.,* 2013). The increase in the percentage of millet in composite flour formation can decrease the GI of the composite flour which may be beneficial for persons suffering from heart disease, diabetes, and hypertension (Saleh *et al.,* 2013). Hence, millet, just like rice or wheat can be used in the production of ready-to-eat convenience cereal to improve its acceptability and other applications such as in composite for breadmaking (Bouis 2000). The oil content in pearl millet is relatively high; linoleic acid represent 4% of the total fatty acids in the oil, it has a higher percentage of n-3 fatty acids and this is better than maize (0.9% linoleic acid) thus, maize is extremely lacking in n-3 fatty acids (Jaybhaye *et al.,* 2014). Terminal diseases causing conditions that affect physiological functions such as platelet aggregation, LDL cholesterol build-up, and the immune system are highly influenced by n-3 fatty acids. Pearl millet bran has a high proportion of soluble dietary fibre and could be used for hypocholesterolemic and hypoglycemic effects (Kaur *et al.,* 2014).

2.1.6.4 Pearl millet proteins and amino acids

Pearl millet grain is high in protein and oil, higher than that of wheat, rice, maize and sorghum (Bora 2014). Under certain conditions of cultivation, some pearl millet cultivars, the type of soil and climate under which they were planted, can influence pearl millet nutrient compositions. Some cultivars (*Pennisetum glaucum, P. americanum, P. typhoideum*) contains an increased level of lysine than most common cereals (rice, maize, and sorghum) (Saleh *et al.,* 2013) (Table 2). Pearl millet cultivars contain crude protein content that ranges between 10 and 15% (Saleh *et al.,* 2013; Kaur *et al.,* 2014). Pearl millet protein consists of prolamins, albumins, globulins, and glutelins (Dendy 1995). The total protein of pearl millet consists mainly of prolamins and glutelins which is about 63%, and they are the most abundant of the grains' protein (Dahiya and Kapoor 1983). The prolamin proteins levels are between 31 to 41% of the available protein in pearl millet (Obilana *et al.,* 2014). The albumins and globulins have the highest percentages of the protein present in pearl millet (25% of the total protein content) (Hadimani *et al.,* 2001). The essential amino acid, lysine, is slightly higher because of high globulin and albumin content in pearl millet (Obilana *et al.,* 2014) (Table 6). In terms of the amino acid score, pearl millet possesses the best protein indices (Almeida-Dominguez *et al.,* 1993). Its protein and the amino acid score are better compared to sorghum, this may be because, of high asparagine, lysine and methionine content compared to sorghum (Dendy 1995). Adeola *et al.* (1995) reported that pearl millet has higher levels of

isoleucine, arginine, threonine, valine, and lysine contents compared to maize. Though, pearl millet is still limited in the essential amino acid lysine (Delgado and Saldivar 2000). Just like other cereals, lysine is the limiting amino acid in millets. The major amino acids in the prolamin fraction are the glutamic acid (16-23%) and leucine (12-22.3%), however, barnyard has a higher amount of alanine (18%) than leucine (Kumar and Parameswaran 1998; Bora 2014). There are variations in the lysine values claimed for pearl millet grain. This may be because of the structure and relative proportion of the germ to endosperm (Obilana *et al.,* 2014). The highest amount of protein is found in the peripheral endosperm, reducing from the exterior to the interior of the kernel (McDonough and Rooney 1989). The endosperm protein exists as a continuous matrix in the peripheral and corneous endosperms (Obilana *et al.,* 2014). Relatively few protein bodies are found in the floury endosperm, and a considerable amount of the pearl millet protein is present in the germ (McDonough *et al.,* 1989).

2.1.6.5 Carbohydrates in pearl millet

The major constituents of cereal grains including pearl millet are carbohydrates, (Table 2). The total carbohydrate varies between 50 to 75% of the grain composition (Hoover *et al.,* 1996; Oshodi 1999; Hadimani *et al.,* 2001; Shahidi and Naczk 2003) closely related to that of sorghum. Carbohydrates present include non-starch polysaccharides starch and free sugars apart from starch (Hadimani *et al.,* 2001). The amylose content is lower compared to sorghum and it ranges from 17 to 21.5% (Obilana *et al.,* 2014). The amylose content of some pearl millet varieties may be as high as 28.8 to 38% which exhibited a variation in the amylose content of pearl millet starch (Hadimani *et al.,* 2001). Pearl millet total soluble sugars are between 2.3 to 2.6% and they include xylose, glucose, raffinose and fructose (Hadimani *et al.,* 2001). Pearl millet has the very same amount of sucrose as sorghum, but its raffinose content (0.23%) is higher than that of sorghum (0.71%) (Obilana *et al.,* 2014). The Fibre (dietary) content in pearl millet is between 8 to 9% (Hadimani *et al.,* 2001). The dietary fibre constituents (hemicellulose, cellulose, pectin, lignin, and gums) are present in the endosperm cell walls and the pericarp of the pearl millet seed (Obilana *et al.,* 2014).

Table 6. The essential amino acid composition of common cereal grains

	Wheat	Rice	Maize	Sorghum	Pearl Millet
Amino acid (g/100g protein)					
Phenylalanine	4.6	5.2	4.8	5.1	5.5
Histidine	2	2.5	2.9	2.1	2
Isoleucine	3	4.5	3.6	4.1	3.8
Leucine	6.3	8.1	12.4	14.2	10.9
Lysine	2.3	3.9	2.7	2.1	2.7
Methionine	1.2	1.7	1.9	1	2.5
Threonine	2.4	3.7	3.9	3.3	3.7
Tryptophan	2.4	1.3	0.5	1	1.3
Valine	3.6	6.7	4.9	5.6	5.5

Adapted from Temba *et al.* (2016)

2.1.6.6 Pearl millet fats

Pearl millet is generally high in lipid content (3–6%) higher than for sorghum and most other common cereals such as maize (Obilana *et al.*, 2014). The reported lipid amounts by FAO (1995) are inconsistent; 3.1%, 4.8% and 4.6% for sorghum, pearl millet, and maize respectively. Pearl millet total fatty acids are approximately 75% unsaturated fatty acids such as a palmitoleic acid (16:1), linoleic acid (18:2), oleic acid (18:1) and linolenic acid (18:3). Stearic acid (18.0) and palmitic acid (16.0) are the saturated fatty acids that constitute the remaining 25% of the total fats. When pearl millet is ground it usually give unpleasant odours and flavours, this is due to the level of unsaturation of the fatty acids present in pearl millet germ (Kaced and Hoseney 1984). The germ size of pearl millet is large and this influences the level of oil (Adeola *et al.*, 1995). Decortication reduces oil content, and this process can reduce it by 1.2% (Hadimani *et al.*, 2001), this directly helps to reduce lipid oxidation and increase the shelf life reasonably in pearl millet flour (Slavin *et al.*, 2000).

2.1.6.7 Minerals and vitamins in pearl millet

Millets are rich in vitamins and minerals and they are present in the aleurone layer, pericarp, and germ. The germ also has high ash content; most time, when pearl millet is refined, it leads to loss of some or parts of the nutrients (Almeida-Dominguez *et al.,* 1993; Chowdhury and Punia 1997; Shahidi *et al.,* 2003). Whole grain millet had free lipid content from 26 to 53 mg/g while finger millet varieties showed the least contents varying from 6.7 to 12 mg/g (Bora 2014). Pearl millet can be used as a vehicle for micronutrients for staple food products due to its richness in all these micronutrients. The major essential micronutrients present in pearl millet are calcium (36 mg/g), iron (9.6 mg/g) and energy (1443 kg/100g) (Temba *et al.,* 2016).

2.1.6.8 Processing and uses of millet

Processing of millet using traditional methods can increase the nutrition, edibility, and sensory characteristics of food products. Processing methods such as mechanical method, fermentation, thermal, soaking, and malting or germination improve the biological availability of nutrients in millet (Saleh *et al.,* 2013). These methods are employed to improve the physicochemical accessibility of micro-nutrients and decrease the content of antinutrients, such as phytates, or increase the content of compounds that improve bioavailability (Hotz and Gibson 2007). Millet grain decortication has been known to be technologically problematic owing to the smallness of millet seeds. It is impossible to dehull millet like other grains such as wheat and maize. Traditional decortication was done on pearl millet manually or application of the mechanical device to remove the seed coat (Saleh *et al.,* 2013). The decortication method expressively reduced the dietary and crude fibre content, total phenols content, minerals and antioxidant capacity but it does not reduce the main constituents (protein and fat content) of pearl millet (Lestienne *et al.,* 2007). Baking of raw pearl millet flour does not lead to a substantial difference in the nutrient content, but milling changes the chemical composition. Heat treatment and milling of pearl millet decreases polyphenols and phytic acid content and improved the protein and starch digestibility to an appreciable level (Chowdhury *et al.,* 1997). Germination or malting usually lead to biochemical changes that produce malt and this increased nutrient availability in pearl millet. Germination is reported to increase the total sugars and free amino acids and causes a decrease in starch content and dry weight of proso millet (Bora 2014). The starch and *in vitro* protein digestibility of pearl millet were increased after germination, this may be because of the decrease of the antinutrients

such as phytic acid, polyphenols, and tannins, which interact with proteins to form complexes (Hassan *et al.,* 2006). Increase in niacin, lysine, sugars, protein fractions, soluble dietary fibre, and *in vitro* availability of calcium, iron, and zinc of food products has also been ascribed to germination (Arora *et al.,* 2011). This is because of the increase in phytase activity, which caused a decrease in the content of phytate in sprouts. The anti-nutrients such as saponins and polyphenols prevent the bioavailability of minerals, after germination, catabolization occurred and this allows improved bioavailability of mineral (Grewal and Jood 2006). Millet grain processed by fermentation preserve, impact flavour and improve the nutritional bioavailability of food products. The number of anti-nutrients in millet grains are decreased by fermentation which causes an improvement in the protein bioavailability, nutritive value and the *in vitro* protein digestibility (Saleh *et al.,* 2013). Fermentation and enzymatic hydrolysis have been reportedly good for processing millet as it impacts high nutritive value into food products (Saleh *et al.,* 2013). Soaking and cooking are used as pre-treatments to decrease the anti-nutritional constituents of millet grains to boost nutrient availability and nutritional constituents of millet foods. Pearl millet has been reportedly used in making fermented bread, ready-to-eat (flaked whole grain) cereal products, biscuits/cookies, muffins, and cake (Osman 2011, Ferriola et al., 1998, Bouis 2000, Keppler et al., 2006, Chang 2004) and other various delicacies. The possibility of using millet for bio-film, ethanol and beer production has also been reported (Wang *et al.,* 2006). Unlike the composite flour, millet alone can be used to make non-gluten bread for celiac patients. Millet has been used in the production and fortification of novel and health focus foods due to its nutritive nature and protein content that is high (Saleh *et al.,* 2013). It is used to form composites flour to make weaning food, biscuits, bread, noodles and in extruded breakfast cereal products (Saleh *et al.,* 2013). Millet milling fractions are convenient raw material in composites to invent foodstuffs with improved nutritive and functional properties which can promote their application for various food products (Saleh *et al.,* 2013). Millet contains bioactive substances and natural antioxidants which are used in food applications and as a nutraceutical and functional food ingredient in health promotion and disease risk reduction (Mohamed *et al.,* 2012). Millet contains some natural inhibitors which are applied in the treatment of clinical sicknesses like diabetes mellitus, cardiovascular diseases, slow aging and control of celiac diseases in gluten intolerance patients (Kaur *et al.,* 2014). Viswanath *et al.* (2009) described that the seed coat of finger millet is used as a food preservative and natural antioxidant. Phenolics extracts and other bioactive components have also been shown to possess

antimicrobial, preservative and therapeutic properties (Siwela *et al.*, 2010). The inclusion of millet in the human diet can also lower the risk of duodenal ulcers, anaemia, and constipation (Jayaraj *et al.*, 2001; Nambiar *et al.*, 2011).

2.1.6.9 Bambara groundnut and millet *in vitro* protein digestibility

The nutritive value of protein known as protein quality depends on its amino acids content and the *in vitro* protein digestibility (IVPD) after consumption (Hahn *et al.*, 1984). The legume proteins are affected by their limited susceptibility to hydrolysis by digestive enzymes. This proteolytic resistance has been attributed to the structural characteristics as well as to the existence of anti-nutritional compounds, for instance, phytic acid, polyphenols and trypsin inhibitors (Hahn *et al.*, 1984). The use of legumes is limited in human and animal nutrition due to the existence of the mentioned factors (Alonso *et al.*, 2000). Legume seeds constitute a cheap source of protein for human nutrition. The susceptibility of native proteins to proteolysis by mammalian digestive enzymes is a factor that is important, and it can add to the nutritive value of seed storage proteins. Different processing techniques can be used to increase the protein and starch digestibility of legumes and their uses. Heat processing methods could cause certain physical or chemical changes in proteins, starch and other constituents of legume seeds thus changing their final nutritional quality (Valle *et al.*, 1994; Alonso *et al.*, 1998; Alonso *et al.*, 2000). Protein digestibility primarily determines the availability of its amino acids (Hahn *et al.*, 1984). Protein digestibility has been historically determined by bioassays using rats or microorganisms. The disadvantages of this procedure were that they were time-consuming and expensive (Hahn *et al.*, 1984). The IVPD in some legumes such as *Lupinus termis*, faba bean, *Dolichos lablab*, cowpea, chickpea, lentil and bambara groundnut ranged between 52.6 to 92.27% (Elamin 1996; Elsheikh *et al.*, 2000; EL Siddig *et al.*, 2002; Awada *et al.*, 2005; Yagoub and Abdalla 2007; Mohamed 2015). Bambara groundnut has been found to contain some anti-nutrients which are common in legumes and these hinder the *in vitro* digestibility. Among these anti-nutrients are polyphenols, phytic acid and enzyme inhibitors (Yagoub and Abdalla 2007). Unprocessed bambara does not support growth adequately because the trypsin inhibitor contained in the seeds prevents proteolysis in the intestines and a large part of the ingested protein is excreted unused (Obasi 1998). Germination as a processing method has been reported as the best method to improve the protein digestibility of bambara (Hardy 2016). The level of anti-nutrients is considerably decreased by thermal treatment methods

which are roasting and cooking. They also improve the *in vitro* starch digestibility of bambara groundnut seeds (Yagoub *et al.,* 2007). Cereals are also known to have some anti-nutritional compounds which reduced the bioavailability of nutrients just like legumes. It has been observed that milling and sieving increased the IVPD of finger millet though there is a reduction in the nutritional composition (Saleh *et al.,* 2013). Pearl millet phytic level was reduced by germination and fermentation and a marked improved mineral availability (Saleh *et al.,* 2013). Fermentation has been identified as a method that reduces the amounts of anti-nutrients in cereal grains and improves the availability of protein, IVPD, and nutritive value (Saleh *et al.,* 2013). Soaking and cooking have been found to increase mineral availability and IVPD of foxtail millet and an appreciable reduction in anti-nutrients such as polyphenols and phytate (Pawar and Macheward 2006). The biological utilisation of a protein is mostly reliant on its digestibility and composite bread from different flour should have a high IVPD for them to improve protein intake in the food product the composite is being used to produce. In this work, the use of bambara groundnut and millet in composite formation with wheat flour to make bread may produce bread with the high digestibility of starch, protein, and minerals. The digestibility of both bambara and millet grains after several processing approaches such as milling, cooking, roasting, sieving, germination/sprouting enzymatic hydrolyzation, and fermentation may be high (Saleh *et al.,* 2013).

2.1.7 Composite flour

Composite flour can be defined as a combination of flours, starches and other ingredients intended to replace wheat flour completely or partially in bakery and pastry products. The flour is either a double or triple mixture of different flours from many sources with wheat flour. It is also literarily the mixtures of two or more flours to get a functional ingredient for food production (Milligan *et al.,* 1981; Shittu *et al.,* 2007). The advantages of composite flour formation in developing economy include foreign exchange savings, promotion of high-yielding, native plant species, a better supply of protein for human nutrition and better inclusive use of domestic agriculture production (Noorfarahzilah *et al.,* 2014). Composite flours are beneficial to African countries in that their use decreases the import of wheat flour and inspires the incorporation of domestically cultivated crops as a source of flour (Hugo *et al.,* 2000; Noorfarahzilah *et al.,* 2014). The FAO (2012) stated that if the importations of wheat could be reduced or even removed in virtually all developing countries and that demand for bakery goods could be addressed using domestically grown products as an

alternative to wheat, then using composites for food production is advantageous (Noorfarahzilah *et al.,* 2014). For upgrading of the nutritious value of food and diet to avoid malnutrition (PEM) and certain diseases, different methods are required to offer adults and children improved nutritious food with cheap and locally grown food formulations (Saleh *et al.,* 2013). Supplementation and fortification of cereals based foods is a dynamic method that proved to combat nutrient deficiencies (Saleh *et al.,* 2013). Compositing wheat flour with legumes can increase the nutritional content of the by-product and produce cheap food products. Additionally, several studies have revealed the advantages of legume flours such as chickpea and millet in increasing mineral bioavailability and lowering of glycaemic response in healthy consumers diet (Noorfarahzilah *et al.,* 2014). Pearl millet is a good crop for supplementation of wheat flour in bread production. Pearl millet is rich in nutrients and other bioactive substances which can contribute to a healthy diet in bread. It also contains some anti-nutrients that can hinder its protein and starch digestibility thus making it undesirable for bread production especially the whole millet grain. Composite flour of barnyard millet and wheat flour made bread with a high protein content and consumer acceptability (Singh *et al.,* 2012). The suitability of oats, sorghum and millet composite flour for making bread was tested, the result showed bread with acceptable qualities and justified that bread made from the composite represent a feasible alternative to making aerated bread with alleviated sensory and technological limits grounded on non-viscoelastic (non-gluten) cereals (Angioloni *et al.*, 2013).

The nutritional composition of food is very important in sustaining the general population physical wellness because nutritional wellness will give sustainable development for health and maximisation of potential for human genetics. Consequently, dietary quality should be considered when solving the problem of deep-rooted food insecurity and malnutrition (Obilana *et al.,* 2014). The baking process can benefit from technological aid materials especially for composite flours to improve breadmaking properties; this includes intensive dough mixing, sponge and dough system, chemical/activated dough development, reduced proofing periods, use of dough conditioners and bread improvers (Angioloni *et al.,* 2013). In composite mixtures of wheat flour, increased level of non-gluten flour usually results in bread with poor quality. Reduction in gas retention capacity and flour strength in the dough is due to a decrease in the level of gluten, which affects taste and sensory likeness of produced composite bread. High levels of grains other than wheat incorporated into baked products are cost-effective and nutritionally advantageous even though technologically

very challenging (Angioloni *et al.*, 2013). Millet has been used as a replacement for wheat composite flour, complementary foods, and food blends seem to be the best method that can be used to prepare nutritional, healthy and safe, high-quality, shelf-stable food products at the domestic level and commercial scales to encourage the utilisation of millet grains (Ratnavathi 2016). In this study, the composite flour formed with bambara groundnut and millet flour will have an insufficient amount of gluten (50% of total flour) which will affect the viscoelastic properties of dough and the bread quality. Improvers such as emulsifiers and pectin will be used to try to improve dough viscoelastic properties and improve the final bread quality.

2.1.8 Bread

Bread is defined as a fermented confectionery product formed primarily from wheat flour, water, yeast and salt by series of processes involving mixing, kneading, proofing, shaping and baking (Dewettinck *et al.*, 2008). One of the major universally accepted food commodities is bread, a convenient form of food that is important to all populations. Bread consumption has been dated to the Neolithic era and still remained one of the most consumed and acceptable staple food products in all parts of the world (Ijah *et al.*, 2014). Bread is a basic and ancient staple food for most households in South Africa and Africa at large, therefore, there is always a need to study ways of improving its suitability as a staple food (Cleasby *et al.*, 2016). Bread is an excellent source of various vitamins and minerals especially phosphorus and copper but it is regarded as nutritionally poor, as the cereal proteins present in wheat flour are deficient in essential amino acids such as lysine, tryptophan, and threonine (Aghamirzaei *et al.*, 2013). Bread is produced from wheat flour, which is low in protein and like all cereals are limiting in lysine (Temba *et al.*, 2016). Diet based on wheat bread alone does not provide the necessary amino acids necessary for staple food balanced diet (Mal *et al.*, 2010) thus, the need to fortify bread with more protein by using highly proteineous but underutilised legumes and cereals such as flours from bambara groundnut and millet (Singh *et al.*, 2012). Bread needs to have desirable characteristics for it to be pleasing to the consumer. For example, bread made from non-gluten cereal such as millet or bread with a low gluten content such as one made from composite flour of bambara, millet, and wheat will not be suitable for industrial processing thus the need to improve its rheological properties using food enhancers or technological aid materials to improve their industrial handling. Breadmaking is mixing of flour with water, yeast (or another leavening agent), with the addition of ingredients,

such as sugar and fat and then baked in preheated ovens at high temperatures (Shewry *et al.*, 2002). The major ingredients of wheat flour are gluten-forming monomeric gliadins and polymeric glutenins, these are the main constituents of wheat. They are about 80% to 90% of the total flour proteins, albumins and globulins occurred in small amounts (Shewry *et al.*, 2002). Glutenins and gliadins play important roles in the functional properties of gluten (Fermin *et al.*, 2003), with gliadins providing viscosity for dough development and glutenins adding strength and elasticity (Toufeili *et al.*, 1999). The interactions of the proteins and starch with water during dough mixing and chemical reactions such as Millard, caramelization, and gelatinization reactions during dough development and bread baking are caused by gliadins and glutenins (Sivam *et al.*, 2011). Gluten from good quality wheat flour usually shows a high elastic modulus (Miller and Hoseney 1999), while poor elastic and more viscous flour is associated with poor quality wheat (Song and Zheng 2007). Fresh bread is valued for its taste, aroma, quality, and texture. Retaining its freshness is important to keep it appetising. Bread that has hardened or dried out is considered stale (Saranraj 2012). During storage, bread undergoes staling, and this is a major cause of concern for manufacturers. Economically, losses to the baking industry from stale unsaleable bread are estimated in the order of 8% of total production (Saranraj 2012). Bread made from functional ingredients such as millet and bambara can improve nutrition and health-related diseases due to the presence of bioactive compounds in their seed. The use of whole millet flour has been found to decrease the glycemic index in bread (Rai *et al.*, 2008). A reported study showed that consumption of sorghum and millet by individuals resulted in lower occurrences of oesophageal cancer compared to those consuming wheat or maize (van Rensburg 1981; Chen *et al.*, 1993). Millet has the attribute and benefits in lowering cholesterol which is better in millet than wheat (Cho *et al.*, 2000). With these attributes, bambara and millet have a vast potential for food production, and for breadmaking.

2.1.9 Rheology of dough

Rheology is defined as the study of how materials deform, flow or fail when force is applied to them (Amjid *et al.*, 2013). The name was derived from the Greek word: ‘rheos’, meaning the river, flowing, streaming which means “flow science”. Rheology does not only encompass flow movement of liquids, but also distortion behaviour of solids (Dobraszczyk and Morgenstern 2003). Measuring the rheological properties of a material means it is subjected to a controlled, précised

and quantifiable distortion or strain over a given time and the material parameters such as viscosity, stiffness, hardness, modulus, strength or toughness are determined by considering the subsequent forces or stresses (Dobraszczyk *et al.,* 2003). Rheology gives a quantitative measurement of stress in the dough which is closely related to the quality of the molecular gluten network (Bloksma and Bushuk 1988). Measurement of rheology gives the physical properties of dough and the objectives of such measurement include; quantitative description of the mechanical properties of the material, gives information connected to the molecular structure and composition of the material and ability to predict the performance of the material during processing and for quality control (Dobraszczyk *et al.,* 2003). Measurements of rheological properties are used to describe and guess the performance of dough during the practical process of mixing, sheeting, proofing, and baking of dough (Dobraszczyk 2004; Amjid *et al.,* 2013). They are also used to predict the final product quality, to examine process conditions, the expected product performance and consumer acceptance, to predict storage and stability measurement and understanding of process design texture. Based on these, knowledge of the rheological and mechanical properties of dough is important (Herh *et al.,* 2000; Amjid *et al.,* 2013). The baking performance of flours is determined by the dough rheology, and rheological analyses have been used to optimise dough formulation (Amjid *et al.,* 2013). Bread dough is a viscoelastic material that shows an intermediate rheological behaviour between a viscous liquid and elastic solid. Dough machinability and textural characteristics of the finished bread are influenced by the viscoelastic network formed (Collar and Armero 1996). There are different methods of rheological properties measurements, mainly the descriptive empirical and the fundamentals measurements (Amjid *et al.,* 2013). Empirical tests involve an easy to carry out process and are often used in practical factory situations. They provide useful data for assessing the performance of dough during processing and for quality control. Empirical rheological instruments include the texturometer, penetrometer, consistometer, farinograph, amylograph, extensigraph, mixograph, alveograph, various flow viscometers and fermentation recording devices (Amjid *et al.,* 2013). The instrument used in this study; the Mixolab equipment falls under the empirical rheological measurement category. Mixolab measures the effect of the dough constituents on the rheological characteristics of the flour and predicts the quality of the final product (Amjid *et al.,* 2013). The use of composite flour in breadmaking usually changes the rheological characteristics of wheat flour and many studies have shown different levels of rheological changes in terms of dough and the final product (Amjid *et al.,* 2013).

2.1.10 Mixolab equipment

The Mixolab® is an equipment used to record dough mixing operations and measures the rheological properties of dough subject to the dual stress of mixing and temperature changes (Pastukhov and Dogan 2014). It measures the torque (in Nm) produced by the dough between two mixing blades (Aprodu *et al.,* 2010). The test is based on the preparation of a constant dough sample weight, hydrated to obtain a target consistency during the first test phase (Pastukhov *et al.,* 2014). In the "Chopin+" protocol, the dough weight is 75 grammes and the target consistency is 1.1 Nm (+/-0.05 Nm) (Pastukhov *et al.,* 2014). The Mixolab allows the characterization of the physicochemical behaviour of dough when submitted to dual mixing and temperature constraints (Rosell *et al.,* 2007). Mixolab can be used to record the mechanical changes due to mixing and heating by simulating the mechanical work as well as the heat conditions that might be expected during the baking operation (Rosell *et al.,* 2007). Mixolab equipment is used to determine the mixing and pasting behaviours of different flours used for bakery products. It allows mixing the dough under controlled temperature and a temperature sweep between 30-90°C followed by a cooling step. It measures in real time the torque (in Nm) produced by the passage of dough between the two kneading arms, thus allowing the study of its physicochemical behaviour (Matos and Rosell 2013). Mixolab is used to ascertain and study flour performance in correspondence to the expectation of customers in finished bakery product (Gedrovica and Karklina 2011). The mixolab equipment can be used to measure varieties of rheological parameters and mostly can be compared to other earlier equipment used in dough rheological testing. It can be used to determine the mixing speed (rpm) and the Mixolab parameters such as (dough consistency during mixing (C1), mixing stability, protein weakening (C2), starch gelatinization (C3), amylase activity (C4) and starch gelling (C5). All these parameters can be checked at different temperatures and speed (Pastukhov *et al.,* 2014). The dough development is a dynamic process where the viscoelastic properties are continuously changing until an optimum level of dough formation is reached (Pastukhov *et al.,* 2014). Dough characteristics are seriously influenced by the way they are mixed. To achieve proper dough development, two basic requirements must be satisfied which are; imparted mixing energy or work input which must be higher than the critical limit of energy needed for gluten formation, and the mixing intensity must be above the critical level for the dough development (Pastukhov *et al.,* 2014). Mixolab also measures dough development time at a constant temperature, this usually occurs at the start of the test and it determines the water absorption

capacity of the flours and measures the characteristics of dough during mixing (stability, elasticity, absorbed power). Protein reduction speed (α) on the mixolab curve is the level on the mixolab when dough temperature increases and dough consistency decreases. The intensity of this decrease depends on protein quality present in the dough. Starch gelatinization speed (β) zone represents the level of gelatinization of the starch present in the dough. The intensity of this level depends on the quality of the starch and, in some cases, on the additives present in the dough (Pastukhov *et al.,* 2014). The enzyme degradation speed or amylase activity (γ) zone on the mixolab curve depends considerably on the endogenous or added amylase activity. The greater the decrease in consistency, the greater the amylasic activity of the dough (Pastukhov *et al.,* 2014). Mixolab analysis is usually carried out using standard 'Chopin+' protocol, which consists of a heating/cooling cycle after a certain mixing time at constant mixing speed (60–120 rpm) in a stainless-steel mixing compartment. The quantity of flour and water for analysis are calculated by Mixolab software according to input values of flour mixtures moisture content along with water absorption (Pastukhov *et al.,* 2014).

2.1.11 Bread improvers and technological aid materials

2.1.11.1 Hydrocolloids and surfactants

Hydrocolloids are water-soluble polysaccharides with different chemical structure providing a range of functional properties that make them widely used in the food industry as gelling and thickening agents, emulsifiers, stabilizers, foaming agents, syneresis inhibitors in freezing-thawing cycles, improvers of water retention and texture properties, and for the control of the water mobility (Rosell *et al.,* 2007). Hydrocolloids have been shown to possess some anti-staling effect which has been seriously studied and described to controlling and maintaining the moisture content, stabilising the dough, and influencing the crust structure (Fadda *et al.,* 2014). Structural changes induced in the key components of wheat flour was used to describe the macroscopic effect of hydrocolloids on wheat dough. Sometimes, the effect is based on the type of hydrocolloid used in the food system (Rosell *et al.,* 2007). For example, carboxymethylcellulose preferentially bound with gluten while hydroxypropyl methylcellulose bound with starch granules. Starch–gluten connections and the creation of physical entanglements is some of the ways hydrocolloids affect dough structure (Rosell *et al.,* 2007). Examples of hydrocolloids include; pectin, guar gum, xanthan gum, carboxymethyl cellulose, alginate, and carrageenan.

Surfactants are molecules with amphiphilic properties, which possess both hydrophilic and hydrophobic parts (Belitz *et al.,* 2009). When present at the interface between two phases (liquid, solid or gas), they decrease the interfacial tension (Belitz *et al.,* 2009). They impact different definite functions in a medium such as detergents, foaming agents, soaps, emulsifiers, etc. Their effects span on a different wide range of systems and can be advantageous for one application while damaging for another (O Brien and Timms 2004). Surfactants in the dough can act as a dough conditioner by interaction with gluten proteins and so strengthen the gluten network by the formation of ordered structures with water thereby supporting the native flour lipids in their foam-stabilizing function (Kokelaar *et al.,* 1995). During purification, filtration, transportation, freeze-drying, spray drying, storage, and delivery of protein solutions, surfactants are used to avoid physical damage (Kerwin 2008). The main divisions of surfactants include dough strengthener that mainly interacts with gluten and crumb softeners or anti-firming agents that can complex gelatinized starch (Stampfli *et al.,* 1996; Delcour and Hoseney 2010). In breadmaking, surfactants are usually added at a level of 0.3–1.0% (Pareyt *et al.,* 2011).

2.1.11.2 Emulsifiers

Emulsifiers are food enhancers and fit into the general class of compounds called surface-active agents or surfactants (Gómez *et al.,* 2004). Emulsifiers are fatty acid substances having equally lipophilic and hydrophilic properties. The surface tension between two normally immiscible phases is reduced by emulsifiers; therefore, the two liquids are able to form an emulsion (Alobo 2001). The chemical nature of emulsifiers enables them to concentrate on the oil/water interphase thus contributing to the increased stability of a thermodynamically unstable system (Forssell *et al.,* 1998). The effect of the emulsifying agents surpasses their emulsifying capacity, as their amphiphilic character provides the prospect of forming complexes with starch and proteins (Forssell *et al.,* 1998). Emulsifiers are also generally used in bakery products to support blending and emulsification of ingredients, to improve the properties of the shortening and to relate with the components of the flour and other ingredients in the mix for softer crumb (Demirkesen *et al.,* 2010). During the kneading process, emulsifiers increase the strength and extensibility of the dough. Emulsifiers improve gas retention and avert dough collapse during the fermentation process (Demirkesen *et al.,* 2010). They improve texture and reduce water loss, and interact with amylose and amylopectin molecules during baking and help in retarding bread aging (Lakshminarayan *et*

al., 2006). Emulsifiers' chemical components can interact and form complexes with starch, shortening, protein, and water. Also, the interaction of emulsifier with the protein can improve the strength and allow better retention of CO_2. In addition, the complex structure formed between the emulsifier and starch may reduce bread staling (Demirkesen *et al.,* 2010). The commonly used emulsifiers in bakery industries include distilled monoglycerides, diacetyl tartaric ester of monoglycerides (DATEM), lecithin, sodium stearoyl fumarate, sodium stearoyl lactylate, polyglycerol esters and sucrose esters (Orthoefer 1997). Sodium stearoyl lactylate (SSL), diacetyl tartaric acid esters of monoglycerides (DATEM), and polyethene sorbitan monooleate (Polysorbate 80, PS80) are generally regarded as safe (GRAS) food emulsifiers and they have desirable properties that improve bakery product appearance (Henriques 2011). According to the required properties in breadmaking, the emulsifiers are normally divided into dough straighteners and crumb softeners, though SSL has the combination of being a strengthener and softener (Sharma *et al.,* 1990). Some of the frequently used emulsifiers for increasing dough strength include DATEM, SSL, CSL, and polysorbate. They impact their effects during fermentation, mechanical handling, shaping, and transport, as well as during the proofing and initial part of the baking period. They usually give higher volume and improved crumb structure of the finished bakery products (Kim *et al.,* 2009; Henriques 2011). No single emulsifier has all the necessary properties needed to make bread or baking product with all the acceptable quality characteristics (Sharma *et al.,* 1990), therefore mixtures of three emulsifiers will be used in this study.

2.1.11.3 Diacetyl tartaric acid esters of monoglycerides

Diacetyl tartaric acid esters of mono and diglycerides (DATEM) is commonly employed in the baking industry to strengthen dough by building a strong gluten network. The exact mechanism still needs more studies, but DATEM seems to network with the hydrophobic sides of the gluten, helping the proteins unfold and form crosslinked structures (Baiano and Terracone 2011). DATEM includes a mixture of glycerol esters of mono and diacetyl tartaric acid and fatty acids of fats present in food. It can be produced by the interaction of diacetyl tartaric anhydride and mono and diglycerides of fatty acids in the presence of acetic acid or by interaction of acetic anhydride and mono and diglycerides of fatty acids in the existence of tartaric acid (Baiano *et al.*, 2011). The two methods of production give basically similar constituents due to inter and intramolecular acyl group exchange, the arrangement is subjected to the relative proportions of the basic raw materials,

on temperature, and on reaction time (FAO/WHO 2001). The major components are a glycerol molecule with a stearic acid residue, a diacetyl tartaric acid residue and a free secondary hydroxyl group (FAO/WHO 2001). DATEM does not form starch complexes but its key role is as a softener unlike other commercially employed dough emulsifiers (Stampfli *et al.*, 1995). Typically, the level of usage is between 0.375 to 0.5% of the total flour weight in most large-scale baking (Jin *et al.*, 1996). DATEM has an E number of E472 and is recognised as a GRAS additive by the US FDA as specified in the Code of Federal Regulations 21CFR184.1101. It dissolves in methanol and ethanol and dispersible in both cold and hot water (FAO/WHO 2001).

2.1.11.4 Sodium stearoyl lactylate

Sodium stearoyl lactylate (SSL) is a surfactant and it is a mixture of salts of connected acids formed by the esterification of commercial stearic acid with lactic acid and neutralised by the sodium salts (FAO/WHO 2001). A non-neutralized palmitoyl and stearoyl lactylic acid emulsifier may also have free fatty acid (majorly palmitic and stearic), free lactic acid and salts of fatty acid esters of lactic and polymerised lactic acid. It is chemically known as Sodium di-2-stearoyl lactate and the major component is the sodium di-(2-stearoyloxy) propionate (FAO/WHO 2001). It is a white or slightly yellowish powder or brittle solid with a characteristic odour and it is insoluble in water but soluble in ethanol. The permitted level is 2 mg/kg in food products (FAO/WHO 2001). SSL is known to improve softness and retain volume in fresh and frozen dough products (Collar *et al.*, 1996). SSL also has the effect of improving the crumb structure and crumb softening (Sluimer 2005). SSL is commonly used in the baking industry and the allowable amount in bakery products ranges from 0.25 to 0.5 g/100 g flour (Sluimer 2005).

2.1.11.5 Polyethene sorbitan monooleate (Polysorbate 80, PS 80)

Polysorbates are amphipathic, non-ionic surfactants comprise of fatty acid esters of polyoxyethylene sorbitan being polyoxyethylene sorbitan monolaurate for polysorbate 20 and polyoxyethylene sorbitan monooleate for polysorbate 80 (Kerwin 2008). Polysorbate 80 (also known as PS 80 or Tween 80) is a non-ionic surfactant and emulsifier used in bakery and food products. They are viscous yellow liquid and soluble in water. It is gotten from polyethoxylated sorbitan and oleic acid (FAO/WHO 2001). The hydrophilic groups in this compound are polyethers which are recognized as polyoxyethylene groups and they are polymers of ethylene

oxide (Kerwin 2008). Polysorbate either comes in mixtures of both the polysorbate 20 or the polysorbate 80, most times the percentage is usually higher in one mixture than the other and that gives the type of polysorbate present (Kerwin 2008). PS 80 has dual hydrophobic/hydrophilic nature and tends to orient itself so that the exposure of the hydrophobic portion of the surfactants to the aqueous solution is minimised (Kerwin 2008). Polysorbates tend to accumulate at interfaces, forming a surface layer of the surfactant oriented way that only their hydrophilic ends are exposed in air-water systems or protein-water systems (Kerwin 2008). Polysorbate is used in food industries as improvers for bakery products, ice creams, dairy products, and cosmetics. PS 80 is considered GRAS and has the E number E433 (FAO/WHO 2001).

2.1.11.6 Pectin

Pectin is a structural heteropolysaccharide existing in the primary cell wall of plants (Keppler *et al.,* 2006). Pectin is an example of a hydrocolloid. It is a structural carbohydrate present in the middle lamellae of the primary cell walls of plants (Correa *et al.,* 2012). Pectin is a water-soluble heteropolysaccharide mostly consisting of a linear chain of D-galacturonic acid and D-galacturonic acid methyl ester residues, which are linked together by α-1, 4 glucosidic bonds. The number of residues per chain range from 200 to 1,000 (Correa *et al.,* 2012). Pectin contains non-sugar substituents such as methanol, acetic acid, phenolics and sometimes amine groups. Pectic substances have an ever-important structural characteristic of esterification of galacturonic acid residues with methanol or acetic acid (Correa *et al.,* 2012). The degree of methylation is defined as the percentage of carboxyl groups esterified with methanol. If more than 50% of the carboxyl groups are methylated the pectin is called high-methoxy pectin (HM), and less than that degree of methylation is called low methoxyl (LM) pectin (Sharma *et al.,* 2006). Pectin is a dietary fibre and they escape hydrolysis, digestion, and absorption in the small intestine (Prosky 1999). They are often used by bacteria in the large intestine, but they produce insignificant net calorie value. They are also attributed to lowering of the plasma LDL cholesterol and animal arteriosclerosis. The intake of pectin can reduce the incidence of degenerative diseases such as coronary heart disease and diabetes (Correa *et al.,* 2012). It is produced commercially as a white to light brown powder, mainly extracted from fruits (orange, apple, mango, etc.) (Kenijz *et al.,* 2013). Pectin is generally regarded as safe by the US legislation; acceptable daily intake level is between 4-5 mg/kg by the Joint Food Experts Committee for the Codex Alimentarius purposes (FAO/WHO 2007). The

addition of pectin to bread usually exerts desirable rheological parameters to dough and bread during the baking process. Pectin improves dough and bread characteristics by forming bonds with gluten proteins which form hydrophobic bonds with the methoxyl groups of the pectin molecule (Correa *et al.,* 2012). High molecular pectin has been found to give a more stable dough that is able to support greater expansion during fermentation and gives a higher volume of final bread. The specific volume of the loaf is usually improved upon significantly when pectin is added to bread and staling was slowed down in the final produced bread (Correa *et al.,* 2012). Sivam *et al.* (2012) and Sun-Waterhouse *et al.* (2011) showed that the addition of pectin to gluten-free bread significantly influenced the physical characteristics of the dough and the finished bread.

2.1.12 Bread shelf-life and sensory evaluation

Shelf-life is defined as the time which the food product will remain wholesome, be sure to retain desired sensory, chemical, physical and microbiological attributes, also when it is still fulfilling label declaration of nutritional data during storage under the recommended conditions (Kilcast and Subramaniam 2000). Shelf life can also be defined as the length of time for which an item (food) remains usable, fit for consumption, or saleable (Additives 1983). Sensory properties determine the consumer preference for bread. The positioning of a product in the market in terms of quality and consumer observations of that quality is associated with shelf-life (Kilcast *et al.,* 2000). The composition of a food product will determine the type of degradation and length on the shelf. The shelf life is even more important if the product contains composites of different raw materials (Kilcast *et al.,* 2000). The major factors that determine the shelf life of a product are basically divided into intrinsic and extrinsic factors (Kilcast *et al.,* 2000). The intrinsic factors are those that are located within the product and they include; water activity, pH value and total acidity; available oxygen, type of acid, redox potential, natural microflora, nutrients and surviving microbiological counts, natural biochemistry of the product formulation (enzymes, chemical reactants), the preservatives used in product formulation (e.g. salt) (Kilcast *et al.,* 2000). The extrinsic factors occurred after production and they include; time; temperature profile during processing, temperature control during storage and distribution, pressure in the headspace, relative humidity (RH) during processing, exposure to light (UV and IR) during processing, storage and distribution, environmental microbial counts during processing, storage and distribution, composition of atmosphere within packaging, subsequent heat treatment (e.g. reheating or cooking before

consumption), and consumer handling (Kilcast *et al.*, 2000). The interaction between the intrinsic and extrinsic factors usually leads to a decrease in shelf life. Both factors can be generally summarised as microbiological degradation, chemical, physical, and temperature related spoilage (Kilcast *et al.*, 2000). Microbial degradation is sometimes caused by the original microbial load at the beginning of storage, the physicochemical properties of the food, such as moisture content, pH, the presence of preservatives; the processing method used in the production of the food, the external environment of the food (surrounding gas composition) and storage temperature. Chemical deteriorative changes include rancidity which occurs in fat-containing foods (oxidative reactions and flavour deterioration reactions), enzymatic reactions, chemical hydrolysis, non-enzymic and Maillard browning. It also consists of exposure to light which can cause colour loss and off flavour development in foods such as bakery products and milk (Kilcast *et al.*, 2000).

Moisture content, water activity, and texture determination can help to establish several new ingredients which will not alter the product significantly from the suitable target value parameters combinations (Henriques 2011). Water activity (a_w) is a major essential property commonly used in food systems. It explains the extent water is present in the food for the involvement in biochemical reactions and growth of microbes, including chemical deteriorative reactions. The safety, stability, and the texture of bread can be influenced by water activity. Physical deterioration includes moisture migration (loss or uptake), freezer burn and chemical migration from food components or packaging materials (Kilcast *et al.*, 2000). Moisture loss or uptake is important in bread shelf life. The moisture content signifies the amount of water contained in a food product and can be expressed as the percentage of dry weight. It can also be used to quantify the freshness and stability of bread (Henriques 2011). The acceptable level of moisture in bread is between 14-17%. The texture is essential for acceptance by the consumer in bread presentation; staled bread will have no attraction to the consumer. Textural instruments are effective tools in product development phases. Textural tests evaluate either comprehensive or tensile forces. Textural profile analyser is commonly used to compressed bread samples with a round plate till a sure percentage of their initial thickness will be gotten (Kim *et al.*, 2009).

2.1.12.1 The shelf life of bread

Bread has a generally limited shelf life and is largely affected by the loss of moisture, staling and microbial deterioration (yeast, mould and bacterial growth) (Gallagher *et al.*, 2003). Baked goods,

produced in the industries arise from the baking process on a surface that is readily sterile but post-bake handling can easily cause fungal and other microbial surface contamination because of exposure to airborne contaminants also equipment contact (Saranraj 2012). Bread staling is one of the reason for substantial financial losses, both for consumers and for manufacturers of bakery products (Gomes-Ruffi *et al.,* 2012). Staling is the loss of freshness in terms of flavour, texture, moisture and other product characteristics (Si and Drost-Lustenberger 2001). The most widely used indicator of staling is the measurement of the increase of crumb firmness, which is the feature recognised typically by consumers. The major theories on the staling mechanism, and the factors affecting bread staling during storage are: (1) starch retrogradation, especially amylopectin retrogradation, which plays an important role, but which alone is not responsible for bread staling; (2) gluten proteins and gluten–starch interactions also play an important role; and (3) moisture migration is also involved in staling (Hui 2006). Staling is the general loss in quality of flavour and texture of bread. Multiple mechanisms operate in bread staling and is a complex phenomenon in bakery products (Gray and Bemiller 2003). The most common factor responsible for microbial spoilage of bakery products is the water activity, initial microbial content, surface contact and handling during baking operation (Saranraj 2012). Mould spoilage is a major and expensive problem for bakery products and losses suffered due to mould spoilage vary between 1-5% depending on the type of products, seasons and methods of processing (Saranraj 2012).

2.1.13 Microbial spoilage of bread

2.1.13.1 Total Aerobic (plate) colony count

They are spoilage micro-organism (bacteria) which grow under aerobic conditions. They are often an indicator of microbial counts or level for quality assessment of foods. The bacteria *Bacillus* species are associated with ropy bread (Saranraj 2012). They are responsible for bakery condition identified as ‘rope’. It is a key economic problem to the bakery industry. Ropiness is a major problem in a bakery after mouldiness, especially during summer periods when the season help the growth of bacteria (Saranraj 2012). Ropiness is caused by *Bacillus subtilis* but *Bacillus licheniformis*, *Bacillus magaterium,* and *Bacillus cereus* have also been implicated with ropy in bread (Saranraj 2012). These bacteria can cause a mild form of illness when present in the bread and ropy ingesting was linked with foodborne illness in the United Kingdom and Canada (Saranraj

2012). The regulation specified in bakery product for total aerobic plate count in a product such as bread is 10^3 logcfu/g (Ijah *et al.*, 2014).

2.1.13.2 Fungal (yeast and mould) count

The main factor reducing the shelf life of high and intermediate bakery products by far is mould growth. The baking process of fresh bread and other baked products generally killed mould spores (Saranraj 2012). The condensation of moisture on the surface of a product, due to packaging preceding to being totally cooled, may be favourable to mould growth (Ellis 1994). It has been reported from the literature that moulds spoilage causes undesirable odours and is frequently found on the surface of bakery products. Common moulds associated with bakery goods are *Aspergillus sp., Rhizopus sp., Monilia sp., Penicillium sp., Mucor sp. and Eurotium sp* (Saranraj 2012). Total fungal counts should not exceed 10^3 logcfu/g in any bakery product (Ijah *et al.*, 2014).

2.1.14 Bread packaging

Food packaging plays a key part in deciding the shelf stability of foods as they act as an oxygen barrier and loss or gain of moisture in foods (Sharma *et al.*, 1990; Khan *et al.*, 2008). Selection of a proper packaging material is essential to ensure optimum product quality during storage to prevent oxidation of lipids and deterioration of the food products (Min *et al.*, 2005). Packaging materials offer ways to protect, preserve, market, merchandise and distribute foods. Packaging materials have an essential function in ways food materials reach the consumers in a harmless and complete way with uncompromising quality (Raheem 2013). The interactions between food and surfaces of packaging materials add to changes that take place over time in food products (Raheem 2013). Any leakage in packaging material for bread can compromise the integrity of such packaging materials and this can cause oxygen to enter which could immediately cause the growth of mould, and cause the product to dry out and become stale (Saranraj 2012). Bread is usually packed in low-density polyethene bags. A typical white bread is packed in a polyethene bag under ambient temperature and it can last up to 7 days on the shelf before spoilage (Kilcast *et al.*, 2000).

CHAPTER THREE: EFFECT OF PECTIN AND EMULSIFIERS ON THE PHYSICAL, NUTRITIONAL QUALITY AND CONSUMER ACCEPTABILITY OF 9WHEAT-MILLET-BAMBARA) COMPOSITE BREAD

3.1.1 Introduction

The problem of over-reliance of African countries on wheat importation for bakery products production has resulted in the loss of foreign earnings (Temba *et al.,* 2016). Protein-energy malnutrition (PEM) represent one of the major challenges faced by the African continent in the production of staple foods need to solve malnutrition occurrences (Temba *et al.,* 2016). These problems are prevalent and according to FAO, they can be reduced by the incorporation of indigenous protein food sources into staple foods such as bread (Noorfarahzilah *et al.,* 2014). Baking with wheat (*Triticum aestivum*) flour alone does not provide the essential amino acids needed for bread as it is limiting in lysine (Mubaiwa *et al.,* 2017). Traditional crops such as bambara groundnut *(Vigna subterranea*) and pearl millet (*Pennisetum glaucum*) have been advocated as potential crops in addressing food security challenges in African countries (Temba *et al.,* 2016). These crops are good sources of protein and essential amino acids. Millet contains significant amounts of methionine (2.5%) (Saleh *et al.,* 2013) which is lacking in wheat grains. Bambara grain is rich in lysine (6-8%) (Hillocks *et al.,* 2012; Arise *et al.,* 2016), and its protein is rich in essential amino acids. Exploring the potentials of bambara and millet grains in bread production could reduce the incident of PEM among the African populace. The substitution of wheat with millet and bambara for bread production can also reduce the over-reliance of African countries on wheat and thus reduce the cost of wheat importation. Previous works on supplementation and fortification of wheat with legumes and other food crops have been successful. However, the addition of non-gluten flours to wheat above 20% in composite flour formulations is known to produce bread with poor quality (Noorfarahzilah *et al.,* 2014; Erukainure *et al.,* 2016). As the addition of other flours (legumes, cereals and tuber flours) increases, the viscoelastic properties of dough formed decreases, and this causes reduced handling properties and poor bread quality. Research works on composite bread are either focusing on the fortification of bread which addresses nutritional significance or supplementation that focused on economic reasons. The combination of both nutritional and economic reasons for the purpose of composite formation will address both issues. Thus, combining the nutritional suitability in bambara

groundnut and supplementation ability of millet into composite formation for staple food such as bread. Active surfactants, including emulsifiers and hydrocolloids, such as apple pectin and other technological aid materials have been successfully employed to improve the rheological properties of dough made from composite or non-gluten flours. Emulsifiers such as sodium stearoyl lactylate (SSL), Polysorbate 80 (PS 80) and diacetyl tartaric acid ester of monoglycerides (DATEM) have the effects of dough strengthening, crumb softening, volume increase and crumb structure improvement on wheat bread (Gómez *et al.*, 2013). Eduardo *et al.* (2014) found that SSL and DATEM increased the specific volume and water absorption of dough of bread made from cassava, maize, and wheat composite bread. Composite bread produced from wheat and pearl millet flour (50% each) produced bread with increased volume and good crumb structure when emulsifiers were added (Schoenlechner *et al.*, 2013). Pectin has also been found to help in retaining gas in the dough, increasing dough volume, retaining structure and slowing down the staling process in bread (Kenijz *et al.*, 2013). Composite flour of wheat, cassava and maize produced bread with increased volume up to 13% when pectin was added (Eduardo *et al.*, 2013). Although the properties of emulsifiers and hydrocolloids on dough and bread quality have been studied, there is a dearth of information on the combined effect of both emulsifiers (SSL, DATEM, and PS80) and pectin on the quality of composite bread containing pearl millet and bambara groundnut flours. Hence, the objective of this study was to investigate the rheological properties of dough made from wheat, millet and bambara composite flour using mixolab after addition of different levels of pectin and emulsifiers and to investigate the quality of the resulting bread.

3.1.2 Materials and methods

3.1.2.1 Materials and sample preparation

Bambara groundnut (*Vigna subterranea*) seeds, wheat flour (*Triticum aestivum*) and other baking ingredients were purchased from a local market in Durban, South Africa. Pearl millet (*Pennisetum glaucum*) (babala type) was purchased from Agricol Pty Ltd, Durban, South Africa. Emulsifiers were purchased from Hangzhou Union Biotechnology Co., Ltd. Xihu District Hangzhou, China. Apple pectin was procured from Sigma-Aldrich St Louis, MO. USA.

3.1.2.2 Flour preparation and blending

Bambara groundnut flour was made according to the process used previously (Mubaiwa *et al.*, 2017). Bambara groundnut (cream colour South African landraces) seeds were cleaned, sorted, and roasted in the oven at 50℃ for 5 hours. The toasted nuts were coarse-milled and winnowed to remove the hull. The dehulled seeds were then milled and sieved (250 µm) to obtain a fine flour. Pearl millet flour was produced according to the process described (Saleh *et al.*, 2013) for millet flour production. Pearl millet grains were sprayed with tap water and shed-dried at room temperature (25℃) for 30 minutes for conditioning. The grains were coarse-milled and dried in an air oven at 60℃ to a moisture content of between 8 - 9%. The coarse grits were winnowed to remove hulls, milled and sieved into a fine flour. Flours were sieved with a 250 µm mesh and then mixed in the ratio 50:25:25 for wheat, millet, and bambara groundnut respectively. A Kenwood blender (BL380 model, Maraisburg, South Africa) was used to mix the flours to get a uniform mixture. Flour thus obtained was packed in a labelled plastic container and stored at 4℃.

3.1.2.3 Preparation of bread samples

The ingredients used for bread preparation were flour (200 g), margarine (10 g), sugar (8 g), salt (2 g), yeast (5 g), and water (100 ml for wheat bread and 110 ml for the composite bread) (Table 7). Water addition was added based on the mixolab water absorption capacity calculation for flour. Composite bread was coded as WMB_{3-7} for different levels of treatment with emulsifiers (SSL, DATEM, and PS80) and pectin. Emulsifiers and pectin were added based on the permitted limit addition to bakery products (FAO/WHO 2001). Pectin (1.0-2.0 g/100 g flour) and emulsifiers namely sodium stearoyl lactylate (SSL) 0.25-0.40 g/100 g flour, polysorbate 80 (PS80) 0.50-0.80 g/100 g flour, and diacetyl tartaric acid ester of monoglycerides (DATEM) 0.10-0.25 g were mixed and added in different proportions (1.0%, 1.3% and 1.6% respectively). Nine bread samples were produced; three samples were developed for treatment with emulsifiers (WMB_{3-5}), three samples for pectin treatment (WMB_{4-6}) and one sample for treatment with both emulsifier and pectin (WMB_7). One sample each was made with 100% wheat (WF) and millet flours (MF) and both serve as a control. Bread samples were made according to the method described by Ceserani (1995) with little adjustments (straight dough method). The dry and wet ingredients were mixed separately in a stainless-steel bowl. A mixer (Kitchen Aid heavy duty – 5K5SS model, Maraisburg, South Africa) was used to mix the dough for 5 minutes. The dough was rested for 10 minutes before

kneading. The dough was proofed at 35℃ for 90 minutes at 85% humidity. The dough was baked in a preheated oven at 200℃ for 25 minutes. Bread samples produced from 100% wheat and pearl millet flour served as controls (WF and MF). Three batches each were produced for all bread samples. The analyses were carried out one hour after bread preparation.

3.1.2.4 Mixolab analysis of dough

The rheological characteristics of the dough were measured using standard Chopin+ protocol according to the manual by the manufacturer. This comprised of a heating/cooling cycle with a constant mixing time speed of 80 rpm at a constant water absorption level calculated by the Mixolab® software based on flour weight and moisture level (Erukainure *et al.,* 2016). The water for dough mixing was calculated by the Mixolab® software. The Mixolab equipment was used to analyse various rheological parameters of the composite flour dough including dough development time (DDT), water absorption capacity (WAC), dough elasticity (DE), torque, and dough stability (Figure 3).

Table 7. Formulations used for bread preparation

Samples	WF	MF	WMB_1	WMB_2	WMB_3	WMB_4	WMB_5	WMB_6	WMB_7
Ingredients	Quantities(g)								
Flour	200	200	200	200	200	200	200	200	200
Margarine	10	10	10	10	10	10	10	10	10
Sugar	8	8	8	8	8	8	8	8	8
Salt	2	2	2	2	2	2	2	2	2
Yeast	5	5	5	5	5	5	5	5	5
SSL	0	0.5	0.3	0.4	0.5	0	0	0	0.5
PS80	0	0.8	0.6	0.7	0.8	0	0	0	0.8
DATEM	0	0.25	0.15	0.2	0.25	0	0	0	0.25
Pectin	0	2.0	0	0	0	1.0	1.5	2.0	2.0

*SSL- sodium stearoyl lactylate, DATEM- diacetyl tartaric acid ester of monoglycerides, PS80- polysorbate 80. $WMB_{1\text{-}7}$; Wheat, millet and bambara bread with different concentrations of emulsifiers and pectin; MF; 100% millet flour; WF- 100% wheat flour.

3.1.2.5 Proximate composition of flours and bread samples

Composite flours and bread samples were analysed for moisture content, crude protein (Kjeldahl method), crude fat (solvent extraction), and ash (AOAC 2000). Determination of carbohydrate was

by simple difference and the value of calorie was calculated using Atwater factors (4, 9, 4 kilocalories for protein, fat, and carbohydrate, respectively).

3.1.2.6 Physical characteristics of bread loaves

The bread volume was determined using the rapeseed displacement method with some changes (millet seed replaced rapeseed) and specific volume was calculated using the loaf volume and weight data (Hallén *et al.,* 2004).

3.1.2.7 Amino acid composition and protein digestibility of bread

The total amino acid content of the bread was determined using Ultra Performance Liquid Chromatograph (UPLC) separation with UV or fluorescence detection after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC). The amino acids were measured in solution either as free amino acids or after hydrolysis of proteins using standard 6M HCl acid digestion. Amino acid separation and detection were performed using UPLC fitted with a photodiode array (PDA) detector. Exactly 1 µl of digested sample was injected into the mobile phase which conveys the derivatized amino acids onto a Waters UltraTag C18 column (2.1 x 50 mm x 1.7 µm) held at 60℃. MassLynx software was used to acquire and control data. Total amino acids were measured as g/100g of the sample (Grobbelaar *et al.*, 2014).

3.1.2.8 Protein digestibility

In vitro protein digestibility (IVPD) was analysed by the method described with some modifications (Ayo *et al.*, 2007). Pepsin enzyme from porcine gastric mucosa supplied by Sigma-Aldrich St Louis, MO.USA was used for digestion. Exactly 0.2 g of the sample was measured into a flask, 1.5 mg of pepsin were added with 35 ml of 0.1 M of phosphate buffer at pH 2.0. The mixture was incubated at 37℃ for 2 hours in a continuous shaking water bath. The digestion was terminated by adding 2 ml of 2 M NaOH. The solution was then centrifuged at 4000 rpm at 40℃ for 24 minutes. The supernatant was discarded and the residue was washed twice with 15 ml of 0.1 M phosphate buffer at pH 7.0 and then centrifuged as previously done. The residue was washed and filtered, then dried in an oven for 2 hours at 80℃. The undigested nitrogen (N) was analysed using the micro-Kjeldahl method, and the digestible nitrogen was calculated as;

% digestibility = (N in sample - Undigested N)/ N in sample x 100.

3.1.2.9 Sensory evaluation

Sensory analyses were carried out 1 hour after bread baking, samples were prepared and were presented in 3 digits coded white cups. The bread samples were evaluated by a 40-panel drawn from staff and students of Durban University of Technology, Durban, South Africa. The nine-point hedonic scale was used by panellists to evaluate the level of likeness (1 = extremely dislike, 9 = extremely liked) (Kilcast *et al.,* 2000).

3.1.2.10 Statistical analysis

All analyses were done in triplicates. Analysis of variance (ANOVA) was performed, means were compared using the Fisher Least Significant Difference (LSD) test ($p < 0.05$).

3.1.3 Results and discussion

3.1.3.1 Mixolab rheological behaviour of composite dough

The addition of bambara and millet flours at 25% levels each significantly increased ($p < 0.05$) the water absorption capacity (WAC) of the resulting composite flour dough (Table 8). Pectin treated dough (PTD) at 1.5% (WMB_5) gave the highest WAC compared to the control (WF). Previous research reported that cryoprotectant such as pectin can improve the water holding capacity of dough (Kenijz *et al.,* 2013). Emulsifiers treated dough (ETD) showed a significant decrease ($p < 0.05$) in WAC compared to the PTD and WF (Table 8). Gómez *et al.* (2013) similarly found that the addition of SSL and PS 80 resulted in a significant decrease in WAC of dough as the level of emulsifiers increased. The time needed for dough development (DDT) and the dough stability was prolonged by addition of emulsifiers (WMB_{1-3}), the effect was significantly increased ($p < 0.05$) when compared with both WF and MF. The same effect was noticed for pectin addition (WMB_{4-6}) but the ETD dough was more significant. Overall, the addition of emulsifier at 1.3% (WMB_1) significantly increased ($p < 0.05$) the DDT of the composite bread compared to both PTD and WF. This observation supported the report that the composite dough treated with emulsifiers may withstand long mixing time without weakening. This may be due to protein-protein networks formed by the emulsifiers with wheat proteins (Gómez *et al.,* 2013). The increased stability time which is a measure of dough strength was significantly increased ($p < 0.05$) for both ETD and PTD but was more prolonged in PTD (WMB_6) and this may be due to the capability of hydrocolloids to form an interface between dough and water present, thus causing increased dough strength and

Table 8. Mixolab parameters for composite dough rheology

	Samples								
Parameters	WF	MF	WMB_1	WMB_2	WMB_3	WMB_4	WMB_5	WMB_6	WMB_7
Torque (NM)	3.9^{f} ±0.09	3.03^{e} ±0.05	1.91^{bcd} ±0.37	2.24^{cd} ±0.09	2.42^{d} ±0.03	1.8^{bc} ±0	2.04^{bcd} ±0.45	1.63^{b} ±0.37	2.38^{d} ±0.05
WAC (%)	43.80^{c} ±0.6	0^{a}	21.46^{b} ±0.01	21.17^{b} ±0.52	21.17^{b} ±0.01	40.15^{c} ±0	44.52^{c} ±4.91	40.17^{c} ±0.03	40.14^{c} ±0.07
DDT(Min)	1.69^{b} ±0.08	0.75^{a} ±0.11	4.89^{e} ±0.26	4.73^{e} ±0.09	4.28^{de} ±0.1	3.38^{c} ±0.01	4.01^{d} ±0.59	3.77^{c} ±0.1	3.35^{c} ±0.74
Stability (Min)	4.75^{b} ±0.38	2.61^{a} ±0.24	5.52^{bc} ±0.48	5.81^{bc} ±0.52	5.14^{bc} ±0.94	5.54^{bc} ±0.27	5.34^{bc} ±0.69	6.22^{c} ±0.31	7.33^{d} ±0.52
DE (NM)	0.15^{c} ±0.01	0.07^{a} ±0.01	0.07^{a} ±0.01	0.07^{a} ±0.01	0.08^{ab} ±0.01	0.1^{ab} ±0.02	0.11^{b} ±0.05	0.097^{ab} ±0.01	0.1^{ab} ±0.04

*DDT– dough development time. *WAC- water absorption capacity, DE- Dough elasticity. WMB_{1-7}: Wheat, millet and bambara bread with different levels of emulsifiers and pectin treatment; MF: 100% millet flour; WF:100% wheat flour. NM- Newton meter, %- Percentage, Min.- Minutes. Values on the same row with different letters are significantly different at $p<0.05$ using Fisher Least Significant Difference (LSD) test.

volume (Correa *et al.,* 2012). EPTD (WMB_7) had the highest stability time among all the dough samples, this may mean that a combination of both emulsifier and pectin improve the composite dough stability. MF dough resulted in no significant improvement in rheological properties despite the addition of both emulsifiers and pectin at the highest level. This could be as a result of lack of gluten and irregular gelatinization of the starch molecules present in the millet grain (Obilana *et al.,* 2014).

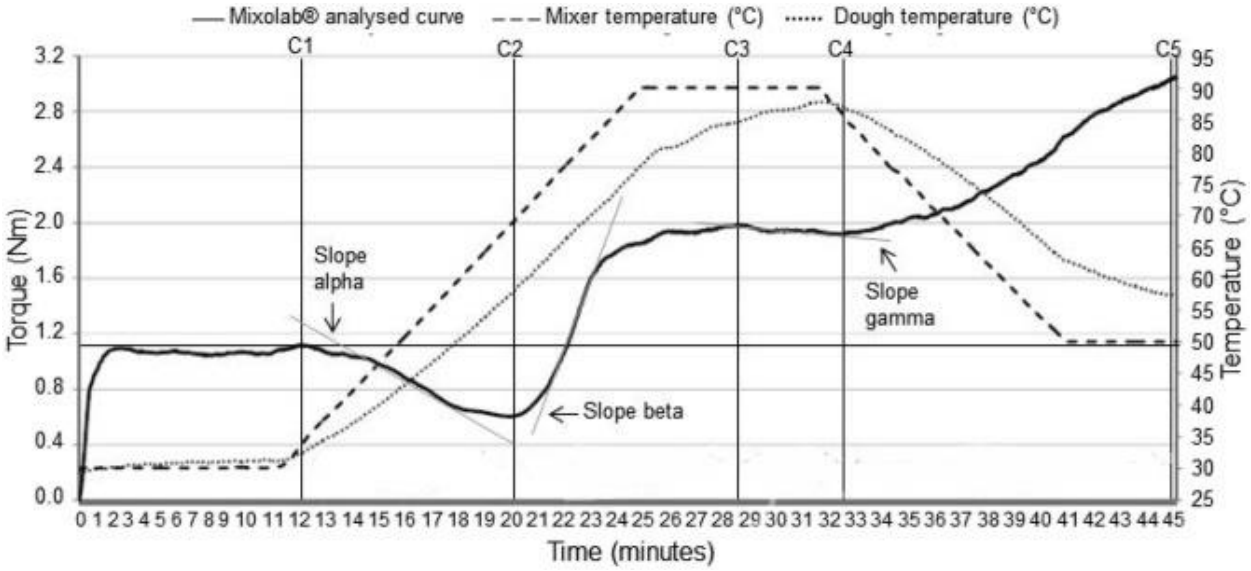

Figure 3. Typical Mixolab curves comparing the rheological behaviours of dough. Description of a Mixolab curve/points obtained. Numbers indicate the different zones detected in the curve according to physical dough changes. C1- dough development time and stability, C2- protein weakening pattern, dough elasticity, C3- maximum viscosity, C4- Amylolysis pattern, C5- Retrogradation. Mixolab dough constitutes behaviour. α: represent protein weakening speed under heat; β: represent starch gelatinization speed and γ: represent enzyme degradation speed. (1) Dough development time. (2) Protein reduction during heating. (3) Starch gelatinization. (4) Amylase activity. (5) Starch gelling due to cooling.

The phases in Mixolab has (C1–C5) each determining diverse rheological characteristics of flour (Table 9). C1 was the highest point on the curve during the dough stability stage (Figure 3) and it records dough development time and stability time. It also measures the protein behaviour of the dough (Table 10). The dough stability increased from 4.75 min in the control (WF) to 5.81 min and 7.33 min for both ETD and PTD. These findings support previous studies where a combination of SSL, DATEM, and PS80 produced high dough stability when proofing time was prolonged (Gómez *et al.,* 2013). The longer stability time observed could also be attributed to the strength of bambara flour protein molecules which are soluble and absorb water easily (Erukainure *et al.,* 2016). The protein weakening pattern (C2) increased significantly ($p < 0.05$) for ETB (WMB_1) and PTB (WMB_6) compared to the control. The increased protein weakening pattern value showed

a good protein pattern present in the composite dough (Table 10). According to, Collar *et al.* (2007) good protein displays stability during heating but becomes weak, and eventually breaks down as heating time increases. The weakening of proteins was further demonstrated by the α slopes of the Mixolab graph, which indicate protein weakening speed for doughs as influenced by heat (Pastukhov *et al.,* 2014) (Table 10).

The viscosity of the dough (C3) showed no significant difference as temperature increased for all composite dough samples and the control (WF). Except for PTD at 2% level of treatment which showed a significant increase in viscosity better than the control. The dough weakened as the mixing temperature increased, suggesting that gelatinization of starch decreased as mixing continued. This may explain the decrease of β slope values for the composite dough compared to the control (Table 10). The Mixolab graph highest point; C3 specifies maximum viscosity and has been attributed to quick rupture of starch granules, leading to lower pasting temperatures and to higher paste consistency (Pastukhov *et al.,* 2014). The increased dough temperature together with water liberated by the denatured proteins (C2) caused the starch granules to swell and burst, thereby inducing an increased dough consistency for all composite dough samples (Aprodu *et al.,* 2010).

At the Mixolab curve peak (C4), there was a decrease in the dough consistency as indicated by the γ slope in Table 10. There was a significant increase ($p < 0.05$) in the level of activities of the amylose and amylopectin of the dough starch of the composite bread samples and these increased more than that of the control WF. At 1.6% and 2% treatment level of both ETD (WMB_3) and PTD (WMB_6), there was a high level of gelatinization better than WF and this signifies that there was a stable increase in the amylase index for both ETD and PTD as the concentration increased (Table 10 and appendix one). This implies that increased concentration of both pectin and emulsifiers reduced the capacity of the starch to withstand amylolysis, and thus a slow rate of staling may be experienced in composite bread (Erukainure *et al.,* 2016). A higher C5 value indicates a longer shelf life for bread (Dhaka *et al.,* 2012) (Figure 3). Conversely, the higher C5 value indicates the greater susceptibility to retrogradation and thus shorter shelf-life of produced bread. The addition of both emulsifiers and pectin did not significantly increase ($p < 0.05$) the retrogradation time of the composite dough (Tables 8 and 9).

Table 9. Rheological values for Mixolab curve (C1 - C5) of wheat-millet-bambara composite dough

	Samples								
Parameters	WF	MF	WMB_1	WMB_2	WMB_3	WMB_4	WMB_5	WMB_6	WMB_7
C1	6.26^{c}± 0.5	2.56^{a} ±0.24	5.71^{b} ±0.24	5.47^{b} ±0.09	5.55^{b} ±0.18	5.58^{b} ±0.24	5.67^{b} ±0.07	6.65^{c} ±0.07	6.91^{d} ±0.32
C2	18.41^{a} ±0.21	20.57^{d} ±0.47	20.01^{cd} ±0.88	18.7^{ab} ±0.14	18.77^{ab} ±0.08	18.59^{a} ±0.05	18.36^{a} ±0.93	19.18^{abc} ±0.88	19.87^{bcd} ±1.04
C3	23.8^{b} ±0.29	0^{a}	23^{b} ±0	23.64^{b} ±0.57	23^{b} ±0	23.98^{b} ±0.09	23.87^{b} ±0.34	25.54^{c} ±2.23	23^{b} ±0
C4	26.96^{b} ±0.12	0^{a}	30^{bc} ±0	27.03^{b} ±2.58	30^{bc} ±0	28.36^{b} ±0.4	26.23^{b} ±2.11	33.35^{c} ±5.17	30^{bc} ±0
C5	45.01^{a} ±0.01	45.02^{a} ±0	45.02^{a} ±0	45.02^{a} ±0	45.02^{a} ±0	45.01^{a} ±0.01	45.01^{a} ±0.01	45.02^{a} ±0	45.01^{a} ±0.01

WMB_{1-7}: Wheat, millet and bambara bread with different levels of emulsifiers and pectin treatment; MF: 100% millet flour; WF:100% wheat flour. C1- dough development time and stability, C2- protein weakening pattern, dough elasticity, C3- maximum viscosity, C4- Amylolysis pattern, C5- Retrogradation. Values on the same row with different letters are significantly different at $p<0.05$.

Table 10. Mixolab dough constituents pattern

	Samples								
*Parameters	WF	MF	WMB_1	WMB_2	WMB_3	WMB_4	WMB_5	WMB_6	WMB_7
α	-0.09^{c} ±0.06	-0.07^{ab} ±0.02	-0.07^{ab} ±0.03	-0.07^{ab} ±0.02	-0.03^{b} ±0.02	-0.10^{a} ±0.02	-0.07^{ab} ±0.03	-0.11^{a} ±0.02	-0.75^{ab} ±0.03
β	0.18^{e} ±0.11	0.0^{abcd}	0.10^{cde} ±0.13	0.00^{abcd} ±0.06	0.08^{bcde} ±0.01	-0.05^{a} ±0.02	-0.04^{ab} ±0.02	-0.03^{abc} ±0.02	0.11^{c} ±0.06
ϒ	-0.05^{a} ±0.01	0.0^{ab}	0.10^{bc} ±0.13	0.0^{ab} ±0.06	0.08^{bc} ±0.01	-0.05^{a} ±0.02	-0.04^{a} ±0.02	-0.03^{a} ±0.02	0.11^{c} ±0.06

* α: represent protein weakening speed under heat; β: represent starch gelatinization speed and γ: represent enzyme degradation speed. WMB_{1-7}: Wheat, millet and bambara bread with different levels of emulsifiers and pectin treatment; MF: 100% millet flour; WF:100% wheat flour. Values on the same row with different letters are significantly different at $p<0.05$.

The retrogradation time of the composite flour doughs has no significant difference ($p < 0.05$) in comparison to WF. However, the emulsifier concentration at 1.6% (WMB_3) gave the highest retrogradation level closer to the control (Appendix one). This result implies that the shelf-life of composite bread and WF may be close. The Mixolab equipment was unable to give a regular dough profile for millet flour dough due to the irregular gelling nature of their flour and absence of gluten (Table 9).

3.1.3.2 Proximate composition of composite flour and bread

The protein content of the composite bread and flour significantly increased ($p < 0.05$) from 10% to 14% (Tables 11 and 12). The chemical composition showed a significant increase in protein. This may be due to the addition of both bambara and millet grain to the composite flour. Also, there was a decrease in carbohydrate content and this may be due to the additive effect of bambara flour protein (Table 11).

Table 11. Proximate composition of composite flours (g/100 g)

Parameters (%)	Moisture	Ash	Protein	Fat	CHO	Energy (KJ)
WF	12.9^{d}±0.04	2.73^{c}±0.15	10.7^{a}±0.1	1.75^{a}±0.08	71.94^{b}±0.18	346.31^{a}±0.83
MF	8.21^{a}±0.05	2.37^{b}±0.15	11.78^{b}±0.15	4.73^{b}±0.21	72.9^{b}±0.12	381.35^{c}±1.6
BGF	9.7^{b}±0.85	2.77^{d}±0.12	22.47^{d}±0.59	5.04^{b}±0.22	59.73^{a}±1.77	374.13^{c}±9.01
WMBF	10.72^{c}±0.02	1.58^{a}±0.26	16.62^{c}±0.14	1.45^{a}±0.12	69.47^{b}±0.2	357.38^{b}±1.24

*WMBF: Wheat-millet-bambara flour, BGF: Bambara groundnut flour, MF: Millet flour; WF: Wheat flour, CHO: Carbohydrate calculated by difference. Values on the same column with different letters are significantly different at $p<0.05$ using the Fisher Least Significant Difference (LSD) test.

Composite bread moisture content was also significantly increased ($p < 0.05$) compared to the control (WF) (14% to 20%). As the addition of emulsifiers increased there was a decrease in the moisture content, the same effect was noticed in the composite bread treated with pectin (WMB_{4-6}). The addition of pectin into bread system has been linked to high water binding of the available water molecules present in the flour. Increased water binding may be linked with the connections between water and hydroxyl, carbonyl or amine groups of the added pectin and the flour proteins (Sivam *et al.,* 2011). The increase in moisture level may be due to the binding of legumes proteins

from bambara flour which has 70 – 90% of their proteins soluble in water (Hallén *et al.*, 2004). Baking at low temperature has been attributed to higher moisture retention in bread and loaf size improvement (Shittu, 2007). The moisture content of the composite bread may also be increased due to relatively low temperature for baking. The fat content of composite bread was also significantly increased ($p < 0.05$), this may be due to a relatively high-fat content of both millet and bambara groundnut flours (Table 12).

Table 12. Proximate composition of wheat-millet-bambara composite bread (g/100 g)

Samples	WF	MF	WMB_1	WMB_2	WMB_3	WMB_4	WMB_5	WMB_6	WMB_7
Moisture	14.78^{a}±0.39	19.45^{e}±0.68	$20.^{22f}$±0.09	19.60^{ef}±0.25	18.03^{cd}±0.17	19.68^{ef}±0.59	18.64^{d}±0.23	17.83c±0.07	16.48^{b}±0.23
Ash	2.68^{c}±0.23	3.22^{e}±0.11	1.70^{ab}±0.39	1.88^{b}±0.22	1.83^{ab}±0.24	1.49^{ab}±0.17	1.47^{a}±0.18	1.87^{b}±0.15	1.51^{ab}±0.17
Protein	10.01^{a}±0.25	10.91^{b}±0.04	14.72^{cd}±0.37	14.86^{cd}±0.16	14.88^{cd}±0.10	14.77^{cd}±0.13	14.59^{c}±0.06	14.96^{d}±0.08	14.89^{cd}±0.06
Fat	1.64^{a}±0.03	4.39^{b}±0.05	5.41^{c}±0.12	6.42^{d}±0.07	6.43^{d}±0.06	6.65^{de}±0.04	6.96^{ef}±0.47	6.49^{d}±0.44	7.08^{f}±0.17
*CHO	70.88^{f}±0.62	62.02^{e}±0.81	57.94^{abc}±0.74	57.24^{a}±0.12	58.82^{c}±0.24	57.41^{ab}±0.80	58.34^{bc}±0.53	58.84c±0.49	60.22^{d}±0.30
Energy (KJ)	328.37^{abc}±15.40	320.33^{a}±16.10	324.42^{ab}26.47	346.15^{abc} ^{d}±1.49	337.7^{abcd}±25.99	348.57^{bc} ^{d}±2.48	354.41^{cd}±1.63	353.62^{cd}±2.35	364.22^{d}±1.23

*WMB_{1-7}: Different concentrations of emulsifiers and pectin; MF: 100% millet flour; WF: 100% wheat flour. KJ; Kilojoule, CHO; Carbohydrate. *Carbohydrate calculated by difference. Values on the same row with different letters are significantly different at $p<0.05$ using the Fisher Least Significant Difference (LSD) test.

3.1.3.3 Physical characteristics of composite bread

The loaf volume of the composite bread significantly increased ($p < 0.05$) as the concentration of emulsifiers and pectin increased (Table13). Overall, emulsifier treated bread (ETB) had a volume increase of 45.6% and pectin treated bread (PTB) 17.42% compared to WF despite the reduction of wheat flour by 50%. This indicates the ability of emulsifiers and pectin to cause volume increase in bread (Correa *et al.*, 2012; Gómez *et al.*, 2013). However, WF gave the highest volume in all the bread samples produced.

Table 13. Physical characteristics of composite bread

	Samples								
Parameters	WF	MF	WMB_1	WMB_2	WMB_3	WMB_4	WMB_5	WMB_6	WMB_7
Volume (cm^3)	851.67^{g} ± 2.89	427.33^{a} ±2.52	550^{d} ±5	616.67^{f} ±15.28	620^{f} ±18.03	476.67^{b} ±7.64	485^{bc} ±5	500^{c} ±10	573.33^{e} ±7.64
Weight (g)	314.1^{g} ±0.44	302.19^{f} ±0.1	298.9^{e} ±0.1	297.7^{d} ±0.36	243.53^{a} ±0.45	292.8^{b} ±0.27	385.03^{h} ±0.15	387.63^{i} ±0.55	295.83^{c} ±1
Specific volume (g/cm^{-3})	2.71^{h} ±0.01	1.42^{b} ±0.01	1.83^{d} ±0.01	2.07^{f} ±0.05	2.55^{g} ±0.07	1.63^{c} ±0.03	1.26^{a} ±0.01	1.29^{a} ±0.02	1.94^{e} ±0.04

*WMB_{1-7}: Wheat-millet-bambara bread at different levels of emulsifiers and pectin; MF: 100% millet flour; WF: 100% wheat flour. Values on the same row with different letters are significantly different at $p<0.05$ using the Fisher Least Significant Difference (LSD) test.

3.1.3.4 Amino acids composition and protein digestibility of bread

A significant increase ($p < 0.05$) was noticed in the lysine content from 0.15 g in WF bread to 0.33g in the composite bread (Table 14). As the levels of emulsifier increased, there was a significant decrease in the level of lysine. The reduction in lysine may be because emulsifiers have been shown to form complexes and networks with gluten proteins to exert their dough strengthening effect (Forssell *et al.,* 1998). Increased levels of complex gelatinization of protein and starch in the presence of bambara and millet proteins had earlier been reported (Stampfli *et al.,* 1995). The reversed trend was noted for PTB, with a significant increase ($p < 0.05$) in lysine content as the pectin level increases. This indicates that the addition of pectin may have improved the bioavailability of essential amino acids of the composite bread. Threonine and lysine showed a significant increase ($p < 0.05$) in emulsifiers and pectin treated bread (EPTB) (WMB_7), this showed that the addition of both emulsifiers and pectin may not affect the availability of amino acids in the composite bread (Table 14). There was a significant increase ($p < 0.05$) in the IVPD for the composite bread (Figure 4). ETB and PTB have an average IVPD of 81% and 76% respectively higher than the control (WF) (67%). This may be because of the inclusion of bambara and millet which have high IVPD (Ayo *et al.,* 2007). The ETB (WMB_{1-3}) gave the best IVPD at a lower level of addition (1.3%), as the concentration of emulsifier increased, *in vitro* digestibility reduced, and this is in line with the trend noticed in lysine and threonine (Table 14). The same trend was also observed for the PTB (WMB_{4-6}). The IVPD of the EPTB (WMB_7) was higher than

that of PTB (WMB_{4-6}) (Figure 4). Generally, in this study, the addition of emulsifiers and pectin significantly increased ($p < 0.05$) the protein digestibility of composite bread.

3.1.3.5 Results of sensory analysis of composite bread

The results showed a significantly high ($p < 0.05$) overall acceptability for PTB at 2% concentration (WMB_6) compared to the control (WF) (Table 15). The addition of emulsifiers and pectin significantly improved the overall acceptability score for composite bread. Millet bread had the lowest score for all the attributes tested. In terms of the colour, all bread samples apart from millet bread scored above the acceptable level (5). The result showed that all composite bread samples (WMB_{1-7}) were significantly different ($p < 0.05$) in taste (4) relative to WF. This may be due to consumer familiarity with bread and this may have introduced bias in panellist ratings (Erukainure *et al.,* 2016). For all the attributes evaluated, all the composite bread samples were judged acceptable as most panellists scored them above five, which is considered the minimum acceptable score on a 9-point Hedonic scale.

Table 14. Amino acid profile of composite bread (g/100 g)

	Bread samples								
Amino Acids	WF	MF	*WMB_1	WMB_2	WMB_3	WMB_4	WMB_5	WMB_6	WMB_7
Histidine	0.31^{a}±0.11	0.31^{a}±0.06	0.24^{a}±0.17	0.5^{b}±0.73	0.47^{b}±0.01	0.46^{b}±0.07	0.26^{a}±0.05	0.44^{b}±0.04	0.46^{b}±0
Serine	0.51^{b}±0.36	0.36^{a}±0.11	0.53^{b}c±0	0.7^{d}±0.16	0.67^{bcd}±0.54	0.54^{bc}±0	0.57^{bcd}±0	0.6^{bcd}±0.03	0.56^{bcd}±0.03
Arginine	0.37^{a}±0.28	0.39^{a}±0.17	0.73^{b}±0.06	0.9^{bc}±0.1	0.74^{b}±0.03	0.83^{bc}±0.01	0.87^{de}±0.03	0.74^{b}±0.18	0.98^{c}±0.32
Glysine	0.62^{b}±0.01	0.47^{d}±0.1	0.72^{bcd}±0.34	0.85^{e}±0.72	0.67^{bc}±0.21	0.73^{c}d±0	0.78^{de}±0.03	0.74^{b}±0.86	0.79^{de}±0.32
Asparagine	0.76^{a}±0.34	0.78^{ab}±0.15	1.26^{c}±0.1	0.99^{abc}±0.17	1.06^{abc}±0	1.22^{c}±0.05	1.2^{c}±0.21	1.09^{bc}±0.18	1.18^{c}±0.11
Glutamine	4.42^{c}±0.56	1.69^{a}±0.25	3.66^{b}±0.15	3.12^{b}±0.3	3.28^{b}±0.52	3.52^{b}±0.16	3.52^{b}±0.6	3.35^{b}±0.29	3.47^{b}±0.4
Threonine	0.42^{a}±0.37	0.43^{a}±0.05	0.53^{ab}±0.04	0.66^{b}±0.18	0.64^{b}±0.08	0.49^{ab}±0	0.55^{ab}±0.01	0.55^{ab}±0.1	0.63^{b}±0.02
Alanine	0.47^{a}±0	0.69^{b}±0.09	0.68^{b}±0.01	0.61^{ab}±0.15	0.63^{ab}±0.04	0.67^{b}±0.04	0.66^{b}±0.07	0.58^{ab}±0.05	0.68^{b}±0.07
Proline	1.71^{d}±0.01	0.73^{a}±0.1	1.32^{bc}±0.05	1.38^{bc}±0.12	1.33^{bc}±0.06	1.41^{c}±0.05	1.29^{bc}±0.06	1.37^{bc}±0.18	1.19^{b}±0
Lysine	0.15^{abc}±0.03	0.09^{a}±0.01	0.33^{d}±0.04	0.13^{ab}±0.01	0.29^{d}±0.08	0.09^{a}±0.01	0.25^{cd}±0.09	0.23^{bcd}±0.06	0.3^{d}±0.02
Valine	0.59^{a}±0.05	0.57^{a}±0.07	0.77^{c}±0	0.75^{bc}±0.13	0.78^{c}±0.04	0.75^{bc}±0.02	0.76^{bc}±0.05	0.74^{bc}±0.11	0.8^{c}±0.1
Isoleusine	0.57^{b}±0.02	0.42^{a}±0.03	0.63^{b}±0	0.64^{b}±0.08	0.64^{b}±0.04	0.63^{b}±0.02	0.64^{b}±0.04	0.63^{b}±0.08	0.67^{b}±0.07
Leusine	0.98^{ab}±0.03	0.94^{a}±0.07	1.17^{abc}±0.01	1.15^{abc}±1.13	1.19^{bc}±0.05	1.16^{abc}±0.08	1.15^{abc}±0.07	1.14^{abc}±0.17	1.23^{c}±0.14
Phenylalanine	0.62^{ab}±0.14	0.51^{ab}±0.46	0.97^{ab}±1.18	1.76^{b}±0.07	0.11^{a}±0.01	0.1^{a}±0.02	0.56^{ab}±1.3	1.01^{ab}±0.87	1.12^{ab}±1.17

*WMB_{1-7}: Wheat-millet-bambara bread at different levels of emulsifiers and pectin, MF: 100% millet flour, WF: 100% wheat flour. Values on the same row with different letters are significantly different at $p<0.05$

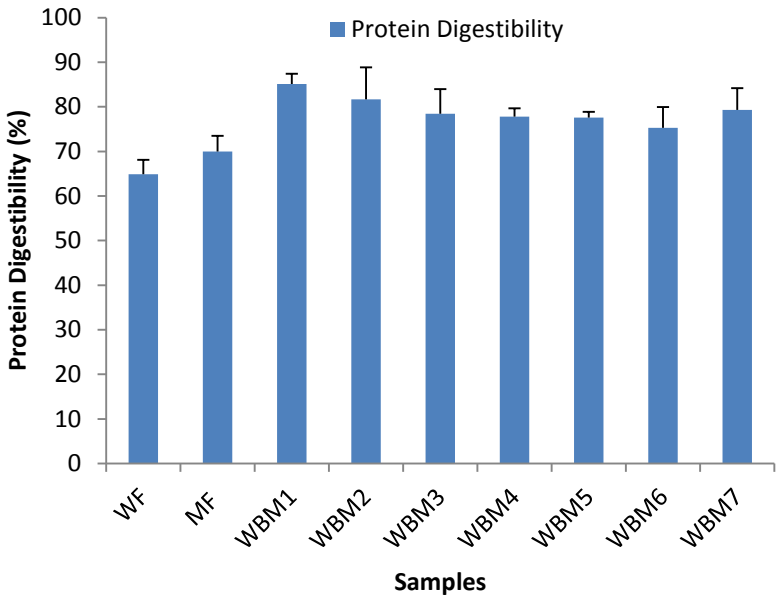

Figure 4. In vitro protein digestibility of composite bread. WMB_{1-7}: Wheat-millet-bambara bread at different levels of emulsifiers and pectin, MF: 100% millet flour, WF: 100% wheat flour.

Table 15. Sensory acceptability of wheat-millet-bambara composite bread

	Sensory attributes				
Samples	Colour	Texture	Taste	Aroma	Acceptability
WF	7.38^{c} ±1.35	7.35^{c}±1.33	6.75^{e}±1.92	6.33^{d}±1.98	7.03^{c}±1.49
MF	4.28^{a}±2.48	3.65^{a}±2.27	3.13^{a}±2.22	4.18^{a}±2.4	4.58^{a}±2.52
WBM_1	5.2^{b}±2.17	5.55^{cd}±2.17	4.33^{bc}±2.41	4.35^{ab}±2.5	6.45^{ab}±2.38
WBM_2	5.73^{b}±1.91	5.83^{d}±1.71	5.38^{d}±1.84	4.73^{abc}±2.01	6.78^{b}±1.87
WBM_3	5.08^{ab}1.93	5.4^{cd}±1.99	5.03^{cd}±1.99	5.38^{bcd}±2.02	6.78^{b}±2.03
WBM_4	5.65^{b}±1.99	4.43^{ab}±2.04	3.95^{ab}±2.03	5.03^{abc}±2.04	6.88^{b}±1.73
WBM_5	5.58^{b}±1.95	4.63^{bc}±1.88	4.45^{bcd}±1.92	5.73^{cd}±2.44	6.7^{b}±1.92
WBM_6	5.53^{b}±2.1	4.25^{ab}±2.1	3.85^{ab}±2.03	5.15^{abc}±2.27	7.13^{ab}±2.03
WBM_7	5.59^{b}±2.1	5.15^{bc}±2.35	4.38^{bcd}±2.33	5.1^{abc}±2.35	6.9^{b}±2.26

WMB_{1-7}: Wheat-millet-bambara bread at different levels of emulsifiers and pectin, MF: 100% millet flour, WF: 100% wheat flour. Values in the same column with different letters are significantly different at $p<0.05$.

3.1.4 Conclusion

The bread made from a composite of wheat-millet-bambara flour showed a significant increase in protein content. The addition of pectin at 1.5% increased the dough stability time which is very important in bread production as shown by the Mixolab. The addition of mixtures of emulsifiers (SSL, DATEM, and PS80) increased dough development time and specific volume at 1.6% level, while pectin caused an increase in the volume of the composite bread. The addition of both pectin and emulsifiers into composite bread resulted in a volume increase that was more than the volume increase in pectin-treated bread. The composite bread treated with emulsifiers at 1.3% yielded the highest lysine content increase. The in vitro protein digestibility of composite bread samples was improved compared to that of wheat flour. Based on the results of the sensory evaluation, composite bread with 2% level of pectin had the highest score for overall acceptability better than the control (100% wheat flour). Bread made with composite flours of wheat, millet, and bambara groundnut had improved protein content, high protein digestibility, and lysine content and may have the potential of solving the problem of protein-energy malnutrition in major food staples. The composite of wheat-millet-bambara flours at 50%: 25%: 25% level respectively, when treated with technological aids materials such as emulsifiers and pectin could produce bread with acceptable rheological properties comparable to wheat dough and bread.

CHAPTER FOUR: INFLUENCE OF DIFFERENT STORAGE CONDITIONS ON TEXTURE AND MICROBIAL QUALITY OF WHEAT-MILLET-BAMBARA COMPOSITE BREAD

4.1 Introduction

Bread is an important food commodity which contributes to the provision of macronutrients (carbohydrates, protein, and fat) and micronutrients (minerals and vitamins) for teaming population of the world (Ijah *et al.,* 2014). Bread production, storage, and distribution face challenges such as high staling rate, loss of moisture, short storage or shelf life, microbial degradation among many other economic problems (Ijah *et al.*, 2014). Bread, like many other processed foods, they face physical, chemical and microbiological spoilage. These spoilage problems cause a reduction in shelf life, loss of moisture, staling and microbial spoilage by bacteria and mould render bread unconsumable thus causing economic loss (Saranraj 2012). The relationship between bread storage, bread firmness, and microbial spoilage has an influence on the total quality characteristics of bread. Bread softness is synonymous with freshness and recently baked, loss of softness results in hardness which is not desirable to consumers (Saranraj 2012). Compositing wheat flour, a major raw material for bread production, with either legume such as cowpea or bambara groundnut flour or supplementation with other cereals such as sorghum, millet, maize and tubers such as cassava and potatoes may lead to other considerations in terms of the economic significance of such compositing (Noorfarahzilah *et al.,* 2014). Replacement of wheat with non-gluten flours such as millet and protein-rich flour such as bambara groundnut flour are expected to affect the shelf-life of the composite bread due to the increased amount of starch which can undergo retrogradation during storage and cause bread firmness to increase and subsequently results in loss of quality (Eduardo *et al.,* 2016). The short-range development of a gel network structure of amylose (crystallization) and a continuing reordering of amylopectin, which is a much slower process involving recrystallization of the outer branches of its polymer takes place during retrogradation of starch (Miles *et al.*, 1985; Ring and Colonna 1987). Starch retrogradation causes the redistribution of water between starch and gluten protein and eventually increase in crumb firmness (Eliasson *et al.,* 1993; Davidou *et al.,* 1996; Purhagen *et al.,* 2012). Reports by Gray *et al.* (2003) implied that amylopectin is a key reason in the retrogradation process but is not only accountable for the observed change in bread firmness or texture.

Cereals and legumes are susceptible to contamination by bacteria and fungi, the latter typically producing mycotoxins, making the foods unwholesome and unsafe for consumption (Temba *et al.,* 2016). The effects of this degradation on the food products produced from flours made from these contaminated flours used for bread may include fast staling rate, reduced shelf life, high microbial activities and high or low water activity (Temba *et al.,* 2016). It has been shown in the literature that bread made from composite flours sometimes have a high moisture content (Noorfarahzilah *et al.,* 2014). This may be due to the additive effect caused by proteins from legumes and carbohydrates from cereals (Hallén *et al.,* 2004). The addition of improvers such as hydrocolloids and surfactants may also affect the water absorption of dough and the final bread moisture content (Gómez *et al.,* 2004). There was a reported link between the starch content of the baked product, moisture content and the rate of bread degradation (Patel *et al.,* 2005). Numerous studies have also documented the mechanisms by which structural changes in starch can be altered to minimise staling of baked products when surfactants and emulsifiers are used as improvers in bakery products (Armero *et al.,* 1998; Stauffer 2000; Patel *et al.,* 2005). High moisture content in bread causes interaction between starch and gluten, this leads to degradation (Patel *et al.,* 2005). Also, microbial spoilage especially moulds and fungi are common due to excess moisture (Saranraj 2012). Mould spoilage is a serious problem in bread as it grows under low water activity and encourages the growth of other microbes (Saranraj 2012). Bread spoilage caused by bacteria is increasing due to non-use of preservatives, the addition of bran and other flours (composite flours) during bread production (Saranraj 2012). The use of hydrocolloids and emulsifiers has been shown to improve breadcrumb and minimised staling process of wheat-cassava-maize bread during storage after four days (Eduardo *et al.,* 2016). The effect of hydrocolloids and emulsifiers on storage quality of wheat-millet-bambara composite bread is limited in the study, thus this study. This work examined the effect of the addition of different improvers (emulsifiers and apple pectin) on the texture of stored composite bread made from wheat, millet and bambara groundnut flours (50%:25%:25%) in different storage conditions: room (± 25℃), refrigeration (4℃) and freezing (-18℃) temperatures. The resulting composite bread was analysed for texture and microbial loads during storage (0 - 7 days).

4.2 Materials and methods

4.2.1 Production of composite flour and bread

Composite flour and bread samples were made as described previously (Chapter 3 and Table 7). Wheat and millet bread at 100% served as control. The bread samples were left for 60 minutes to cool to room temperature (±25℃), wrapped in aluminium foil and placed in ziploc bags for storage. The bread samples storage method in this study simulates the home handling of bread after baking or purchase by consumers. Bread samples stored at room temperature were packaged in a polyethene/ziploc bag. The bread samples were stored on the shelf in the laboratory at ±25℃, in the refrigerator at 4℃ and freezer at -18℃ for 7 days. Bread samples were baked in triplicates to give a total of 27 loaves for nine bread sample types, each loaf was divided into four parts to represent each day of storage for all bread sample types at each storage condition.

4.2.2 Bread colour determination

Colour measurement of the composite bread was carried out using colour flex (A60-1014-593; Hunter Associates Laboratory, Reston, VA, USA) based on lightness (L*), red-green (a*) and yellow-blue (b*) values. Change in colour of the breadcrumb and crust was determined by the method described by Arise *et al.* (2015). The equipment was calibrated against white and black colour tiles before colour measurement. The total colour difference (DE) was calculated as shown below:

$$\Delta E = [\ (\Delta L^*)^2 + (\Delta a^*) + (\Delta b^*)^2]^{1/2}.$$

4.2.3 Bread firmness and compression energy analysis

Bread samples were cooled for 60 minutes after being removed from the oven. Bread firmness is defined as the force required for a compression of 25% of a sample of bread of 25 mm thickness (Gomes-Ruffi *et al.*, 2012). The texture of bread samples was determined using the method described by Gómez *et al.* (2013) with little adjustments. The speed of the texture analyser was adjusted to 2 mm/s and the bread slices were 2.5 cm thick. Bread samples that were stored at both 4℃ and -18℃ were placed on a clean stainless-steel tray and allowed to equilibrate with room temperature (±25℃) before analysis. Bread firmness and compression energy were recorded for all bread samples throughout the storage periods. The values of bread firmness and compression

energy were obtained using an EZ-SX texture analyser (Shimadzu compact table top, Shimadzu Co. Japan) software.

4.2.4 Bread storage

Shelf-life studies were conducted by packing the bread samples into materials such as aluminium foil and Ziploc bags (polyethene bag). The bread samples (WF, MF, and WMB_{1-7}) were individually packed loaf and stored at various temperatures (±25℃, 4℃, and -18℃). Three loaves of bread were made for each storage conditions for each day of storage. The bread samples were stored at room temperature (±25℃) on the shelf in the laboratory, refrigeration temperature (±4℃) and freezing temperature (-18℃) and were observed for 7 days. Bread samples were checked for spoilage by visual observations for mould growth. Visual analysis for the presence of mould growth was carried out at intervals on the samples stored under all storage conditions (Malomo *et al.*, 2012).

4.3 Microbiological analysis of bread samples

Total aerobic plate count (viable bacteria) and fungi counts (yeast and mould counts) were carried out on the bread samples to determine the microbial load of the samples as described by Egbuta *et al.* (2015) with some modifications. Bread samples were prepared by mashing and mixing in ringer solution. The ringer solution was prepared by dissolving one ringer tablet in 500 ml of sterilised water. Samples were dissolved serially in 5 aliquots and plated on nutrient agar (NA) for aerobic bacteria plate count and acidified potato dextrose agar (PDA) at pH 3.5±1 for the total yeast and mould counts. The NA were incubated at 35℃ for 48 ±2 hours and the PDA plates were incubated at 25℃ for 5 to 7 days. The colonies were then counted and expressed as log of colony forming units per gramme (log cfu/g). All plating and counting were done in triplicates using the Stuart Scientific Colony Counter.

4.3.1 Statistical analysis

All analyses were performed in triplicates. Analysis of variance (ANOVA) was performed and means were compared using the Fisher Least Significant Difference (LSD) test ($p < 0.05$).

4.4 Results and discussion

4.4.1 Bread colour

There was a significant difference ($p < 0.05$) in the composite breadcrumb colour as shown in Table 16. The colour difference may be due to the influence of colour from pearl millet grain which could have imparted a slight grey colour to the composite flour mixture. There was a significant increase ($p < 0.05$) in crumb colour of the composite bread as both pectin and emulsifiers addition increased. The composite bread showed a significant increase ($p < 0.05$) in the crust colour. This may be due to an increased level of milliard reaction at high temperature during baking. The increased level of protein in the composite bread increased the level of Milliard reaction in the bread hence, deep crust colour occurred in the composite bread samples (Zhang *et al.*, 2007). The colour change (ΔE) of millet bread is significantly different ($p < 0.05$) in terms of crumb and crust colour compared to WF. The emulsifier treated bread had the least significant difference ($p < 0.05$) in colour change among the composite bread samples. In this study, emulsifier and pectin-treated composite bread samples showed no colour change in the crumb based on the visual observation of all bread samples during all storage conditions after 7 days.

4.4.2 Bread texture

In this study, there was a significant difference ($p < 0.05$) in the firmness and compression energy of the composite bread samples (WMB_{1-7}) compared to the control (WF) one hour after leaving the oven. The firmness is the degree of hardness while the compression energy is the force required to cut the 2.5 cm bread sample using the texture analyser (Malomo *et al.*, 2012; Gómez *et al.*, 2013). The pectin treated bread (PTB) had the lowest firmness (2.12 nm) at 2% level compared to WF (2.59 nm) followed by emulsifiers treated bread (ETB) (2.1 nm) and combination of both emulsifiers and pectin treated composite bread (EPTB) (2.20 nm) have a better firmness than both (PTB and ETB) one hour after coming out of oven (Figure 5). This shows that composite bread is softer than the control and thus indicated the ability of pectin and emulsifiers as bread softeners (Gómez *et al.*, 2013). The compression energy was significantly increased in the PTB (WMB_{4-6}) and the EPTB (WMB_7) compared to the ETB (WMB_{1-3}) samples (Table 16). This means that emulsifiers reduced the energy needed to cut composite bread and thus improved the cutability of the ETB samples. Emulsifiers have been shown to improve the cutability (slicing) of bread

(Stampfli *et al.*, 1995). MF showed the highest compression energy needed to cut through as shown in Table 6, one hour after coming out of the oven.

In this study, as the number of storage days increased, both bread firmness and compression energy increased and this was more pronounced in the room temperature storage (Figures 5 and 6). There was a significant difference ($p < 0.05$) in WF firmness compared to the composite bread samples in room temperature storage at 3 days storage.

Table 16. Composite bread instrumental crumb and crust colour

Parameters								
	Crumb colour				Crust colour			
Samples	L*	a*	b*	ΔE	L*	a*	b*	ΔE
WF	62.72^{g} ±0.58	2.52^{a} ±0.0	22.01^{h} ±0.01	0	46.78^{g} ±0.01	12.73^{d}±0.01	26.45^{f}±0.02	0
MF	49.47^{a} ±0.06	4.63^{h} ±0.01	19.69^{d} ±0.01	13.28^{a}±0.48	53.79^{i} ±0.02	4.07^{a}±0.03	20.21^{a}±0.02	8.05^{a}±0.04
WBM_1	55.42^{d} ±0.35	3.22^{c} ±0.06	19.37^{a} ±0.02	7.73^{e}±0.07	45.97^{f} ±0.02	13.96^{g}±0.01	26.67^{g}±0.04	0.91^{e}±0.02
WBM_2	60.40^{f} ±0.38	2.57^{b} ±0.10	19.64^{c} ±0.02	3.3^{g}±0.02	50.30^{h} ±0.01	13.69^{f}±0.01	30.40^{i}±0.05	5.2^{c}±0.06
WBM_3	53.95^{c} ±0.06	3.29^{d} ±0.01	19.54^{b} ±0.01	9.08^{c}±0.28	40.48^{c} ±0.01	13.58^{e}±0.01	22.44^{e}±0.03	7.42^{b}±0.06
WBM_4	54.11^{c} ±0.06	4.08^{f} ±0.01	21.97^{g} ±0.02	8.47^{d}±0.13	39.66^{b} ±0.01	12.09^{c}±0.01	21.94^{d}±0.04	8.45^{a}±0.07
WBM_5	50.58^{b} ±0.01	7.07^{i} ±0.01	23.63^{i} ±0.02	11.14^{b}±0.29	40.88^{d} ±0.01	16.90^{i}±0.01	29.08^{h}±0.01	3.24^{d}±0.05
WBM_6	49.25^{a} ±0.02	4.37^{g} ±0.01	20.11^{e} ±0.01	13.48^{a}±0.3	35.93^{a} ±0.05	14.91^{h}±0.01	20.39^{b}±0.02	4.04^{d}±0.03
WBM_7	56.36^{e} ±0.02	3.94^{e} ±0.01	21.43^{f} ±0.02	6.23^{f}±0.05	44.34^{e} ±0.01	11.43^{b}±0.01	21.82^{c}±0.03	5.39^{c}±0.05

WMB_{1-7}: Wheat-millet-bambara bread at different levels of emulsifiers and pectin treatment, MF: 100% millet flour, WF: 100% wheat flour. Lightness (L*), red-green (a*), yellow-blue (b*), Change in colour (ΔE). Values on the same column with different letters are significantly different at $p<0.05$ using the Fisher Least Significant Difference (LSD) test.

ETB (WMB_4) at 1% treatment level had the closest firmness to the control (WF) at 3 days and MF had the highest compared to WF after 3 days storage at RT. ETB firmness decreased, as the level of addition of emulsifiers increased after 3 days storage at RT. This supported the ability of pectin and emulsifiers in improving bread firmness during storage (Stampfli *et al.*, 1995). EPTB resulted in less significant ($p<0.05$) firmness after 3 days of storage compared to PTB and ETB after the same number of storage days at RT. This means that a combination of emulsifiers and pectin at

this concentration may not decrease the firmness of composite bread made from wheat, millet, and bambara groundnut.

All bread samples showed an increase in both firmness and compression energy across the storage days, as the storage days increased the bread firmness and compression energy increased at room temperature (RT) (Figure 7).

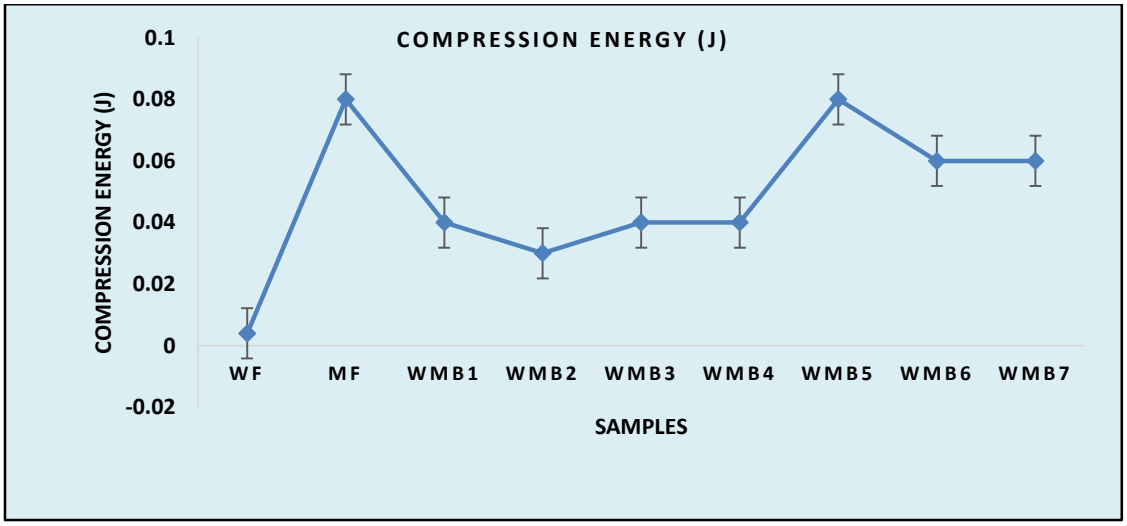

Figure 5. Composite bread compressed energy one hour after leaving the oven. $WMB_{1\text{-}7}$: Wheat-millet-bambara bread at different levels of emulsifiers and pectin treatment, MF: 100% millet flour, WF:100% wheat flour.

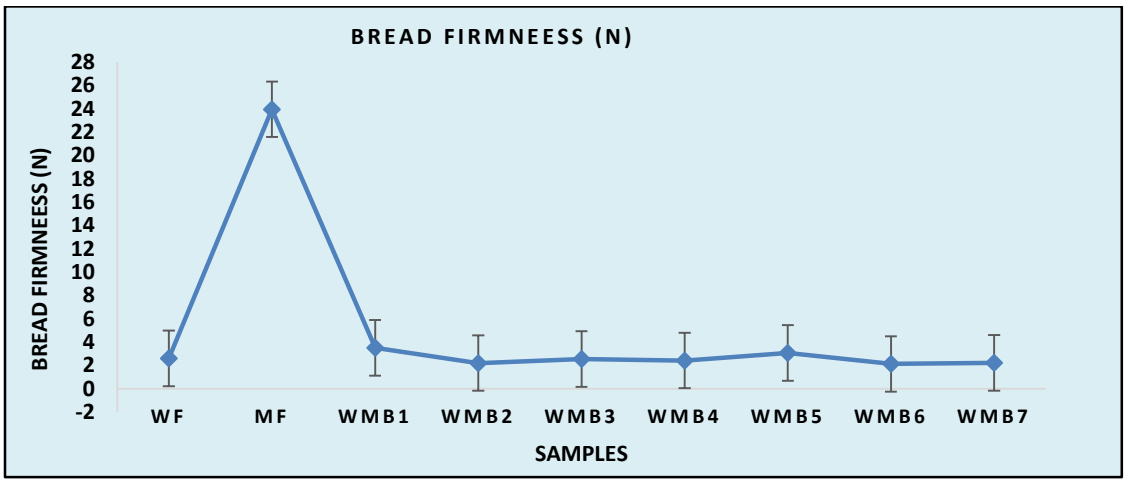

Figure 6. Composite bread firmness one hour after leaving the oven. $WMB_{1\text{-}7}$: Wheat-millet-bambara bread at different levels of emulsifiers and pectin treatment, MF: 100% millet flour, WF:100% wheat flour.

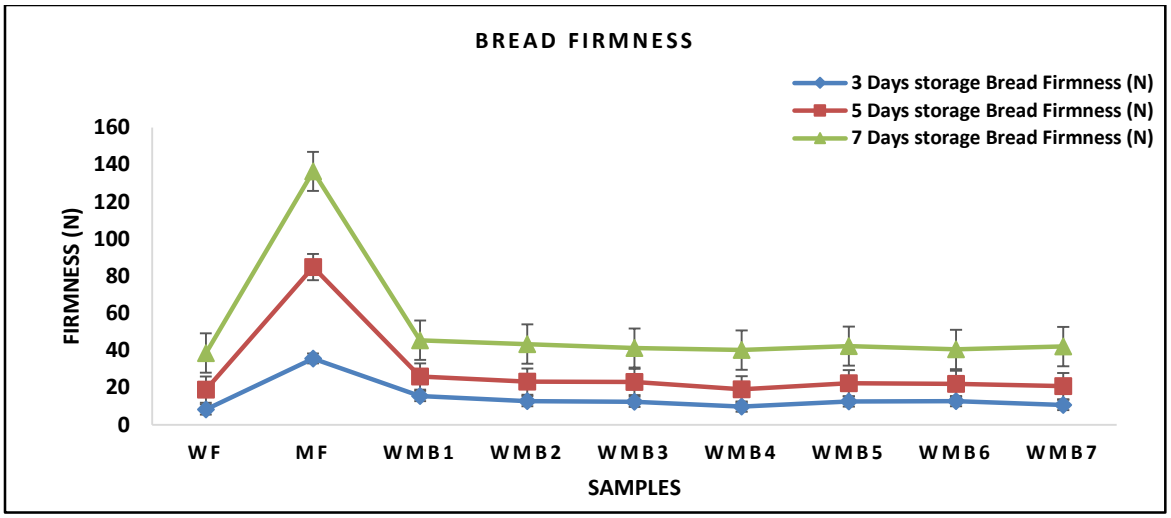

Figure 7. Composite bread firmness during room temperature (±25℃) storage condition. WMB_{1-7}: Wheat-millet-bambara bread at different levels of emulsifiers and pectin treatment, MF: 100% millet flour, WF:100% wheat flour.

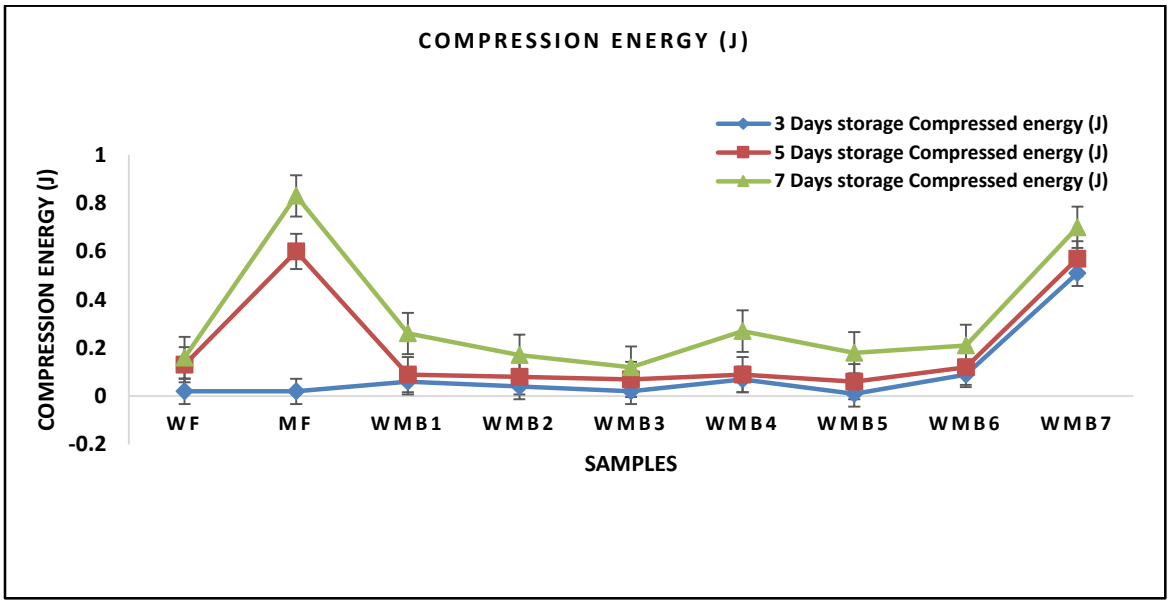

Figure 8. Composite bread compressed energy during room temperature (±25℃) storage. WMB_{1-7}: Wheat-millet-bambara bread at different levels of emulsifiers and pectin treatment, MF: 100% millet flour, WF:100% wheat flour.

The result of bread firmness of all samples stored at refrigeration temperature (4℃) (FRI) is significantly different ($p < 0.05$) compared to the control as the number of storage days increased (Figures 8 and 9). Bread stored at FRI had a high rate of firmness increase, this may be due to the storage temperature (4℃) which according to reported works increased the rate of staling in bread due to the high rate of amylose retrogradation of starch molecules (Saranraj 2012). The addition

of emulsifiers and pectin did not affect the rate at which the composite bread became hardened (loss of firmness) at this (FRI) storage condition (4℃) (Figure 9). The rate of both firmness and compression energy increased as the number of storage days increased in bread samples stored at FRI for all bread samples after each of the days (3, 5 and 7) of storage.

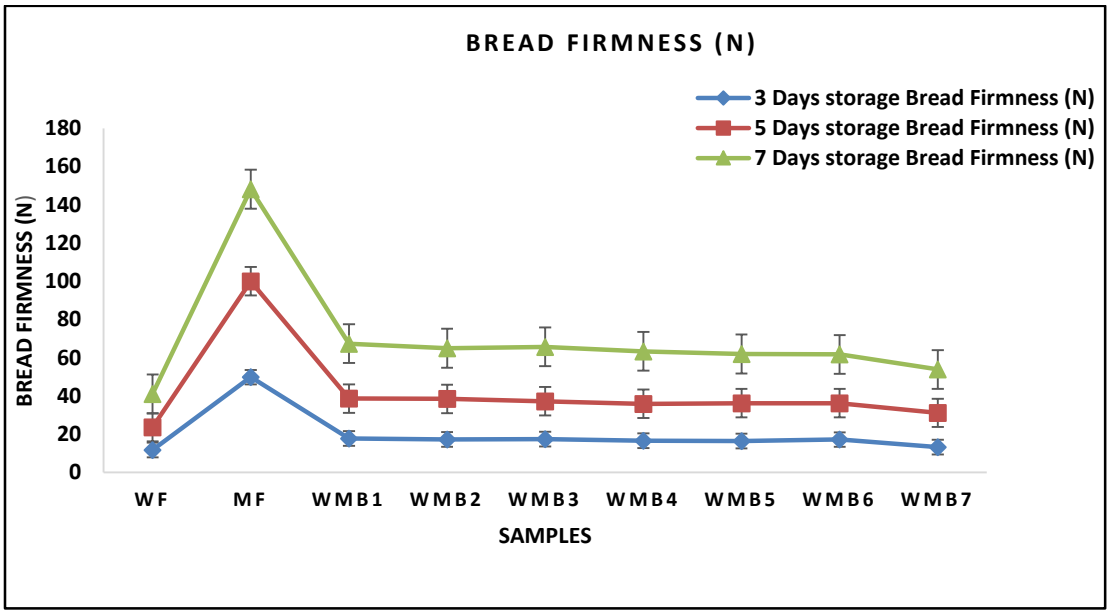

Figure 9. Composite bread firmness at room temperature (4℃) storage condition. $WMB_{1\text{-}7}$: Wheat-millet-bambara bread at different levels of emulsifiers and pectin treatment; MF: 100% millet flour; WF:100% wheat flour.

Composite bread stored at freezing temperature (FET) showed a significant difference ($p < 0.05$) in firmness and compression energy during storage (Figures 10 and 11) compared to the control. The PTB (WMB6) at the highest level of treatment showed a significant decrease ($p < 0.05$) in firmness and compression energy compared to the control (WF) at -18℃ storage condition after 3 days. This supported the reported work that pectin can hold molecules of water to protein, starch and oil molecules together thus causing the breadcrumb to hold water and causing a softening effect after defrosting to room temperature (Kenijz *et al.*, 2013; Ngouémazong *et al.*, 2015). Aguirre *et al.* (2011) also confirmed that during bread storage, there is the presence of moisture equilibration between crumb and crust and this verified that storage at -18℃ resulted in very restricted water transfer when compared to bread stored at 4℃ and 25℃.

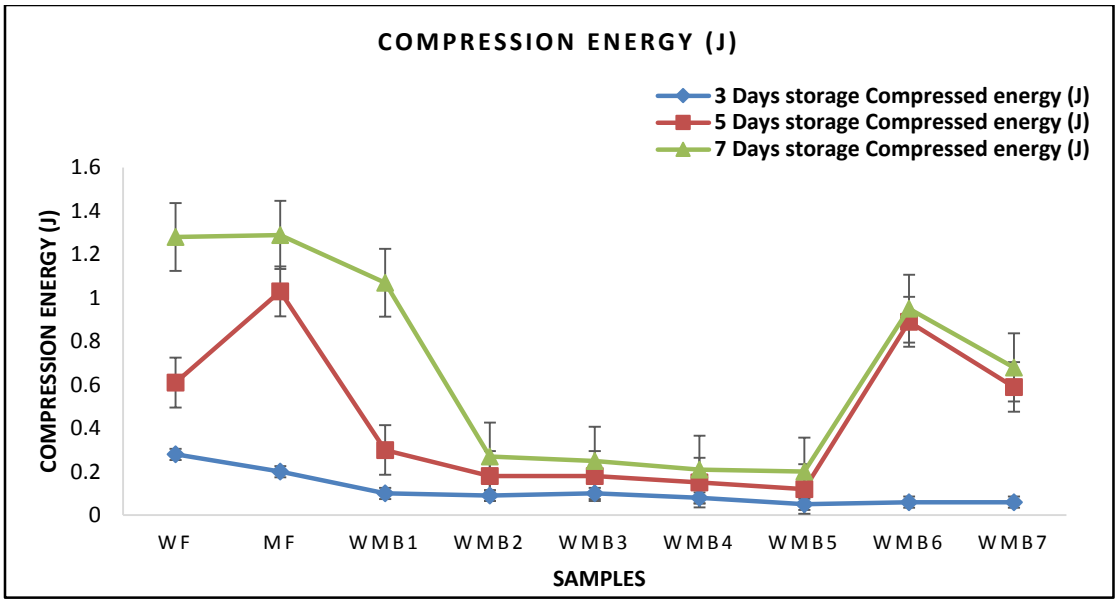

Figure 10. Composite bread compressed energy during room temperature (4℃) storage condition. WMB_{1-7}: Wheat-millet-bambara bread at different levels of emulsifiers and pectin treatment, MF: 100% millet flour, WF:100% wheat flour.

The rate of firmness decreased as the level of treatment with emulsifiers and pectin increased in the composite bread samples (WMB_{1-6}) (Figure 11). This may indicate that pectin and emulsifiers are good softness retainers in bread stored at freezing temperature. The addition of both emulsifiers and pectin to composite bread (EPTB) (WMB_7) did not significantly ($p < 0.05$) increase composite bread softness when compared with individual treatment (PTB and ETB) at all storage temperatures. Millet bread (MF) showed a significant increase ($p < 0.05$) in the level of firmness and compression energy at FET (-18℃) (Figures 11 and 12) storage condition. Overall, all bread samples had a decreased firmness and compression energy during storage at FET (-18℃) as the number of days increased.

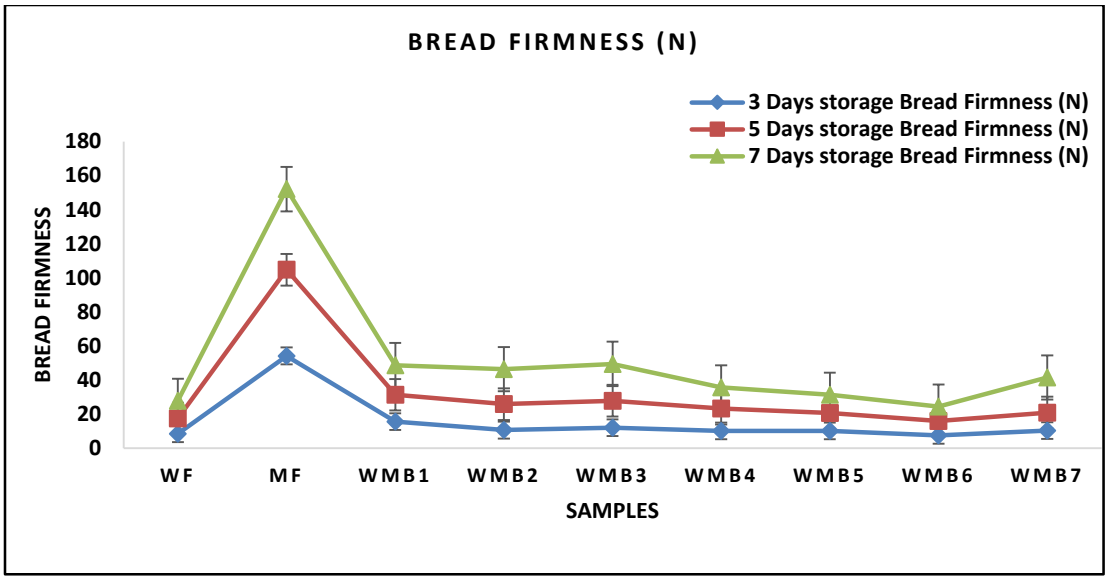

Figure 11. Composite bread firmness during room temperature (-18℃) storage condition. WMB_{1-7}: Wheat-millet-bambara bread at different levels of emulsifiers and pectin treatment, MF: 100% millet flour, WF:100% wheat flour.

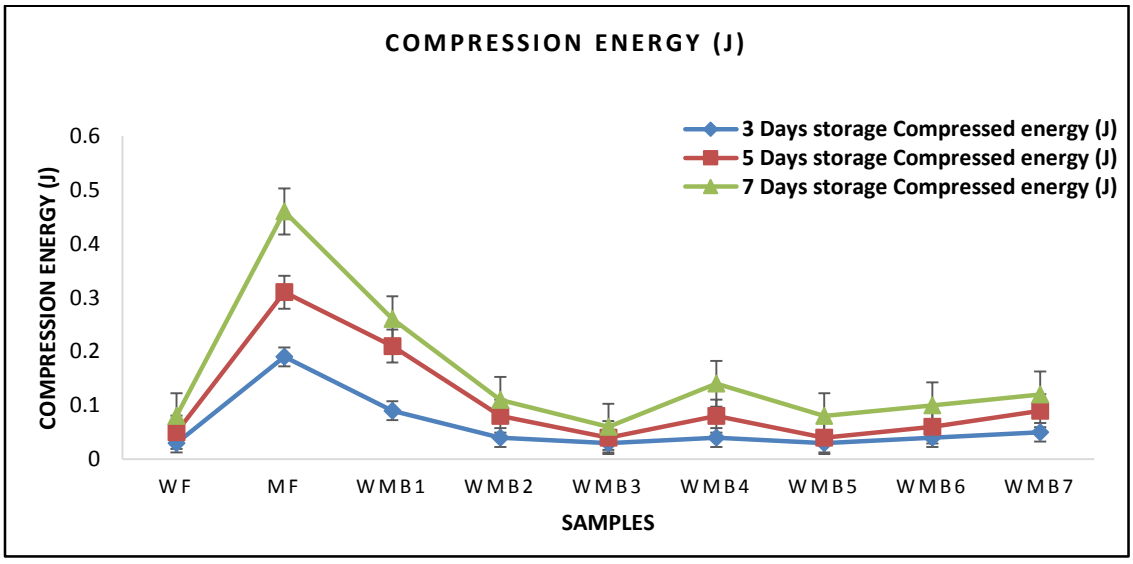

Figure 12. Composite bread compressed energy during room temperature (-18℃) storage condition. WMB_{1-7}: Wheat-millet-bambara bread at different levels of emulsifiers and pectin treatment, MF: 100% millet flour, WF:100% wheat flour.

4.4.3 Microbial Analysis results

4.4.3.1 Visual observation for mould spoilage

The composite bread samples stored at room temperature on the shelf in the laboratory were observed visually for mould growth. The bread samples lasted for 5 days before obvious spoilage

was noticed (Table 17). The WF and the PTB samples showed mould growth on the 5th day of storage, the ETB and the EPTB had no mould growth until the 7th day of storage at ±25℃. There was no mould growth observed in the EPTB at the highest level of emulsifier and pectin treatment of the composite bread. Also, this was noticed in the millet bread which also showed no mould growth at ±25℃. This suggests that at this level (1.6% and 2%) of treatment, mould growth might have been delayed. Bread samples stored at FET did not show any signs of mould growth after the 7th day of storage. Spoilage of bread samples was indicated with black and green colouring of bread samples (suspected to be mould growth) (Ijah *et al.,* 2014).

Table 17. Spoilage (Visual observation of mould growth) at various storage temperatures for composite bread samples

	Room temperature storage at ±25℃				Refrigeration temperature storage at 4℃			Freezing temperature storage at -18℃		
Days	0	3	5	7	3	5	7	3	5	7
Samples										
WF	Nil	Nil	Nil	MD	Nil	MD	MD	Nil	Nil	Nil
MF	Nil	Nil	Nil	MD	Nil	MD	MD	Nil	Nil	Nil
WMB_1	Nil	Nil	Nil	MD	MD	MD	MD	Nil	Nil	Nil
WMB_2	Nil	Nil	Nil	MD	Nil	Nil	MD	Nil	Nil	Nil
WMB_3	Nil	Nil	MD	Nil	Nil	MD	MD	Nil	Nil	Nil
WMB_4	Nil	Nil	MD	MD	Nil	MD	MD	Nil	Nil	Nil
WMB_5	Nil	Nil	Nil	MD	Nil	MD	MD	Nil	Nil	Nil
WMB_6	Nil	Nil	Nil	Nil	Nil	Nil	MD	Nil	Nil	Nil
WMB_7	Nil	Nil	Nil	MD	Nil	Nil	MD	Nil	Nil	Nil

*WMB_{1-7}: Wheat-millet-bambara bread with different levels of emulsifiers and pectin treatment, MF: 100% millet flour, WF:100% wheat flour. Nil- Mould not detected, MD- Mould detected.

4.4.3.2 Microbial counts or loads

It was observed that microbial growth increased as the number of storage days increased for composite bread stored at RT (±25℃) (Table 18). The total aerobic bacterial count (APC) for all bread samples ranged from 3.02 log cfu/g to 6.19 log cfu/g and fungal count (FC) ranged from 3.48 log cfu/g to 4.86 log cfu/g. The PTB had the highest APC (6.19 log cfu/g) at ±25℃ storage while it also had the lowest APC (3.02 log cfu/g) at the same storage temperature. This may be due to the microbial content of the baking ingredients such as flour, margarine, and sugar. Some species of bacteria have been known to survive high temperatures that occur during baking (Saranraj 2012; Ijah *et al.*, 2014). ETB had high bacteria counts and lower fungal counts compared to the control (WF) during storage at both room and refrigeration temperature. The microbial level in bread has not been affected by the addition of emulsifiers and pectin to bread samples. Bacteria

and fungi were not detected in the bread samples that were analysed immediately after baking. This is expected due to high temperatures which may have killed all the spores and microbial cells present in the bread before baking. All bread samples after one hour from oven had their APC and FC within the set limit for mould, yeast and bacteria counts in bread. According to the South African Bureau of Standards (SABS), it must be lower than 4 logcfu/g for bakery products. This means the bread samples are safe for consumption. The high bacteria population noticed in some bread samples could be due to a high moisture content of the composite bread sample and the presence of nutrients from both bambara groundnut and millet flours, which provides favourable conditions for growth of microbes (Ijah *et al.,* 2014). PTB composite bread (WMB_4) had the highest bacteria content and this may be because of the microbial content of the raw ingredients used (flour, sugar etc.). Some bacteria spores (*Bacillus sp*.) can survive unfavourable conditions such as baking temperature (Saranraj 2012). *Staphylococcus* species have been reported to be widely distributed in the environment and they are found on the skin and nostrils of humans and can thus contaminate food. Irregular detection of bacteria in bread samples may also be due to microbial pick-up from surfaces and equipment used for baking (Saranraj 2012) (Table 17).

Table 18. Microbial counts at room temperature storage for composite bread samples

	Bacteria (log cfu/g)				Fungi (log cfu/g)			
Samples	0 day	3 days	5 days	7 days	0 day	3 days	5 days	7 days
WF	ND	ND	4.48	5.72	ND	ND	4.48	4.6
MF	ND	5.7	ND	4.3	ND	ND	ND	4.7
WMB_1	3.11	6.12	5.22	5.88	ND	ND	ND	ND
WMB_2	3.3	3.85	5.09	5.94	ND	ND	ND	ND
WMB_3	ND	5.82	3.48	4.23	3.48	ND	4.11	ND
WMB_4	3.02	5.4	6.19	5.97	ND	ND	3.48	4.83
WMB_5	ND	ND	ND	4.6	ND	ND	ND	3.48
WMB_6	ND	ND	ND	3.48	ND	ND	ND	ND
WMB_7	3.48	ND	ND	3.48	ND	ND	ND	4.78

*WMB_{1-7}: Wheat-millet-bambara bread at different levels of emulsifiers and pectin treatment; MF: 100% millet flour; WF:100% wheat flour. ND; Not detected.

The high fungal population in composite bread samples (WMB_7) may be due to contamination of raw materials, processing, handling, and storage materials used for baking (Ijah *et al.,* 2014). Fungi such as *Aspergillus*, *Penicillium*, *Rhizopus*, and *Mucor* species have been identified as spoilage fungi which could be responsible for bread spoilage and they could have been introduced at the

different stages of bread production since they are present in the air and around bakery area (Saranraj 2012).

Refrigeration storage of bread samples showed a significantly ($p < 0.05$) lower microbial growth in both APC and FC compared to RT storage (Table 19). Microbial growth was lower for bread samples stored at FRT as the number of days increased. This indicates that microbial growth may have been slowed down by low temperature (4℃).

Table 19. Microbial counts at refrigeration temperature storage for composite bread samples

Samples	Bacteria (log cfu/g)			Fungi (log cfu/g)		
	3 days	5 days	7 days	3 days	5 days	7 days
WF	4.9	4.48	4.0	ND	ND	3.48
MF	ND	3.48	4.86	ND	3.85	4.48
WMB_1	ND	5.22	5.01	3.48	ND	ND
WMB_2	ND	ND	5.18	ND	ND	ND
WMB_3	4.36	3.48	ND	ND	4.36	ND
WMB_4	4.63	4.82	ND	ND	4.11	ND
WMB_5	ND	4.11	ND	ND	ND	ND
WMB_6	ND	3.48	ND	ND	ND	4.48
WMB_7	4.78	3.48	ND	3.85	ND	4.2

*WMB_{1-7}: Wheat-millet-bambara at different levels of emulsifiers and pectin treatment, MF: 100% millet flour, WF:100% wheat flour, ND: Not detected.

Bread samples stored at freezing temperature (FET) (-18℃) showed significantly lower ($p < 0.05$) bacteria and fungal counts as the number of days compared to RT and FRT storage (Table 20). Generally, bread samples stored at freezing temperatures (-18℃–22℃) had a slow rate of staling and microbial increase and can store for more than one year (Saranraj 2012). Bread samples stored at FET show better microbial deterioration compared to other storage temperatures (4℃ and ±25℃). It has been confirmed from the reported work that bread stored at freezing temperatures (-18℃) usually keep for a longer period of time than any other method of storage (Saranraj 2012). Freezing temperature storage of bread have the challenge of crumb wetness during thawing but this can be overcome by using the microwave oven method of thawing. Freezing storage has been shown to be the best method of storage of bread to retain freshness and reduce microbial growth (Saranraj 2012). This result showed that composite bread of wheat-millet-bambara treated with emulsifiers and pectin can store well under freezing conditions just like the WF bread.

Table 20. Microbial counts at freezing temperature storage for composite bread samples

Bacteria (log cfu/g)			Fungi (log cfu/g)			
3 days	5 days	7 days	3 days	5 days	7 days	
WF	4.95	4.43	ND	ND	ND	ND
MF	5.51	5.49	5.19	ND	ND	4.36
WMB_1	5.4	5.11	3.48	ND	ND	4.72
WMB_2	5.54	3.48	4.08	ND	ND	ND
WMB_3	5.31	3.78	4.23	ND	4.23	3.48
WMB_4	5.33	5.18	ND	ND	4.3	3.48
WMB_5	ND	4.78	ND	ND	ND	ND
WMB_6	ND	ND	ND	ND	ND	4.48
WMB_7	ND	ND	ND	ND	ND	4.86

*WMB_{1-7}: Wheat-millet-bambara bread at different levels of emulsifiers and pectin treatment, MF: 100% millet flour, WF:100% wheat flour. ND; Not detected.

4.4.4 Conclusion

There was no significant difference between the colour of composite bread and that of the wheat bread. There was a decrease in the firmness of composite bread treated with pectin (2%) at -18°Cstorage. At room temperature storage, bread texture became softer in both emulsifiers and pectin treated bread significantly improved compared with wheat flour bread. All bread samples had an acceptable level of both bacteria and fungal counts ($<10^4$ cfu/g) one hour after production. Emulsifiers and pectin treated bread samples had low microbial loads compared to wheat flour bread. Visual observation revealed no mould growth until the 7^{th} day of storage in all bread samples except wheat flour bread, which showed mould spoilage from the 3^{rd} day. The results of this study favour storage of composite bread made from wheat-millet-bambara groundnut flours at freezing temperature. This research also showed that improvers such as emulsifiers and pectin can be used to improve the quality characteristics of wheat composite bread. Furthermore, storage at a freezing temperature can significantly delay spoilage of composite bread.

CHAPTER FIVE: GENERAL DISCUSSION

The composite flour made from wheat millet and bambara groundnut flours showed an improved dough and bread quality despite the reduction of gluten in the composite mixture up to 50% substitution. The level of viscoelastic properties of the dough produced from these flours was improved by emulsifiers and pectin. The use of composite flour in the production of staple foods such as bread most especially in developing countries can solve the problem of protein-energy malnutrition and may reduce the over-reliance of Africa countries on the use of wheat in bread production.

In the first part of this study where the rheological properties, nutritional and sensory analysis of bread made from composite flour of wheat-millet-bambara groundnut were studied, it was found that there was a significant increase in water absorption, stability and dough development time, which is very important in breadmaking. Bread made with composite flours of wheat, millet, and bambara groundnut have improved protein content, high digestibility, and lysine content and may have the potential of solving the problem of protein-energy malnutrition in developing regions. The consumer acceptability study of the composite bread showed the highest score in overall acceptability for the pectin treated bread (2%), compared to the control (100% wheat flour). Emulsifier treated bread scored average (≥5) overall acceptability but its acceptability was lower than that of the control. The emulsifiers and pectin treated composite bread (WMB_7) had low acceptability as it received a slightly higher score than the emulsifiers and pectin treated samples (WMB_{1-6}). In terms of colour, all bread samples apart from millet bread scored above the average acceptable level (5). The results showed that the composite bread samples (WMB_{1-7}) were scored low (4) in terms of taste. This may be due to consumer familiarity with wheat bread and this may have introduced bias in the panellist scoring.

This means that the composite of wheat-millet-bambara flours at 50%: 25%: 25% level respectively, when treated with emulsifiers and pectin could produce bread with quality that is comparable to that of wheat bread.

The second part of this study showed the influence of storage on bread texture and the microbial load of bread when the bread was stored in the room, fridge and freezing temperatures. There was an improvement in the texture of composite bread treated with pectin at -18 ^{o}C storage. At room temperature storage, bread texture in both emulsifiers and pectin treated bread were better than the

wheat flour bread. All bread samples had the acceptable level of both bacterial and fungal counts ($>10^4$ logcfu/g) level an hour after production. Emulsifiers and pectin treated bread resulted in low microbial loads compared to wheat flour bread. Visual observation revealed no mould growth until the 7th day of storage in all bread samples except wheat flour bread which showed mould spoilage from the 3rd day. The result of this study favours storage of composite bread made from wheat-millet-bambara groundnut flours at freezing temperature as it gives the best texture (lowest firmness) and reduced staling characteristics of stored composite bread.

This research showed that technological aid improvers such as emulsifiers and pectin can improve bread quality characteristics during storage at room, refrigeration and freezing temperatures. The research also showed that when mixtures of emulsifiers and pectin are used as improvers in bread production, the resultant bread may have a better texture and longer shelf life compared to wheat flour bread.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

The bread made from a composite of wheat-millet-bambara flour showed a significant increase in protein content. There was an increase in the proximate composition results of the composite flour and bread due to the addition of millet and bambara flour. The mixolab rheological parameters analysed showed an improvement in the pectin treated dough in terms of stability, water absorption and dough development time. Emulsifier treated composite bread gave a decreased water absorption when compared to wheat flour and the highest dough development time at the low level of addition of emulsifiers. Composite dough with the addition of both pectin and emulsifier at the highest level gave the best stability among all the treated dough. Composite bread volume was increased despite gluten reduction in the composite mixture up to 50% substitution. Emulsifiers treated dough resulted in volume increase among the composite bread samples. The composite bread treated with emulsifiers at 1.3% yielded the best lysine content. The *in vitro* protein digestibility of composite bread samples was increased compared to that of wheat flour and millet bread. Based on the result of the sensory evaluation, composite bread with 2% level of pectin had the highest score for overall acceptability better than the controls (100% wheat and 100% millet flour).

The storage stability studies showed that there was no significant difference in the composite bread colour compared to the wheat bread, but there was a significant difference compared to millet bread colour. There was an improvement in the texture of composite bread treated with pectin (2%) at -18 ^{o}C storage after 7 days. At room temperature storage, bread texture in both emulsifiers and pectin treated bread significantly improved compared with wheat flour bread. All bread samples had an acceptable level of both bacterial and fungal counts ($<10^4$ cfu/g) one hour after production. The level of microorganisms increased for all bread samples as the number of storage days increased for all bread samples at all storage conditions (25 ^{o}C, 4 ^{o}C and -18 ^{o}C). Emulsifiers and pectin treated composite bread had low microbial loads compared to wheat flour bread across all storage conditions. Visual observation revealed no mould growth until the 7th day of storage in all bread samples except wheat flour bread which showed mould spoilage from the 3rd day.

Conclusively, bread made with composite flours of wheat, millet and bambara groundnut had improved protein content, high digestibility, and improved lysine content and may have the potential of solving the problem of protein-energy malnutrition in developing regions. The

composite of wheat-millet-bambara flours at 50%: 25%: 25% (WMB) level, when treated with improvers such as emulsifiers and pectin could produce bread with acceptable quality that is comparable to wheat dough and bread. This research also showed that dough improvers such as emulsifiers and pectin can improve composite bread quality characteristics during storage at room, refrigeration and freezing temperatures.

The use of composite flour in the production of staple foods is vast. The use of bambara and millet in the supplementation and fortification of staple foods is applicable to various staple foods and health focus food products. Future studies can focus on the quality characteristics of the composite mix/flour to predict their application in various food products. The composite flour of wheat, millet, and bambara can also be investigated for their suitability to produce other baked goods (cake, doughnuts, etc.), extruded products and as an infant formula. Future studies based on this work can investigate and identify the spoilage microorganisms present in the stored bread at various storage temperatures and the effect of mixtures of emulsifiers and pectin on the staling process of the stored composite bread.

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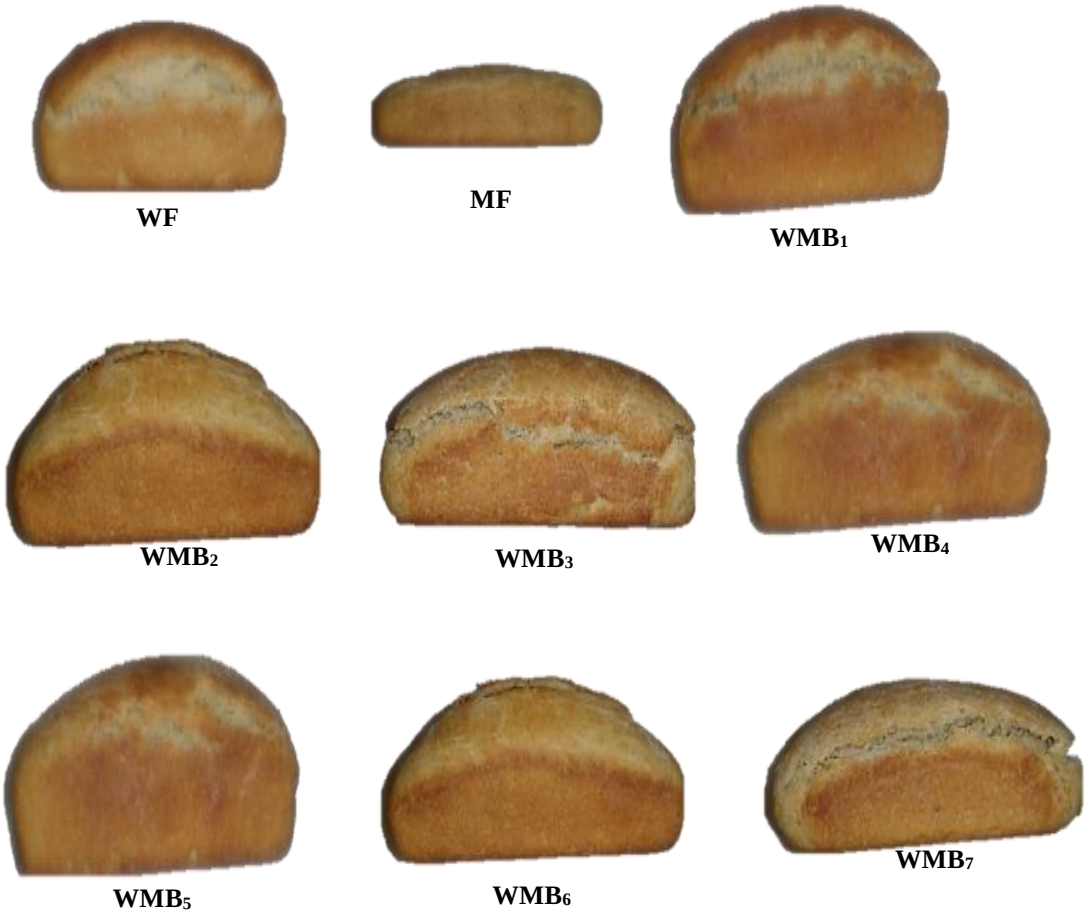
APPENDIX ONE: EFFECT OF INCLUSION OF MILLET AND BAMBARA FLOURS ON THE FUNCTIONAL PROPERTIES OF WHEAT COMPOSITE DOUGH (CHAPTER THREE)

Parameters	WF	MF	WMB_1	WMB_2	WMB_3	WMB_4	WMB_5	WMB_6	WMB_7
Retrogradation	9.0^{d} ±0.58	0.0^{a}	6.67^{bc} ±0.58	7.0^{bcd} ±0.0	8.0^{cd} ±0.0	6.0^{bc} ±0.0	7.00^{bcd} ±2.65	5.0^{b} ±1.73	7.33^{cd} ±1.16
Amylase	9.33^{f} ±0.58	0.0^{a}	6.67^{cde} ±0.58	6.67^{cde} ±0.58	7.0^{de} ±0.0	5.0^{b} ±0.0	5.67^{bc} ±1.16	6.0^{bcd} ±0.00	7.33^{e} ±1.16
Viscosity	8.33^{c} ±0.58	0.0^{a}	1.67^{b} ±0.58	2.0^{b} ±0.0	2.0^{b} ±0.0	2.0^{b} ±0.0	2.33^{b} ±0.58	2.0^{b} ±0.0	1.67^{bc} ±0.58
Gluten	2.67^{d} ± 0.58	0.0^{a}	2.33^{cd} ±0.58	2.0^{cd} ±0.0	2.0^{cd} ±0.0	1.0^{b} ±0.58	2.0^{cd} ±1.0	1.0^{b} ±0.0	1.67^{bc} ±0.58
Mixing	4.0^{bcd} ±0.0	0.0^{a}	3.0^{b} ±0.0	3.0^{b} ±0.0	3.0^{b} ±0.0	5.0^{d} ±0.0	4.33^{cd} ±1.16	5.0^{d} ±0.0	3.67^{bc} ±1.46
Absorption	1.0^{ab} ±0.0	0.0^{a}	1.0^{ab} ±0.0	1.0^{ab} ±0.0	1.0^{ab} ±0.0	1.0^{ab} ±0.0	3.33^{b} ±4.04	1.0^{ab} ±0.0	1.0^{ab} ±0.0

Effect of inclusion of millet and bambara flours on functional properties of wheat composite dough. $WMB_{1-7:}$ Wheat, millet and bambara bread with different levels of emulsifiers and pectin treatment, MF: 100% millet flour, WF: 100% wheat flour. Values on the same row with different letters are significantly different at $p<0.05$ using Fisher Least Significant Difference (LSD) test.

APPENDIX TWO: COMPOSITE BREAD ONE HOUR AFTER LEAVING THE OVEN (CHAPTER THREE).



Composite bread samples one hour after leaving the oven. WMB_{1-7}: Wheat, millet and bambara bread with different levels of emulsifiers and pectin treatment (50:25:25), MF: 100% millet flour, WF:100% wheat flour.