KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY KUMASI

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DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

SHELF LIFE STUDIES ON WHEAT-CASSAVA COMPOSITE BREAD STORED UNDER DIFFERENT CONDITIONS BY BREAD STAKEHOLDERS

A thesis submitted to the Department of Food Science and Technology of Kwame Nkrumah University of Science and Technology in partial fulfilment of the requirements for the award of the degree of Master of Philosophy (Food Science and Technology)

BY

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DECLARATION

I hereby declare that this submission is my own work towards the MPhil degree in Food Science and Technology and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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DEDICATION

To my wonderful family and all my friends, you are the reason why I didn’t give up!!

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ABSTRACT

The advocacy to substitute indigenous flour in 100% wheat bread is becoming more apparent as bread consumption keeps increasing. However the storage conditions to which bread is subjected could affect its shelf life and stability. The main objective of this study was to determine the shelf life of wheat and cassava-wheat composite bread at different storage conditions used by bread stakeholders (**retailers, consumers and bakers**) as obtained through a baseline survey. 10% substituted High Quality Cassava Flour (HQCF) bread (tea and sugar), 100% wheat bread (tea and sugar) were stored under room temperature conditions (25-31°C) and elevated temperature conditions under the sun (26-35°C) for 7 days. Data from chemical (moisture, water activity, free fatty acid), microbial (yeasts and mould, total plate count) and physical analyses (descriptive sensory evaluation using a 10 point ordinal scale) were obtained and three parameters (yeasts/mould, water activity and descriptive sensory evaluation) were used to predict the overall shelf life through simple and multiple regression modelling. Bread stored at room temperature had a longer shelf life; between 6 and 8 days whereas bread stored at elevated temperature conditions had a shelf life between 5 and 7 days. The results also showed that the sugar bread samples had a better storage life of between 5 and 8 days than the tea bread samples which had a shelf life of 5 and 7 days probably because the more sugar in sugar bread served as a preservative. Composite bread had a shelf life of between 5 and 7 days whilst the 100% wheat bread had a shelf life between 7 and 8 days.

KEY WORDS

HQCF composite bread, predictive shelf life model, regression analysis, storage conditions, descriptive sensory evaluation

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LIST OF ABBREVIATIONS

IITA- International Institute of Tropical Agriculture

HQCF- High Quality Cassava Flour

1FST- Institute of Food Science and Technology

KNUST- Kwame Nkrumah University of Science and Technology

IFAD- International Fund for Agricultural Development

CSIR-FRI- Council for Scientific and Industrial Research - Food Research Institute

SPSS- Statistical Package for the Social Sciences

LDPE- Low Density Polyethene

AOAC- Association of Analytical Chemists

PDA- Potato Dextrose Agar

TPC- Total Plate Count

NA- Nutrient Agar

FFA- Free fatty acid

TAGs- Triacylglycerides

CFU- Coliform Forming Units

CHAPTER 1

1.1 BACKGROUND

Bread is a staple food both in the developed and developing world (Noorfarahzilah *et al*., 2014). It is widely accepted and consumed by people of all ages and nationalities including Ghana. It is available in different forms and consumed in different ways (Khatkar, 2011). It can be taken alone as a snack or with hot beverages (tea, cocoa and coffee), cold nourishing drinks (chocolate drinks, milk shakes, etc.) and refreshing drinks (fruit drinks, fruit juices and carbonated drinks). Bread, may also be consumed with breakfast cereals such as corn and millet porridge, 'tom brown', etc.

The use of wheat flour in the Ghanaian diet has come to stay as there is no home where wheat product is not consumed (personal communication, Philomena Arthur, 24/8/2016, 12:30pm). It is consumed in the form of biscuits, breads, noodles, pizzas, ball float ('bofrot' in Ghanaian language), cookies & pastries, and other breakfast cereals. Ghana and Nigeria import over one million tons of American red winter wheat annually, making them one of the largest importers of American red winter wheat in the world ((Saranraj & Geetha, 2012; Ohimain, 2014 and Length, 2007). This situation has placed a huge financial burden on the Ghanaian economy, as the government often has to import this commodity in foreign currency. Another challenge that has been associated with the continuous use of wheat in bread making is the development of celiac disease (Ohimain, 2014). The author reiterated that an alternative source of flour that can be used to produce bread that is similar to or better than bread produced with wheat flour will be a welcome development.

Cassava (*Manihot esculenta Crantz*) is one of the most important crops in Africa, and Nigeria is the leading producer globally but in Ghana it is an important perennial crop with a per capita rate of consumption of 152.9 kg/yr (Addo *et al*., 2010). Cassava tubers can be kept in the ground for up to two years prior to harvesting, but once harvested; they begin to deteriorate because of the high moisture content of the fresh roots (Eleazu *et al*., 2014). Cassava is traded in its processed form due to its bulkiness and early deterioration. Aside using fresh cassava tubers, the commodity is often processed into intermediate products such as 'gari', 'konkonte' (fermented cassava flour) etc. and recently into High Quality Cassava Flour (HQCF), which is a major intermediate product.

In 2003, a presidential initiative was launched in Nigeria with the aim of adding HQCF (10% w/w) to the wheat flour used in bread. The purpose of this initiative was to restrict the outflow of funds for the importation of wheat (Noorfarahzilah *et al*., 2014) and to encourage research on cassava/wheat composite breads. In previously published works, different wheat flours were composited with various proportions of cassava starch and flour (Eduardo *et al*., 2013). Shittu (2007) and Beloved *et al*. (2012) reported that the 30% (w/w) inclusion of cassava flour into wheat flour could yield an acceptable fresh loaf of bread, depending on the source of the composite flour used. According to (Shittu, 2012), bread from various flour composites from roots and tubers such as cassava and sweet potato have also been shown to have higher yield and profit margins making it a viable product to improve livelihoods and boost national economies.The International Institute of Tropical Agriculture (IITA) recounted that 40% cassava flour bread that possessed comparable eating qualities to 100% wheat flour bread was produced (IITA, 2012).

Previous investigations into the incorporation of HQCF into wheat flour for bread making have concentrated on the acceptability of the composite bread to consumers (Eleazu *et al.*, 2014). Eleazu *et al*. in 2014 recounted that bread stakeholders gave numerous explanations for the slow adoption of HQCF in bread because of the life span of cassava bread owing to its high microbial load and moisture content. The authors correlated the microbial count of 100% wheat bread with substitution levels of 10%, 20%, 30% and 40% cassava composite bread and found high microbial counts in the cassava composite breads.

Kilcast & Subramaniam (2000) stated that with the exceptions of wine and cheese, the sensory characteristics of most foods tend to decline throughout storage. However, most consumers tolerate this change provided the foods remain safe. The Institute of Food Science and Technology (IFST) Guidelines (1993) states that, “shelf life is the period of time during which a food product will remain safe, be sure to retain desired physical, sensory, chemical and microbiological characteristics and also conform to any label declaration of nutritional data when stored under the recommended conditions. This definition takes into consideration the storage conditions on the shelf-life of products. Kilcast & Subramaniam stated in 2000 that the storage characteristics of most food products are usually measured in controlled environmental condition but this is seldom done in practice after the product has left the producer to the retail point.

According to IFST (1993) factors that affect the shelf-life of food products can be classified as extrinsic and intrinsic. Stored bread undergoes changes such as redistribution of moisture, starch retrogradation, loss of flavour and aroma and increase in product firmness (El-khoury, 1999). Microbial spoilage of bakery products is mostly characterized by the onset of staling and ropiness and these indicate the end of the shelf life (Bhise, 2013).

A lot of methods have been applied in the estimation of shelf life of food products. Regression models can be applied to products stored at given condition (Dalgaard *et al*., 1997). The authors recounted also that predictive models give the best shelf life estimation than other applied shelf estimation models due to its accuracy by taking into consideration the decrease or increase in the quality of a product with respect to time. This study seeks to apply predictive shelf life models in the estimation of the shelf life of composite bread stored under the different conditions by stakeholders in the bread value chain in Ghana.

1.2 PROBLEM STATEMENT

The fluctuating temperature and humidity conditions of the keeping environment to which bread is exposed in Ghana could affect the shelf stability of bread. Research has been conducted on how fluctuating temperature and humidity conditions can affect the shelf life of bread using laboratory settings. However there is a dearth of information about the shelf life of composite bread from wheat and HQCF stored under conditions by the various bread stakeholders such as retailers, bakers and consumers in Ghana. There is also a gap in the use of predictive models in the estimation of shelf life of composite bread from HQCF and wheat flour.

1.3 JUSTIFICATION

The promotion of cassava-wheat composite bread in Ghana will be improved. This is because bread stakeholders will have information on the best storage conditions to subject composite bread. The study would provide information on the shelf stability of wheat composite cassava bread which will help in informing the government on policies that centre on the inclusion of HQCF in bread. This will lead to the subsequent reduction of wheat flour importation into the country. This will also promote the cultivation and use of indigenous roots and tuber crops such as cassava which will create employment and generate income for farmers. The resultant estimated shelf life of wheat-HQCF composite bread which would be provided will also go a long way to control postharvest losses. Information on the best storage conditions for wheat-cassava composite bread types will also be outlined. It is expected that this study will also promote the use of predictive shelf life models in the estimation of shelf life of foods.

1.4 OBJECTIVE

To determine the shelf life of wheat-HQCF composite bread under different storage conditions.

1.5 SPECIFIC OBJECTIVES

1. To determine the preference and storage conditions of bread types among stakeholders.
2. To determine the changes in the moisture, water activity, sensory, free fatty acid content and microbial quality of wheat-HQCF composite bread under room (about 25^{o}C) and fluctuating temperature conditions over a six day period.
3. To determine the shelf life of wheat-HQCF bread types using a predictive model.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bread and types

Bread is not only consumed in all countries of the world but is also the most acceptable form of food. It is an important staple food in the world and its consumption is increasing. It is made from wheat flour which is imported to Africa and Ghana for that matter, making it relatively expensive. Due to its nutritional, sensorial and textural characteristics, ready to eat convenience as well as cost competitiveness it makes it an appealing food product (Beloved *et al*., 2012).

Different bread types exist in Ghana; it could be butter, sugar or salt based. These could all be made from either whole wheat or composite flours. Nonetheless, they all have wheat as the main flour base.

2.3 Composite bread

Composite breads are made from blends of wheat and non-wheat flours (Beloved *et al*. 2012). Several researchers have reported on different composite bread from various composite flours. The Table 1 below shows various composite flour that have been used for bread in the world.

Table 1: Composite bread developed from various flour composites in the world

Composite bread	Reference
Fababean, cotton seed, sesame, corn and barley- wheat composite bread	Annon, 2011
Cassava, plantain and soybean- wheat composite bread	Shittu, 2007
Pumpkin, canola seed flour- wheat composite bread	Ariffin *et al*., 2015
Soybean-wheat composite bread	Beloved *et al*., 2012
Full fat and defatted cocoa powder-wheat composite bread	Bhise, 2013
Peanuts, sunflower seed flour-wheat composite bread	Beloved *et al*., 2012
Beinseed-wheat composite bread	Ababa, 2000
Sweetpotato-wheat composite bread	Callejo *et al*., 2016
Coconut-wheat composite bread	Trinidad *et al*., 2006

All these ingredients impart sensorial attributes and nutritional value which may be favourable in bakery product recipes and other food products. The composite flours are an advantage to developing countries because the use of locally made flour could reduce wheat imports and increase the potential use of locally grown crops (Abdelghafor *et al*. 2011). The idea of substituting part of wheat with other starchy crops is not new. Several institutions have carried out research designed to find ways of partially substituting wheat flour with other sources of flour or replacing wheat altogether (Eriksson, 2013). With the constant increasing consumption of bread and other baked products in many countries, the composite flour programme promises to save significant amount of foreign exchange, provide a traditional nutritious food to more people at lower cost and to utilize indigenous crops to a greater extent (Eleazu *et al*., 2014). Bread is basically made from hard wheat flour, yeast, fat, sugar, salt and water. The consumption of bread in Ghana as a staple food has steadily been on the increase, especially with explosions in population and changing life style patterns (Beloved *et al.*, 2012).

2.3.1 Studies on the utilization of composite flour in Africa

According to Eleazu *et al*. (2014) three main market ventures exist for the cassava. The first and major business venture is the substitution of HQCF for wheat flour in baking. Another market opportunity is the use of cassava starch as a raw material for both food and non-food industries. Cassava can also be used to feed livestock in the form of chips and it can also be exported. Eriksson (2013) stated that if wheat flour is substituted with 15% cassava, Nigeria would be able to save US$14.8 million per annum. Cassava processors would be able to make an income of US$ 12.7 million whilst 4.2 million US dollars will also go to farmers. The findings from a survey conducted in Côte d'Ivoire and Nigeria showed that the bulk of bread eaten was made from composite flours of wheat and either maize, sorghum or cassava. Adeniji (2015) also stated that in order to expand the use of cassava in Tanzania, most bakery products such as cakes, biscuits, chin-chin and croquettes were made with cassava flour in place of wheat flour. Eriksson *et al*. (2014) stated that a lot of research is being conducted to study replacing wheat flour with other flours to help alleviate the problem of gluten allergy in certain populaces in the world.

2.3.2 Studies on composite bread in Ghana

A study was made by Eriksson (2013) on the effect of the replacement of wheat flour, at 0%, 15% and 30% with cassava starch, wheat starch and cassava flour, on the mixing requirements. The gas retention capability during fermentation and baking stages of the composite flour dough was also studied. The authors concluded that the degree of wheat flour replacement and type of replacement used greatly influenced the quality of composite bread produced. The 15% composite gave better bread qualities such as larger bread volume than the 30% composite bread. Beloved *et al*. (2012) also studied the use of different additives to increase the potential of cassava flour as a substitute for wheat flour in baking. Xanthan gum and whisked egg white with table margarine were used in little quantities as additives in wheat-cassava composite bread. These additives helped improve the composite bread quality by increasing the bread volume and improving the crumb characteristics. The additives used were xanthan gum, whisked egg white and table margarine. The selected African panellists accepted the taste of the bread that resulted from the study and the bread also had acceptable storability. Udofia *et al*. (2013) studied acceptability of 10%, 20% and 30% composite bread from cassava and wheat using sensory attributes such as aroma, colour, texture, purchasing preference and overall acceptability. However, there was no significant difference in the sensory attributes, the purchasing preference and overall acceptability of the 10% and 20% cassava-wheat composite bread and the 100% wheat bread. Another observation made by the authors suggested that, labelling which indicated the nutritional information of the per cent cassava flour did not affect the acceptability of the composite bread. This is because there was consistency in the scores of unlabelled and labelled samples. The study further proposed that a composite bread recipe which has 10-20% cassava flour could be a sustainable substitute for wheat bread which will have acceptable economic value and be used to improve food security. The authors recommended that there was a need to get a bread package with an in depth nutritional info for commercial composite bread from cassava and wheat.

2.4 The impact of other flours and additives on the dough properties of bread

Uzomah & Ibe (2011) in their research used chemically modified and fermented starches from various varieties of cassava to produce bread. Their major objective was to study the pasting and functional properties of these starches. They also studied the effect of these flours on baking. Their findings showed that the starches which were acetylated had outstanding functional properties and its resultant bread also had a high expansion volume than the resultant bread from cassava starch treated with lactic acid. Though various modifications of these starches presented varied effects on the sensory properties of resultant bread, those from acetylated and unfermented starches had high scores for overall acceptability. Jolaosho (2010) studied the impact of bromate on the specific volume of composite bread from maize and wheat; wheat and cassava; soy and wheat and wheat only. The author concluded that potassium bromate had a detrimental impact on the loaf volume of the cassava-wheat composite bread by impacting on the specific loaf volume. The specific volume of the composite bread was also inversely proportional to the bromate level. The author further suggested that the government should effectively implement the substitution of wheat flour with 10% cassava flour at source to help promote the commercial use of composite flour from cassava and wheat.

2.5 Shelf life of bread

In the domestic environment and distribution chain, thermal abuse is a common thing. Usually in the kitchen, room temperature conditions differ extensively. This makes controlling temperature in the domestic freezer and refrigerators very poor. Food manufacturers therefore have the mandate to understand the characteristics of products during storage and the fluctuating conditions that their products are likely to face in the supply chain (Kilcast & Subramaniam, 2012).

Health safety and ideal sensory properties are the main benchmarks used to assess the quality of bakery products. The resilience of bakery products with medium and high humidity content is usually narrowed by mould growth (Steele, 2004). Mode of product preparation, season and product type affects the growth of moulds in most products and these accounts for a percentage loss of between 1 and 5% in bakery products globally (Butt *et al*., 2004). According to Tarar & Ur-Rahman (2010) after 48 hours of producing bread, it begins to spoil resulting in substantial losses to the manufacturers. Mouldiness and ropiness are among the main kinds of spoilage in bread due to microbes. The formation of rope in bread is usually caused by mould species such as *Fusarium* sp., *Penicillium* sp., *Claslosporium* sp., *Rhizopus* sp., and *Aspergillus* sp. (Vlášek *et al*., 2013). On the other hand, *Bacillus subtilus* and *Bacillus licheniformis* are accountable for the formation of rope in spoilt bread. These microbes can be prevented if the hygiene practices in bakeries are improved and preservatives are used in bread (Kilcast & Subramaniam, 2012).

Vlášek *et al*., (2013) stated in their research that extending the shelf stability and shelf life of food has recently been highlighted. Unit operations of food processing such as sterilization and pasteurization have been employed to achieve this. On the contrary, this cannot be applied in bakery products. According to Fik (2004) bread is categorized as one of the foods which have a very short shelf life. Ahmad *et al.* (2016) stated that bread has two main structures which are the crust and crumb. The crust has a lower water activity and dry matter than the crumb thus it forms a protective primary barrier that prevents moisture loss and attack of microorganisms. Mir *et al*. (2014) also added that for products such as bread, boundary shifting of the crumb and crust of bread is a very vital parameter to look out for in determining shelf life. The authors added that ensuring proper sanitation of the working environment and the use of quality raw materials could go a long way to extend shelf life. The use of acceptable high temperature during processing and also new and quality technology such as the use of chemical additives and part baking could help avert spoilage of bread for an appreciable period of time. Water activity as stated by Zou *et al*. (2014) regulates the accessibility of water for the proliferation of microorganisms. A water activity value of 0.6 is known to be very critical. The water activity value of bread crust is 0.822 whereas that of the crumb is between 0.971 and 0.976 and above (Abellana *et al*., 2000). This water activity value is solely dependent on the bread type, the time, environmental conditions and age of the bread (Ahmad *et al*., 2016; Vlášek *et al*., 2013). Vlášek *et al*. (2013) stated that due to the composition and high water activity, bread is known to be an appropriate substrate that promotes the growth of microorganisms such as moulds. Khatkar (2011) reported that another possible factor affecting the shelf life of bread is humidity. During the orthodox baking process, time and temperature are directly proportional to the moisture content and crust colour of bread. A high humidity is known to affect the crust colour and final quality of bread by increasing its moisture content and volume. Vlášek *et al*. (2013) recounted that most of the studies done on the integration of humidity in baking emphasize mostly on the characteristics of the product subsequently after baking. There is a gap however in the study of the outcome of baking and storing bread under a particular temperature and humidity proceeding to a shelf life study. According to Punturug & Netiwaranon (2013) gluten in bakery products is affected significantly by moisture content. The authors added that one of the impure protein systems in bread is gluten. The rheology of gluten describes the protein as having connections between proteins even though the protein components have major effects. The gluten network is made up of two major sub-fractions which are glutenins and gliadins. The gliadins are responsible for impairing the viscosity of the dough whereas the glutenins strengthen the dough. According to Vlášek *et al*. (2013) there still has to be a lot of research in the rheology of gluten. After wheat flour is mixed with water, the gliadins act like a viscous liquid whilst the glutenins behave as its coherent having a flexible structure. The quality and amount of glutenins present in particular wheat flour affects its dough properties. The wheat flour used for baking bread has a high proportion of glutenins to gliadins.

2.5.1 Factors affecting shelf life of bread

The internal properties of a final product affecting its shelf life are termed as its intrinsic factors. These factors comprise of the redox potential (Eh), pH value, acid type, total acidity, existing oxygen, water activity, product biochemistry (chemical reactants and enzymes), preservatives (acids and their salts) used when formulating the product, the live microbes present and the natural micro flora. The formulation, structure, raw material form and quality of the final product usually affect these intrinsic factors (Kilcast & Subramaniam, 2012). On the other hand, the external environmental factors that the final product is likely to meet as it travels through the food chain are termed as the extrinsic factors. Among these factors are the following:

- How the consumer handles the final product.
- The microbes present in the environment during the product's storage, processing and distribution.
- How the product is exposed to ultraviolet and infrared rays during secondary processing, storage and distribution.
- The relative humidity of the environment when the product is being processed, stored and distributed.
- The amount of pressure present in the headspace and the profile of the time and temperature.
- The control of temperature when storing and distributing the final product.
- Consequent heat treatment given to the product before consumption.
- Atmosphere configuration present in the package.

These extrinsic factors stated above however function in a product by interacting with each other in random patterns. There should be research into the possible interactions between these extrinsic factors (Kilcast & Subramaniam, 2012; Steele, 2004). The 'hurdle effect' which is described as regulating the interaction between two or more extrinsic factors by reducing one and increasing the other and vice versa could be used to limit the growth of microorganisms. Examples include the use of antioxidants, treating the product with mild pressure and temperature, controlled atmosphere packaging, etc. (Vlášek *et al.*, 2013). Manufacturers usually apply the hurdle effect in the quest to extend a product's shelf life. They do so by keeping in mind how to retain the nutrients and the sensory qualities of the product (Kilcast & Subramaniam 2012). Shelf life is further limited if the extrinsic and intrinsic factors interact by stimulating various processes. The processes are categorized as physical, microbiological, temperature and chemically related (Steele, 2004). According to Kilcast & Subramaniam (2012) the proliferation of a particular microorganism when a product is stored rests on various factors. The vital factor is the microbial load of a product at the beginning of storage; the temperature, relative humidity and gas composition in the surrounding environment that the product is exposed to in the external environment during storage. Another important factor is the physicochemical characteristics of the food which include its pH, the method used in processing the food, presence of preservatives and moisture content.

2.5.2 Effect of water activity, pH and storage temperature on the shelf life of bread

The shelf life of most bakery products is often affected by moulds such as *Aspergillus* sp., *Fusarium* sp., *Claslosporium* sp., *Rhizopus* sp. and *Penicillium* sp. These moulds usually play key roles during the spoilage of foods that are high in moisture. There is always a competition between bacteria and moulds during spoilage. The relationship between bacteria and pH tend to be more fastidious as compared to that between moulds and pH. An acidic pH between 3.5 and 5.5 tends to favour the growth of moulds. This further implies that moulds are more susceptible to cause spoilage of foods with a pH less than 4.5 as compared with bacteria (Saranraj & Geetha, 2012). Bread has a pH range of 4.0-5.8 making is susceptible to yeasts and mould spoilage (Kilcast & Subramaniam, 2012). In their study, Abellana *et al*. (2000) developed a procedure to study the growth of xerophillic fungi on bakery products. They also determined the effect of temperature, water activity ($_{aw}$), isolates and its relationship with the growth of mycelia of *Eurotium* sp. Their findings showed that water activity, temperature and isolate type had an effect on the growth and interaction between the different isolates used at 99% confidence interval. It also showed in their results that at water activity of 0.90 and a temperature of 30 ^{o}C there was optimum growth than at a water activity of 0.75. Mould growth and germination of spores depends solely on optimum temperature. A temperature range of 18.3 and 29.4 ^{o}C is known to support growth of most moulds. Thus when bakery products are cooled to ambient temperature they become very susceptible to mould spoilage (Salim-ur-Rehman *et al*., 2007). According to Abellana *et al*. (2000) reducing storage temperature from 27 ^{o}C to 21 ^{o}C could considerably reduce mould growth and double the shelf life of cake. It may therefore, be necessary to give attention to the care of bakery products such as cakes during supply and storage. The authors used sponge cake equivalents as substrates to investigate the effect temperature and water activity had on the mycelial growth and interactions of *Penicillium corylophilum*, *Aspergillus flavus*, *Penicillium aurantiogriseum* and *Penicillium chrysogenum*. Growth rates depended on temperature and water activity as anticipated. Nonetheless, there were no significant differences between the growth rates of the various isolates used for the study. It was concluded from the study that a water activity of 0.90 and a temperature above 15.8 ^{o}C favoured the growth of *Aspergillus flavus* but *Penicillum* sp. needed a water activity range of 0.85 to 0.90 to grow. Another conclusion that was drawn based on the studies using sponge cake equivalent was that fungal growth on other bakery products apart from cakes could be prevented if water activity is maintained at 0.85. In their study in 2013, Vlášek *et al*. studied the resistance of five strains of fungi which were xerophytes against preserving agents and elevated temperature. The fungi strains they identified and isolated were *Wallema sebi*, *Eurotium rubrum*, *Eurotium amstelodomi*, *Eurotium herbariorum* and *Eutorotium chevalier*. The authors concluded that the resistance of the *Eurotium* sp. against preservatives and elevated temperature was stronger than the fungi strain *Wallemia sebi*. They further recommended the use of Sorbic acid as a preservative against fungi that are known to be xerophytes instead of Calcium propionate.

In another study of the effect of Carbon dioxide (CO_2) levels, pH and water activity on the growth of xerophillic fungi, Ogunsakin *et al*. (2015) used sponge cake equivalents to study common spoilage fungi in bakery products. The common fungi that cause spoilage in bakery products like cakes and bread such as *Aspergillus flavus*, *Penicillium carylophilum*, *Aspergillus niger* and *Eurotium* sp. were used for this research. The authors stated that the interaction between those fungi, water activity and CO_2 levels were among the major factors that affected the proliferation of fungi. Their findings also showed that water activity between 0.80 and 0.90 significantly influenced the growth of fungi. It also influenced the CO_2 concentration necessary to avert spoilage in the sponge cake analogue. There was a two-fold increase in the lag phase when there was a 70% increase in the CO_2 level in the headspace. However, a 100% CO_2 level and temperature of 25 ^{o}C seemed not to have affected the growth of fungi during the 28-day incubation period and regardless of the water activity level.

"Chalk mould", *Pichiaa butonii* is the most recurrent and bothersome yeast in bakery products. This kind of yeast can proliferate quickly on bread even before the mould growth. A concentration of between 0.125% and 0.3% of Sorbic acid has been proven to be effective in controlling the growth of moulds in bakery products (Ehavald, 2009). In contrast to spoilage of bread by moulds, yeast spoilage is characterized by contamination of bread after baking by practices such as poor hygiene handling of bread coolers, racks, conveyor belts, slicing machines, etc. (Saranraj & Geetha, 2012).

2.6 Shelf life modeling of foods

Salvador *et al*. (2010) stated in their research that the major idea of survival analysis was to focus the approximation of shelf life on a consumer rejecting a product than it deteriorating. The authors added that a consumer observing the product's deterioration should be linked to the product's possibility of spoilage. The authors also reiterated that log-normal distribution and Weibull distribution shelf life models could be applied in studying a product's life span. These models usually assume a parametric model status if the survival functions exclusively rests on the parameters of the model if the data is adjusted.

2.6.1 Types of models used in estimating shelf life

A lot of approaches have been applied in estimating shelf life of foods. Storage temperature which can be correlated with several measured responses such as sensory, microbial, biochemical and chemical factors to help calculate shelf life of foods. Regression models can be effectively applied to food products that are stored at a particular condition (Dalgaard *et al*., 1997). Tsironi & Taoukis (2014) in their research used a multivariate statistical method to correlate chromatographic and spectroscopic measurements of food products with sensory properties. Dalgaard *et al.* (1997) also stated that predictive models give a better estimation of shelf life of frozen foods such as fish than regression models hence Giannoglou *et al.* (2014) applied predictive models in the estimation of shelf life of frozen sea foods. The authors stressed the reliability of predictive models in shelf life prediction. In the research, they studied the deterioration of sea foods by measuring food spoilage indices such as sensory and chemical parameters. Ofosu *et al*., (2011) used polynomial regression models to estimate the shelf life of avocado fruit spread. The authors measured deteriorating factors such as the peroxide value, sensory properties and microbial quality of the avocado fruit spread and fitted the data using polynomial regression curves. The shelf life of the avocado fruit was estimated by applying Polhemus (2005) deduction which states that " the largest number of weeks for which the degrading parameter has reached 90% must be used in predicting the shelf life of a product". Tsironi & Taoukis (2014) applied predictive models in estimating the effect of processing and storage temperature on the shelf life of gilthead sea beam fillets that had been dehydrated osmotically.

2.7 Bread staling

Bread staling is also defined as any change usually physical, sensory or microbiological that occurs in bread during the post-baking period making it less acceptable to the consumer. Besbes *et al*. (2011) defined bread staling as a complex process which accounts for various changes in bread affecting its chemical properties such as the tearing properties, firmness and moisture distribution and causing physiochemical changes in the texture and even sensory properties. These reactions or changes can result in substantial reduction in bread tearing force and time during bread storage. Bread staling is the major cause of spoilage of bread. There has been a lot of research into this occurrence. Research has indicated that the migration of water from the crumb to the crust, the cross linking of starch and gluten and the retrogradation of starch accounts for the staling of bread (Fadda *et al*., 2014; Hojjati *et al*., 2013; Hug-Iten, 2000).

2.7.1 Methods used in the extension of bread shelf life

Various bread additives and scientific methods such as part baking, toasting, etc. have been used to delay the staling of bread (Majzoobi *et al*., 2011). Storing in a frozen state for between three to four weeks is one of such methods. It is usually used as a long term storage technique. Bosmans *et al*. (2013) stated in their research that freezing changes the water in bread into an inactive form thereby restricting the proliferation of microorganisms and also retarding the enzymatic decay of bread. Phimolsiripol (2009) also reported in his work that after frozen bread has been defrosted, it will not be able to recover all the qualities as it was baked fresh. According to Besbes *et al*. (2011) bread can only last for 48 hours after production if no shelf life extension methods are applied.

Part-baking is also a method used in the extension of bread shelf life. This method was made known in 1990 and it was referred to as interrupted or par-baked bread. In this method the bread is partly baked and the process is interjected just before the crust colour forms that is before the bread crust caramelizes and Millard reaction starts (Majzoobi *et al*., 2011; Fadda *et al*. 2014). Consumers can then store the part baked bread at either room temperature for a few days or store in the fridge as a long term storage method and finish baking when needed. A few challenges with this method are quick staling after full baking, decrease in the volume of bread and flaking of the crust of the bread (Ortolan *et al*., 2015; Ahmad *et al*., 2016; Majzoobi *et al*., 2011). However, part baking has been applied effectively in producing various kinds of loaves of bread and lately for cakes in India (Majzoobi *et al*., 2011; Hojjati *et al*., 2013; Renata *et al*., 2015; Ahmad *et al*., 2016). A lot of research has been done to investigate the suitable temperature and time for part baking (Anca *et al*., 2015; Ortolan *et al*., 2015), microbial quality (Majzoobi *et al*. 2011; Bhise, 2013) and properties and quality of part baked bread after full baking (Besbes *et al*., 2011). According to Majzoobi *et al*. (2011) part baking is a better alternative to the orthodox technique of freezing in restricting bread staling.

2.7.2 Additives used in the extension of shelf life of bread

According to Saranraj & Geetha (2012), the only way to prevent the spoilage of bakery products by fungi largely depends on the use of preservatives. They added that preservatives aid in the reduction and prevention of food wastage due to microbial spoilage. The extension of shelf life goes a long way to help the storage of various products at home. Tarar & Ur-Rahman (2010) recounted in their research that the proliferation of mould growth can be controlled by chemical preservatives. These preservatives act by physically damaging the cell membrane, discontinuing metabolism and also by the denaturation of the protein of cell membranes of spoilage microorganisms such as moulds. Sorbic and propionic acid and their salts were among the mentioned preservatives and these have been proven to extend the shelf stability and shelf life of most bakery products such as bread and pastries. The authors used 0.1% Calcium propionate and 0.2% of propionic acid as bread preservatives. Their findings showed that the formation of rope in bread was inhibited and also the growth of moulds was repressed for more than 2 days.

This current study seeks to determine the shelf life of composite and wheat bread stored under different conditions by the bread stakeholders by using predictive models by applying regression analysis.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 RESEARCH DESIGN

A survey was performed using semi-structured questionnaires. This was to find out the different storage conditions used by major bread stakeholders; retailers, bakers and consumers and to determine the perception of bakers on the inclusion of HQCF in bread making. Some respondents (illiterates) were assisted by the researcher through interpretations in order to enable them understand some of the questions.

A 2x3x2 factorial design was used for the laboratory shelf life study. In this design, 2 storage conditions (i.e. room and fluctuating temperature in the sun) were used. Three types of bread were used (a 100% wheat baker's control, 100% wheat bread from a standard recipe and 10% HQCF composite bread). Two of the types; 10% HQCF composite bread and 100% wheat bread, were developed from standard recipes from the Department of Food of Science and Technology, KNUST (see Appendix 1 for recipe development of standard recipe of bread) and the 3^{rd} was a baker's bread which is 100% wheat used as a control. Two bread kinds, tea and sugar bread (see Table 3.2) was used for this study.

3.1.1 Population for Survey

The target population for the study were bread stakeholders in Fante Newtown and Kwadaso in the Ashanti Region; Suhum, Nkawkaw and Koforidua in Eastern Region; Dakuman, Kaneshie and Tema in the Greater Accra Region of Ghana.The population comprised mainly of females (72.3%) and males (27.7%).

3.1.2 Sample and Sampling Technique for Survey

The sample size for the study was one hundred and fifty (150) respondents from the Eastern, Greater Accra and Ashanti Regions of which 50 respondents were interviewed respectively from each Region. The respondents interviewed comprised of 150 bread consumers, 43 bakers and 39 retailers (Table 3.1). The selection of the subjects for the study was done using the snow balling, convenience and purposive methods. The snow balling method was employed in the selection of the respondents who were bakers because it helped the researcher identify the other members of the sample. The researcher identified one baker in each of the communities (Table 3.1) and that baker led in identifying the other respondents in the community. Bakers who work with Council for Scientific and Industrial Research-Food Research Institute (CSIR-FRI) on pilot projects on composite bread were interviewed to get first-hand information on composite bread. Some consumers, retailers and bakers in Kwadaso were also interviewed for convenience. The purposive sampling technique was employed in the selection of the regions for the study. This is because Eastern Region is known to be the bread basket of Ghana, because it supplies bread to almost all the regions of Ghana and Greater Accra and Ashanti Regions are also known to be cosmopolitan areas. The researcher also purposely selected respondents from Fante Newtown because it is the bread basket of the Ashanti Region. Respondents from KNUST campus were also interviewed because on campus most workers and students consume bread almost every day.

Table 2: Study areas used for baseline survey

Greater Accra		**Ashanti**		**Eastern**	
Region	Number	Region	Number	Region	Number
Kaneshie	15	Kwadaso	14	Koforidua	25
Darkuman	29	Kwadaso SDA	12	Suhum	25
Tema	6	KNUST	14		
		Fante Newtown	10		
Total	**50**	**Total**	**50**	**Total**	**50**

3.1.3 Instrument for Data Collection

Semi-structured questionnaires and oral interviews (in certain cases) were used to elicit information on the demography of the study participants (age, years, marital status, level of education, sex, household size etc.), years in the bread business and the perception of consumers, bakers and retailers on the storage conditions and perceived shelf life of bread. The respondents were also interviewed on the impact of composite flour on bread quality and their perception of the inclusion of composite flour especially HQCF in bread making.

The data obtained were analyzed using SPSS version 20 on the 3 most produced bread types.

3.1.4 Validation of Questionnaires

The questionnaires were validated by two experts at the Food Science and Technology Department of the Kwame Nkrumah University of Science and Technology. The lecturers were given the purpose of the study, the research questions and instrument to check whether the items were relevant and capable of eliciting the right responses from the respondents. Some sentences were restructured for clarity.

3.2 SOURCE OF HIGH QUALITY CASSAVA FLOUR (HQCF) AND PRODUCTION OF COMPOSITE FLOURS

HQCF was purchased from a small scale business company, Josma Agro processing Limited at Mampong in the Ashanti region of Ghana. The cassava used was *esam bankye* variety. Other ingredients for baking were obtained from a reputable supermarket (Opoku Trading Limited) in Kumasi. The HQCF was sieved with a 60 mesh sieve and was mixed with wheat flour using the KitchAid dough maker at speed 3 for 1½ minutes in batches of 1 kg.

3.3 Shelf life studies on bread samples

3.3.1 Sample preparation

The bread used for the shelf life study was prepared according to a standard recipe developed by the Department of Food Science and Technology, KNUST. The ingredient compositions are presented in Table 3.2.

Sugar bread was prepared by weighing 4,125 g of composite flour (10% was made up of HQCF), 475.2 g of margarine, 19.8 g of salt, 707.85 g of sugar, 2 g of yeast, 9.9 g nutmeg as flavouring and 2,145 mL of water (see Table 3.2). A control was prepared by using 100% wheat flour with all other ingredients constant as used for the composite sugar bread.

Tea bread was also prepared by weighing 4,125 g of composite flour (10% was made up of HQCF), 475.2 g of margarine, 29.7 g of salt, 224.4 g of sugar, 2 g of yeast, 9.9 g nutmeg as flavouring and 2,145 mL of water. A control was prepared by using 100% wheat flour and the same quantity of ingredients as used for the composite tea bread.

Table 3.2: Standardized recipe for the bread types

Ingredient	Type of bread	
	Tea	Sugar
Composite flour (g)	4,125	4,125
Margarine (g)	475.2	475.2
Salt (g)	29.7	19.8
Sugar (g)	224.4	707.85
Yeast (g)	2	2
Nutmeg (g)	9.9	9.9
Water (mL)	2145	2,145

Source: Department of Food Science and Technology, KNUST.

Plate 1: Baking of bread samples in a local bakery

The baking process used for preparing samples for analysis was done by under studying the traditional methods used for baking bread by bakers interviewed during a survey conducted by the Department of Food Science and Technology, KNUST in 2015. The bread was prepared by kneading the dough by hand for about 25 minutes. The loaves were weighed with a spring balance and put in aluminium baking cans and allowed to proof at room temperature (27^{0}C) for 12 hours. The proofed loaves were baked at the same time in a traditional swish oven at about 200^{o}C for 20 minutes (Plate 1). Twenty loaves per each bread type (sugar and tea) was allowed to cool to room temperature before it was packed in Low Density Polyethene (LDPE) bags for further analysis. Twenty loaves each of sugar and tea bread were taken from a local bakery and used as a second control for the shelf life studies. Samples were coded according to the bread type, type of analysis to be run on it and storage condition (room temperature conditions (25-31ºC) and elevated temperature conditions in the sun (26-35ºC).

3.3.2 Storage of bread during shelf life study

The coded bread samples were stored in plastic baskets under room temperature (as done by bakers and consumers) (Plate 3.3) and elevated temperature conditions (as done by retailers) in the sun for 7 days (Plate 3.2). Samples stored at elevated temperature conditions were brought back into the room after sunset as normally done. Visual observations were made daily on the stored bread samples, to check for mould growth and samples were taken daily for analysis in the morning. Temperature and humidity readings for both room and elevated temperature conditions were taken thrice daily (morning, afternoon and evening) using a temperature and humidity gauge (Sinometer, model HTC-2, China).

Plate 2: Bread stored under elevated temperature conditions

Plate 3: Bread stored under room temperature conditions

3.3.3 Microbiological assessment of the shelf stability of bread samples produced

The microbiological analysis run on the bread samples during the shelf life study were Total plate count (TPC) and Fungi count (yeasts and mould) as described by American Public Health (2010). This was carried out on fresh bread samples and daily for both the total mesophilic (total viable bacterial counts) and fungi (yeasts and mould count). Samples taken were stored at -20^{o}C in a freezer prior to analysis. . The bread samples were mashed using a Kenwood dry sample blender. Ten grams of the mashed bread was mixed with Peptone water and sub samples were serially diluted decimally 4 times and 0.1 mL aliquots were spread plated on Potato Dextrose Agar (PDA) and Nutrient Agar (NA) for the enumeration of fungi and viable aerobic bacteria respectively. The PDA plates were inverted and incubated at room temperature (28 ± 2 ^{o}C) for 3-5days. The NA plates were also incubated at 37 ^{o}C for 24-48 hours. The colonies for both PDA and NA plates were counted and expressed as colony forming units per gram (cfu/g) of samples. All counts were done in triplicate using a colony counter (New Brunswick Quebec Darkfield, Model C110W, made in Edison, New Jersey, USA).

3.3.4 Quality evaluation of bread

Moisture content of the bread samples was determined as described by AOAC (1995).

Free fatty acid content was also determined as described by AOAC (1995).

Water activity was determined using Aqualab (made in Virginia, USA) water activity meter. An amount of 2g of bread was mashed and filled into the sample cup and put into the water activity meter for reading. The readings were taken in duplicate for each bread type (Quevauviller and Maier, 1999).

3.3.5 Sensory evaluation

3.3.5.1 Screening and training of sensory panel

Twenty-five sensory panellists were selected and screened using a Triangle test. Fifteen subjects made up of students and Teaching assistants in the Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology (KNUST) were selected for the Quantitative Descriptive Analysis (QDA). The subjects generated and agreed on the descriptors to be used for the test. These assessors were then trained an hour each day for an eight-day period using model products such as stale bread, fresh bread, mouldy bread and hard foam to classify the selected parameters for this study: crumb colour, springiness, smell and crumb texture as defined in Table 3.3.

Table 4: Description of sensory attributes used in QDA

Sensory attribute	**Descriptor definitions for bread samples adopted**	**Reference**
Crumb texture	This is described as the feel of the crumb between the fingers	Not soft (hard foam (0) to very soft (soft foam) (9)
Springiness	Force with which the sample returns to its original size/shape, after partial compression. From (very springy to not springy)	(Very springy) soft foam (0) to (not springy) dry bread (9)
Crumb colour	The creamish white colour of the bread crumb as perceived by the human eye	No off colour (creamish white) (0) to any off colour of either green, ash, orange or any other colour apart from the creamish white colour of bread crumb (9)
Crust colour	The brown colour of the bread crust as perceived by the human eye	No off colour (brown) (0) to any off colour of either green, ash, orange or any other colour apart from the brown colour of bread crumb (9)
Bread aroma or smell	The aroma associated with smell of freshly baked bread	Very strong (fresh) (0) fresh bread aroma to very low (stale) (9) decrease in the aroma of fresh bread

3.3.5.1 Sensory evaluation

The sensory evaluation was conducted by 12 of the trained panellists using a ten-point number scale from 0-9. A general factorial design with two factors: products (random scale) and panellists (coded from P1-P12) were used to plan the serving order for the sampling of the 12 different products. Each serving order was made up of four samples. Panellists were given 5 minutes of break between the tests.

3.3.6 Statistical methods

Microsoft Excel 2010 was used to calculate the averages and standard deviation of all data collected (Water activity, moisture, sensory evaluation, free fatty acid content, yeasts and mould count and total plate count). These averages were then fitted into models that could describe trend of the data in predicting the shelf life of the stored bread. Coefficients of the models such as the regression –(R^2), prediction regression –(pred. R^2), adequate precision –(adeq. precision) and adjusted regression –(adj. R^2) were studied.

Analysis of Variance (ANOVA) studies of the fitted models was performed and further diagnostic plots were obtained by analysing the normality of the studentized residuals using normal probability plots. The studentized residuals were studied against the predicted values to check for constant errors. Outliers and influential points in the studentized residuals were also determined externally. Model graphs were then plotted after the entire model statistics calculated were deemed fit.

The statistical tool (Stat Point, 2008) was used to plot the simple regression using each parameter meter (Y-axis) and the number of days (X-axis) of the data collected. The model with the largest R-squared (at 95% confidence interval) which was inferred as a percentage of the various models suggested by the statistical tool was chosen to fit the data of each parameter determined to predict the shelf life of each sample. In addition, based on Polhemus (2005) deduction which states that in every shelf life prediction method, the largest number of days (or weeks) for which a degrading parameter has gotten to 90% must be interpreted into a product's shelf life, the shelf life of each bread type per each measured parameter was calculated.

A multiple regression model using a chemical parameter (water activity), physical parameter (descriptive sensory evaluation) and microbial parameter (yeasts and mould count) was plotted against the number of days for each bread type. The limits from the simple regression models of the selected measured parameters were then substituted into the multiple regression model equation to calculate for the overall shelf life of each bread type. The shelf life of the bread was then calculated in days, hours, minutes and seconds.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Socio-economic characteristics of respondents

4.1.1 Total number of respondents interviewed

Table 4.1 shows the socio-economic characteristics of respondents interviewed. A total of 150 respondents were interviewed from the Eastern, Ashanti and Greater Accra Regions. Majority of the respondents were females. The sample size interviewed had a lot of females in the bread business than males.

Majority of the retailers interviewed were single. The results from Table 4.1 showed that females consumed more bread than males. The results from the sample size also showed that more females were in the bread business than males. Most males in Africa are not used to learning trades relating to cooking because of tradition (Majzoobi *et al*., 2011).This further implied that the baking profession is mostly done by females than males. According to a recent survey conducted by the Department of Food Science and Technology, KNUST in 2015 in the Ashanti Region, the elite are also not into bread baking due to the stress involved in the trade. According to this sample size most of the respondents have not studied bread baking as a Science because it is mostly passed on.

Most of the consumers interviewed from the three regions were less than 25 years of age. (Table 4.1). This implied from the results that the youth between the ages of 25 and 45 years were regular bread consumers. Bread is usually consumed as a snack or as part of breakfast (Correia *et al*., 2015). Most of the respondents above 56 years complained of constipation anytime they consumed bread, thus the percentage of 9.3 of bread consumers aged above 56 years. This result is also similar to the findings of Adepoju and Oyewole (2013) during a survey in Oshodi, a suburb in Lagos, Nigeria that most bread consumers were found to be people less than 59 years old.

The results from Table 4.1 showed that majority of the retailers were less than 25 years of age.

Majority of the bakers according to this sample size were above 56 years (Table 4.1). The results showed that each of the respondents had formal education in one form or the other. Majority of the respondents had at least basic education. This implied that most of the respondents interviewed lived in the urban areas. The study areas chosen for this research were mostly found in cosmopolitan areas (Table 3.2) were most people are expected to have had at least basic education. These results explained why most respondents had some knowledge of bread baking. Some of the answers they gave were the mode of bread preparation, the ingredients used in bread making and some of the health implications associated with consuming a lot of bread confirmed these findings from the survey.

The mean household size of the consumers was 6.5 having a standard deviation of 3.1, whereas the retailers interviewed had a mean household size of 6.4 with a standard deviation of 2.8. The bakers had a mean household size of 7.9 with a standard deviation of 3.2. The results from Table 4.1 suggested that most of the respondents had a family size between 7 and 8 members. This showed that their nuclear family size was quite large and very characteristic of the African family system where some members from the extended family usually lived together with the nuclear family. This could affect the rate at which bread was consumed since more bread could be consumed within a day or at sitting during meal times. Nuclear families with small number of members could have left over bread whilst families with a large size would hardly have left over bread due to the consumption rate.

The mean years in the bread business for retailers interviewed was 8.1 with a standard deviation of 7.2 whereas that of the bakers was 18.7 with a standard deviation of 11.4 (Table 4.1).

The mean number of employees for the retailers was 2.1 with a standard deviation of 1.2 whereas the bakers had a mean number of employees of 11.7 with a standard deviation of 10.7. From Table 4.1, most bread retailers were between 25 and 45 years. Only a few bread retailers were above 56 years. This could also be due to the reason that most of the youth were involved in a bread business that had been handed over from older family members. It could also be due to the reason that the bread business was their main occupation. On the contrary, most bakers were above 45 years. Most of these bakers had also been in the bread business for more than 10 years. This implied that most of the youth were not actually interested in baking. Some of the dangers such as the risk of getting burnt during the baking process and the risk of losing a whole batch of bread if the correct quantity of some ingredients such as yeast and salt were not used were some of the concerns mentioned. Most of the bakers interviewed used traditional baking methods which needed a lot of expertise and experience. The number of years in business for bakers was above 18 years which implied that most of the bakers were experienced. The average number of employees that the bakers had was also above 11 implying that most of them had large bakeries and produced bread in large quantities.

Table 5: Socio-economic characteristics of respondents

Variable	Consumers		Retailers		Bakers	
	Freq	**Percent**	**Freq**	**Percent**	**Freq**	**Percent**
Gender						
Male	41	27.3	9	23.1	7	16.3
Female	109	72.7	30	76.9	36	83.7
Marital status						
Single	80	53.3	21	53.8	15	34.9
Married	56	37.3	14	35.9	21	48.8
Separated/Divorced	7	4.7	2	5.1	2	4.7
Widowed	7	4.7	2	5.1	5	11.6
Age						
Less than 25 years	47	31.3	15	38.5	4	9.3
26-35 years	38	25.3	6	15.4	10	23.3
36-45 years	33	22	11	28.2	9	20.9
46-56 years	18	12	6	15.4	9	20.9
More than 56 years	14	9.3	1	2.6	11	25.6
Educational level						
None	8	5.3	4	10.3	2	4.7
Basic	78	52	26	66.7	25	58.1
Secondary	28	18.7	6	15.4	8	18.6
Tertiary	21	14	1	2.6	7	16.3
Postgraduate	15	10	2	5.1	1	2.3
Mean household size	**6.5 (3.1)***		**6.4 (2.8)**		**7.9 (3.2)**	
Years in business	**-**		**8.1 (7.2)**		**18.7 (11.4)**	
Number of employees	**-**		**2.1 (1.2)**		**11.7 (10.7)**	
Total	150	100	39	100	43	100

Plate 4: A picture showing how bread is stored by retailers in recent times

4.1.2 Bread storage conditions and perceived shelf life of bread by retailers in the three regions

The results from Table 4.2 show the shelf life and storage practices of bread retailers in the Eastern, Greater Accra and Ashanti Regions. Retailers in the Greater Accra and Eastern Regions were noted for storing left over bread in the fridge. The results from Table 4.2 show that the common practice done by retailers was to store bread in boxes. Table 4.2, showed that in the Greater Accra Region the most common storage condition used for left over bread was the use of polythene. This practice was very common amongst bread retailers who sold bread on the roadside. Retailers who sold bread in shops used shelves and tables as storage places. The storage of leftover bread in the fridge was not common among retailers in the Greater Accra and Ashanti Regions but was prevalent among retailers in the Eastern Region. Some of the reasons the retailers gave for keeping leftover bread in the fridge were that they wanted to keep the bread fresh and delay bread staling. According to Belitz and Grosch (2009) temperatures below 14°C also inhibits staling by decreasing the rate of amylopectin retrogradation by a factor of 4 and improving the crumb freshness. Retailers in the Eastern and Greater Accra Regions were known to sell bread on a large scale and probably have opted to go for a storage method that will help them better. Retailers in the Ashanti Region mostly stored leftover bread in wooden boxes and baskets. Some of the retailers stated that they requested for bread which could be sold within a day or two and returned the leftover to the bakers for reheating. Reheating bread is known to restore the elasticity of the crumb by the reformation of the three dimensional network of the amylose-lipid complexes (Coultate, 2003). However this process is not effective because the rate of bread staling is increased as water quickly leaves the crumb resulting in the formation of crystalline structures in the amylopectin network according to Kilcast & Subramaniam (2012).

Table 4.2 also shows the perceived shelf life of bread according to retailers. Fourteen retailers representing 35.9% responded that the shelf life of bread was more than 4 days, 5 retailers (12.8%) of the pooled results said that bread lasted for 2 days and only 8 retailers across the three regions reiterated that the shelf life of bread was just four days. The table also shows the corresponding shelf life of bread as perceived by retailers in the three regions. The perceived shelf life of bread by retailers in the Greater Accra and Ashanti Regions was three days whilst that for retailers in the Eastern Region was more than four days. This was expected because according a recent survey conducted in 2015 in the Eastern Region by the Department of Food Science and Technology, KNUST, the use of bread additives is more common than in the Ashanti and Greater Accra Regions. The storage of leftover bread in the fridge by retailers in the Eastern Region could also have contributed to extension of the shelf life of bread. The common practice of storing leftover bread in polyethene by retailers in the Ashanti Region could also have accounted to the short perceived shelf life of bread. This is because there is generation of heat in the bread within the polyethene which causes the formation of droplets of moisture on the surface of bread. This moisture encourages the growth and proliferation of microorganisms which cause mouldiness in bread.

Table 6: Shelf-life and storage practices of bread by retailers in the Eastern, Greater Accra and Ashanti Regions

Variable	Greater Accra Freq	Percent	Ashanti Freq	Percent	Eastern Freq	Percent	Pooled Freq	Percent
Place of storage								
Polyethene	5	50	2	13.3	1	7.1	8	20.5
Shelves	3	30	5	33.3	2	14.3	10	25.6
Boxes	1	10	6	40	6	42.9	13	33.3
Fridge	1	10	-		5	35.7	6	15.4
Other	-		2	13.3	-		2	5.1
Perceived shelf-life of bread								
2 days	1	10	3	20	1	7.1	5	12.8
3 days	4	40	6	40	2	14.3	12	30.8
4 days	2	20	4	26.7	2	14.2	8	20.5
More than 4 days	3	30	2	13.3	9	64.3	14	35.9
Total	10	100	15	100	14	100	39	100

Table 6 shows the composite bread often requested and the perceived shelf life features of composite bread as perceived by retailers in the 3 regions. The table shows that composite bread from cassava, corn, sweet potato, wheat bran, coconut, chocolate and honey were often requested by retailers in the three regions. Wheat bran bread was the most requested having a frequency of 14 and corresponding percentage of 42%. It was closely followed by cassava bread with a percentage of 18 and frequency of 6. The least common composite bread requested was honey bread with a percentage and frequency of 3% and 1 respectively. The results from Table 6 show that the most common composite bread requested by retailers was wheat bran bread. Some of the reasons the retailers gave for requesting for this type of bread was that most consumers were becoming health conscious and they preferred bread which had a lot of fiber. The request of cassava bread was common among retailers in the Eastern Region. The least common composite bread requested by retailers in the three regions was honey bread. Retailers complained about the short shelf life and stability of honey bread. Bread retailers in the Greater Accra and Ashanti Regions stated however that chocolate bread was the most shelf stable composite due to the high amount of sugar it contained and the presence of cocoa powder. They stated that most consumers did not prefer this type of composite bread so they requested for it on a small scale. Sweet potato bread was only requested by retailers in Tema, which is in the Greater Accra Region. The retailers stated that it was newly introduced composite bread by CSIR-FRI and they were yet to promote its consumption. They stated that only a few consumers who knew its health benefits patronized it hence its limited request from their suppliers.

The Table 6 also shows the perceived shelf life of composite bread by retailers. Fifteen retailers (45%) interviewed in the three regions stated that the shelf life of composite bread was 3 days. The least number of retailers who chose the 4 days options were only 3 (9%). The results from the table showed that the perceived shelf life of composite bread was three days. This short shelf life of the bread could be due to the presence of other flour in the bread. Another reason could also be that retailers requested for composite bread in small quantities for fear of running at a loss due to the perceived high rate of moldiness in composite bread than the other wheat bread types such as tea, sugar and butter bread. The retailers recounted various experiences of discarding mouldy composite bread which were not purchased after four days and this could account for these results.

Table 7: Composite bread often requested by consumers and shelf-life features of composite bread as perceived by retailers in the Eastern, Greater Accra and Ashanti Regions

Composite bread type	Frequency	Percent
Cassava	6	18
Corn	3	9
Sweet potato	2	6
Wheat bran	14	42
Coconut	2	6
Chocolate	5	15
Honey	1	3
Shelf-life of composite bread		
2 days	8	24
3 days	15	45
4 days	3	9
More than 4 days	7	21
Total	33	100

4.1.3 Bread storage conditions and perceived shelf life by bakers interviewed from the three regions

Table 8: Storage days and conditions for left-over bread for bakers in the Eastern, Ashanti and Greater Accra Regions of Ghana

Variable	Greater Accra		Ashanti		Eastern		Pooled	
	Freq	Percent	Freq	Percent	Freq	Percent	Freq	Percent
Storage days for left-over bread								
1 day	10	55.6	2	16.7	-		12	27.9
2 days	5	27.8	8	66.7	8	61.5	21	48.8
3 days	3	16.7	2	16.7	5	38.5	10	23.3
Storage conditions for bread								
Room temperature	11	61.1	3	25	7	53.8	21	48.9
Fridge	1	5.6	-		-		1	2.3
Polythene	6	33.3	9	75	6	46.2	21	48.8

Table 8 shows the storage days and conditions for left over bread for bakers in the Eastern, Ashanti and Greater Accra Regions of Ghana. Twelve bakers across the three regions stated that they only stored bread for a day, 21 answered 2 days and 10 bakers said they stored left over bread for 3 days representing 27.9%, 48.8% and 23.3% respectively. The results from Table 8 show that most bakers in the Greater Accra Region only stored leftover bread for a day. Some of the reasons the bakers gave was that they only baked bread upon the request from their customers. They kept proper records of the request pattern of customers so they hardly had leftover bread. However bakers in the Eastern and Ashanti Regions could still have leftover bread after two days of baking. The bakers in the Eastern Region were not worried about keeping leftover bread for a long time at their bakeries due to bread additives they added to the bread to keep it fresh.. Generally the bakers interviewed stated that they made sure to sell leftover bread first before selling the freshly baked bread that is why they hardly had bread staying for more than three days at their bakeries. They stated however that they intentionally kept leftover bread to observe its shelf life and stability after having complaints from retailers so that they could improve upon the quality of the bread. This practice was seldom done. The bakers also stated that some consumers also preferred bread that was about two to three days old so they sometimes kept leftover bread just to meet customer demand.

The most common storage condition used by bakers across the three regions was room temperature on shelves having a percentage of 48.9%. It was followed closely by storage in polythene with a percentage of 48.8. Only 2.3% stored leftover bread in the fridge which was only predominant in the Greater Accra Region. The common storage condition of bread by bakers was on shelves at room temperature as shown in Table 4.4 and Plate 4. The bakers stated during the survey that they allowed bread to cool at room temperature before packaging it in plain polyethene bags. They then arranged the bread neatly on shelves in their store rooms used for storing baking equipment and baking ingredients (Plate 4). They reiterated that they stored leftover bread in this location because that was the only available space and also to prevent mice from destroying it. Only a few bakers kept leftover bread in the fridge. This was seen in bakers with large established bakeries and bakers who had their bakeries situated in their place of abode. This practice was common amongst bakers in the Eastern and Greater Accra Regions.

Table 9: Observed shelf life and bread type that stores better as perceived by bakers in the three regions

Variable	**Greater Accra**		**Ashanti**		**Eastern**		**Pooled**	
	Freq	**Percent**	**Freq**	**Percent**	**Freq**	**Percent**	**Freq**	**Percent**
Bread that stores better								
Butter	-		1	8.3	-		1	2.3
Sugar	18	100	11	91.7	12	92.3	41	95.3
Tea	-		-		1	7.7	1	2.3
Shelf life of bread								
2 days	1	5.6	-		-		1	2.3
3 days	1	5.6	2	16.7	-		3	7
4 days	3	16.7	5	41.7	4	30.8	12	27.9
More than 4 days	13	72.2	5	41.7	9	69.2	27	62.8
Total	18	100	12	100	13	100	43	100

Plate 5: A picture showing how left over bread is stored by bakers

Most of the respondents stated that the bread that stores better is sugar bread. This is because according to the respondents sugar bread has more sugar than all the other bread types. This implies that most of the bakers have basic knowledge of sugar retarding the growth of microorganisms. This could also be attributed to the educational level and the years of baking experience these bakers had (Table 7).

Majority of the bakers interviewed from the three regions reiterated that the shelf life of bread was more than four days (Table 9). However, only 1 (5.6%) baker from the Greater Accra Region stated that bread only lasted for 1 day (Table 9). The perceived shelf life given by the bakers interviewed was longer as compared to the duration given by retailers interviewed from the three regions. This could be due to the storage conditions to which bread was subjected to by these retailers as shown also in Plate 4. The results from this survey were used as a baseline in designing the shelf life study.

Table 10: Shelf life of composite bread as perceived by bakers in the Greater Accra, Eastern and Ashanti Regions

Variable	**Greater Accra**		**Ashanti**		**Eastern**		**Pooled**	
	Freq	**Percent**	**Freq**	**Percent**	**Freq**	**Percent**	**Freq**	**Percent**
Shelf-life of composite bread baked (for only those who use composite flour)								
2 days	1	5.9	1	9.1	1	10	3	7.9
3 days	5	29.4	4	36.4	6	60	15	39.5
4 days	-		3	27.3	-		3	7.9
More than 4 days	11	64.7	3	27.3	3	30	17	44.7

A greater percentage of the bakers interviewed from the three regions stated that the shelf life of composite bread was 4 days (Table 10). However the findings from Table 4.6 showed that the perceived shelf life of composite bread according to bakers from the Greater Accra Region was more than four days. This could be due to the proper storage conditions used by bakers in that region. Composite bread was very common in the Greater Accra Region due to its cosmopolitan nature. Bakers from this region usually collaborated with CSIR-FRI during their pilot studies so they might have received training on bread storage conditions that will extend its shelf life. Another reason could also be due to the use of bread additives which could extend the shelf life of the bread. However, most bakers in the Ashanti and Eastern Regions stated that the perceived shelf life of composite bread was just three days. This could be due to the small scale of production of composite bread in these two regions. Bakers in Tema in the Greater Accra Region also stated during the interview that sometimes they pre-treated leftover composite bread using processes such as toasting. They reiterated that the toasted composite bread could last on the shelf for about two weeks without going bad. Toasted bread could help reduce the water activity and moisture needed for growth of microorganisms such as moulds and yeasts (Latif *et al*., 2005). They stated further that toasted sweet potato composite bread could last for more than two weeks before going mouldy.

4.1.4 Bread storage conditions and perceived shelf life as stated by consumers from the Eastern, Greater Accra and Ashanti regions of Ghana

Table 11: Storage and shelf-life issues of bread for consumers in the Eastern, Ashanti and Greater Accra Regions

Variable	Greater Accra		Ashanti		Eastern		Pooled	
	Freq	Percent	Freq	Percent	Freq	Percent	Freq	Percent
Condition of point of bread purchase								
Under a shed	22	44	22	44	26	52	70	46.7
In a shop	18	36	15	30	16	32	49	32.7
In the sun	9	18	10	20	4	8	23	15.3
In a van	1	2	3	6	-		4	2.7
Table top	-		-		4	8	4	2.7
Usual storage place of storage of bread								
At room temperature	30	60	30	60	34	68	94	62.7
Refrigerator	18	36	13	26	13	26	44	29.3
Do no store	2	6	7	14	3	6	11	8
Length of bread storage before final consumption								
One day	8	16.7	8	17.4	16	32	32	22.2
Two days	13	27.1	15	32.6	4	8	32	22.2
Three days	9	18.8	14	30.4	6	12	29	20.1
Four days	9	18.8	7	15.2	16	32	32	22.2
Five days	4	8.3	1	9.1	6	12	11	7.6
More than 5 days	5	10.4	1	9.1	2	4	8	5.6
Observed shelf-life of bread								
2 days	2	4.2	3	6.2	8	16	13	8.9
3 days	15	31.2	13	27.1	6	12	34	23.3
4 days	7	14.6	18	37.5	4	8	29	19.9
Five days	10	20.8	10	20.8	7	14	27	18.5
More than 5 days	14	29.2	4	8.3	25	50	43	29.5
Total	50	100	50	100	50	100	150	100

The findings from Table 11 showed that most consumers from the three regions bought bread displayed under a shed whilst 49% of the consumers bought bread from retail shops. The practice of buying bread from vans was only common in the Greater Accra and Ashanti Region. This practice was the best on condition that these vans had shelves on which the bread could be arranged neatly. Consumers stated during the survey that the reason why they did not prefer to purchase bread from vans was because some of these vans did not have compartments or shelves thus the bread was just spread on polythene lining in the boot of the vans. Consumers who had tertiary education gave various hygiene complaints on the above reason so they preferred to buy from retail shops such as malls, supermarkets or even at source from bakeries even though sometimes they had to pay a high cost for the bread. The display of bread on table top was only prevalent in the Eastern Region. Bread was mostly displayed on table top to attract travellers plying the Eastern corridor road linking the Greater Accra and Ashanti Region of Ghana.

The usual storage place of bread by consumers according to Table 4.7 is at room temperature, represented by 94 consumers (62.7%). Some reiterated that they only bought what they could consume within a short period of time and they did not have microwaves or toasters to heat leftover bread if they kept it in the refrigerator. This was prevalent among consumers who had just secondary, basic or no formal education. Consumers with large household sizes also added that due to their family sizes, a whole loaf of bread was consumed within a short period of time such as within a day or two. Forty-four consumers out of the 150 interviewed stated that they stored their leftover bread in the refrigerator. Some of the reasons why for this practice could be that they had small family sizes so they could not consume a whole loaf of bread within a day or two. Another reason given by the consumers was that they did not like bread so much so after consuming a little slice, they just kept the rest in the refrigerator. Some of the consumers also stated that they kept bread in fridge to preserve it and also to prevent insects such as ants and rodents such as mice from destroying it. Only eleven consumers representing 8% said they did not store bread. Those consumers reiterated that they preferred to consume only fresh bread so they only bought a slice of fresh bread that they could consume within a day from retail shops. Some of the consumers also stated emphatically that they only bought bread which they use as an accompaniment for carbonated drinks, breakfast cereals and beverages.

Majority of the consumers interviewed stored bread for a day. Most of the consumers stored bread from between one day to five days. The reasons that they gave were that, it was difficult to consume stale bread so they tried to consume bread within a day or two. Those who said they consumed bread within a week said they kept leftover bread in the fridge and reheated it using either a microwave oven or toaster each time they wanted to consume bread. This practice was seen to be very common amongst consumers with very small family sizes. Some of the consumers added that they did not really like bread due to constipation issues so anytime they purchased a loaf of bread they either kept it on a table top at room temperature or left it in the refrigerator and sometimes even forgot about it. One surprising reason a consumer gave was that she intentionally kept wheat bran bread frozen and consumed it very early in the morning to enable her have free bowels.

The observed shelf life of bread for consumers across all the three regions was more than 5 days which accounted for 43 consumers representing 29.5%. Only 13 consumers out of the 150 respondents interviewed said that bread lasted for only 2 days. Those who stated that bread lasted for only 2 days probably said this because bread they purchased from retailers had already been there for two or three days under harsh conditions of the weather. It can therefore be inferred that such bread would not last long on the shelf in their homes. The consumers who stated that bread lasted for more than 5 days probably chose that option because they purchased fresh bread at source from bakeries and kept it under refrigerator conditions. Cold temperature is known to inhibit the proliferation of microorganisms such as mould and yeasts that cause bread spoilage according to (Fadda *et al*., 2014). Another reason could be that they bread they purchased had additives that could help extend the shelf life as cited by Besbes *et al*. (2011) in their research on impact of the conditions of baking on bread staling.

Table 12: Shelf life of composite bread as perceived by consumers in the Eastern, Ashanti and Greater Accra Regions

Variable	Greater Accra		Ashanti		Eastern		Pooled	
	Freq	**Percent**	**Freq**	**Percent**	**Freq**	**Percent**	**Freq**	**Percent**
Observed shelf-life of composite bread (for those who have ever consumed composite bread)								
2 days	-		-		2	4.3	2	1.6
3 days	4	8	5	14.3	21	45.7	29	22.8
4 days	22	44	17	48.6	7	15.2	45	35.4
Five days	14	28	7	20	6	13	26	20.5
More than 5 days	10	20	6	17.1	10	21.8	25	19.8

A greater percentage (35.4%) of the consumers interviewed across the three regions stated that the shelf life of composite bread was 4 days (Table 12). Some of the reasons for above findings could be the presence and quality of other flours other than wheat flour used in baking these bread as reiterated by Eleazu *et al*. (2014). Most of these composite flours such as HQCF-wheat contained a lot of moisture and had high water absorbing capabilities that could affect the shelf of bread by encouraging the growth and multiplication of spoilage microorganisms such as yeasts and mould at stated by Eduardo *et al*. (2013). Consumers who stated that the shelf life of composite bread was more than 5 days said so because composite bread such as chocolate bread could stay on the shelf for more than 5 days. This is because that bread type had high sugar content and cocoa powder which cause it to last long on the shelf. Another reason could be that they kept composite bread under refrigeration conditions after purchase.

4.1.5 Correlation of bread storage conditions used by bread stakeholders and the shelf life of bread

Table 13: Correlation of bread storage conditions by bread stakeholders in the three regions and shelf life of bread

Parameter	Bakers	Retailers	Consumers
Pearson's Chi square	15.123 (0.235)	11.774 (0.464)	20.308 (0.207)
Likelihood ratio	14.949 (0.244)	14.944 (0.244)	25.083 (0.068)
N	39	39	37

Figures in bracket show the significant difference at 95% confidence interval level. N represents the number of observations.

The bread storage conditions used by the bread stakeholders (bakers, retailers and consumers) were correlated using Pearson's Chi square. There was no significant difference between the storage conditions used by retailers, bakers and consumers at 95% confidence interval. The Pearson's Chi square value for bakers, retailers and consumers was 15.123, 11.774 and 20.308 respectively. The Likelihood ratio for bakers, retailers and consumers was 14.949, 14.944 and 25.083 respectively. There was no significant difference between the Likelihood ratio of bakers and retailers but for consumers there was a significant difference at 95% confidence interval. There was a weak positive correlation between the storage conditions of bread stakeholders in the three regions and the shelf life of bread according to Table 13. This showed that each of the bread stakeholders stored bread in a different way and this affected the shelf life of bread differently. The reason why consumers had a high Pearson's Chi Square value than retailers and bakers was because their way of the bread storage really affected the shelf life of bread as compared to the other two groups of bread stakeholders (bakers and retailers). This is also evident in the Likelihood ratio of 25.083 of the bread consumers' storage conditions affecting bread shelf life. The overall results from Table 13 showed that there was a correlation between how bread was stored and its perceived shelf life. The low Pearson's Chi Square value of 11.774 for retailers showed that their storage conditions of bread greatly reduced the shelf life of bread.

4.2 Chemical shelf life models of 100% wheat and 10% HQCF composite tea and sugar bread stored at fluctuating and room temperature conditions

The Table 14 shows the shelf life of 10% HQCF composite, 100% wheat and a baker's control sugar breads stored at room and elevated temperature conditions according to water activity. The shelf life results according to water activity showed that all the bread samples had a water activity limit between 0.87 and 0.94 therefore they all had high probability of being prone to destruction by microorganisms such as yeasts and mould. With the exception of the stored 100% wheat sugar bread, which had a water activity limit below 0.88; the other bread samples had a water activity of high moisture foods (Aw above 0.89) (Steele, 2004).

Table 14: Shelf life of 10% HQCF composite and 100% wheat tea and sugar bread stored at fluctuating and room temperature according to water activity

Type of bread	Storage condition	Model	Data limit	Estimated shelf life
100% wheat sugar	Room	$Aw = \frac{1}{1.1466 - 0.000160618 \times \sqrt{Days}}$	0.874396	5 days 16 hours
	Elevated	$Aw = \frac{1}{1.08594 + 0.00040524 \times Days^2}$	0.936056	4 days 11 hours
10% HQCF composite sugar	Room	$Aw = \frac{1}{1.07874 - 0.0032857 \times \sqrt{Days}}$	0.941634	4 days 15 hours
	Elevated	$Aw = \frac{1}{1.08326 - 0.00193904 \times Days}$	0.936225	4 days 10 hours
Baker's sugar	Room	$Aw = \frac{1}{1.14163 - 0.00650029 \times \sqrt{Days}}$	0.906056	5 days 7 hours
	Elevated	$Aw = \frac{1}{1.11178 + 0.000903825 \times Days^2}$	0.922113	5 days 6 hours
100% wheat tea	Room	$Aw = \frac{1}{1.094661 - 0.00690966 \times \sqrt{Days}}$	0.931634	4 days 18 hours
	Elevated	$Aw = \frac{1}{1.08594 + 0.000405254 \times Days^2}$	0.936056	4 days 11 hours
10% HQCF composite tea	Room	$Aw = \frac{1}{1.07874 - 0.00332857 \times \sqrt{Days}}$	0.941634	4 days 15 hours
	Elevated	$Aw = \frac{1}{1.08326 - 0.00193904 \times Days}$	0.936225	4 days 10 hours
Baker's tea	Room	$Aw = \frac{1}{1.07331 - 0.0078157 \times \sqrt{Days}}$	0.956056	4 days 10 hours
	Elevated	$Aw = \frac{1}{1.10711 - 0.0171386 \times \sqrt{Day}}$	0.9440869	4 days 3 hours

**Elevated temperature is indicative of fluctuating temperature condition

This is evidenced in the 100% wheat sugar bread stored at room temperature recording the longest shelf life of 5 days 16 hours. The fluctuating temperature to which the bread samples were exposed also could have contributed to high levels of water activity due to the absorption of moisture from the atmosphere unto the bread. Since moisture and water activity are directly proportional all the sugar bread samples which had high moisture content had a subsequent high water activity and reduced shelf life. Sugar bread samples which had a moisture content limit ranging from 21.003 to 24.3063% had a subsequent water activity limit ranging between 0.87396 and 0.94163 and shelf life ranging between 4 days 15 hours and 5 days 17 hours respectively (Table 14, 15). Even though water activity specifically is the available moisture for microbial growth whereas moisture content gives the total amount of moisture or water a food sample contains, the moisture content could also roughly indicate the water activity of a food sample. The high moisture content of the 10% HQCF composite bread could be due to hydroscopicity of the cassava flour (Ohimain, 2014). This affected the shelf life of the bread by encouraging microbial growth and bread spoilage. It was observed during the shelf life study that after 3 days of storage of the composite bread there was the development of yeasts and mould on the crust and crumb of the bread. This was in accordance of the findings stated by Ohimain, (2014) that composite bread from HQCF had a high moisture content, faster staling rate and shorter shelf life as compared to 100% wheat bread. However the shelf life of the composite sugar bread was seen to be longer than the baker's control. This could be due to the different quantity of water used by the baker for mixing the dough and other ingredients such as eggs and milk added which were absent in the 100% wheat and composite tea and sugar bread (Baker's recipe shown in Appendix 4). The ratio of the shortening to sugar used for the 100% wheat and 10% HQCF composite tea and sugar bread was higher as compared to that used by the baker in her recipe. The increase in the amount of sugar could also have served as a better preservative by reducing the retention of moisture and decrease in water activity and subsequent increase in shelf life of the sugar bread. According to Adeniji (2015) high amount of sugar in bread can delay staling by reducing the rate of starch retrogradation and gelatinization. This was evident in the longer shelf life of the sugar bread samples than the tea bread. The water activity limits values recorded for all the tea bread samples were higher as compared to that of the sugar bread samples. The water activity limit ranged from 0.93 to 0.95 and this explains the comparatively shorter shelf life of the tea bread samples.

4.2.2 Shelf life according to Moisture content

Table 15: Shelf life of 10% HQCF composite and 100% wheat tea and sugar bread stored at fluctuating and room temperature according to moisture content

Types of bread	Storage conditions	Model	Moisture content (%) limit (y)	Shelf life (x)
100% wheat sugar	Room	$Moisture = \sqrt{}(504.59 - 0.613878 \times Days^2$	21.0035	5 days 18 hours
	Elevated	$Moisture = \dfrac{1}{0.0457337 + 0.000228988 \times Days}$	21.1518	5 days 13 hours
10% HQCF composite sugar	Room	$Moisture = \sqrt{(653.838} - 55.3893) \times \sqrt{Days}$	22.4085	5 days 8 hours
	Elevated	$Moisture = \sqrt{(591.189} - 33.5424 \times \sqrt{Days}$	21.4085	5 days 6 hours
Baker's sugar	Room	$Moisture = \dfrac{1}{0.0379696 + 0.000429634 \times Days}$	24.3063	5 days 17 hours
	Elevated	$Moisture = \sqrt{}(505.943 + 3.36642 \times Days^2$	22.4085	5 days 12 hours
100% wheat tea	Room	$Moisture = \dfrac{1}{0.0404604 - 0.000633484 \times \sqrt{Days}}$	24.2042	4 days 10 hours
	Elevated	$Moisture = \sqrt{}(668.152 - 77.2993 \times \sqrt{Days}$	21.6127	4 days 5 hours
10% HQCF composite tea	Room	$Moisture = \dfrac{1}{0.037297 + 0.0000713366 \times Days^2}$	24.4085	4 days 11 hours
	Elevated	$Moisture = \dfrac{1}{0.0375245 + 0.000167172 \times Days^2}$	21.8169	4 days 4 hours
Baker's tea	Room	$Moisture = \dfrac{1}{0.0371272 - 0.00161278 \times \sqrt{Days}}$	26.5106	4 days 6 hours
	Elevated	$Moisture = \sqrt{}(756.747 - 11.6832 \times Days$	24.5106	3 days 21 hours

**Elevated temperature is indicative of fluctuating temperature condition

The shelf life of the tea bread samples according to moisture content ranged between 3 days 21 hours and 4 days 18 hours (Table 15), a duration shorter than the one recorded for the sugar bread samples which was between 4 days 3 hours and 4 days 18 hours. This difference could be due to earlier stated reason of the high amount of sugar present in the sugar bread samples acting as a preservative. The baker's control tea bread recorded a slower staling rate but a faster rate of mouldiness. This could be justified by the presence of high amount of water used in the baker's tea bread and other additives such as milk and eggs. The high water activity values recorded for the tea bread samples further suggest that it contained a high amount of available water for microorganisms to grow and proliferate quickly than the sugar bread samples. The tea bread samples contained less amount of sugar than the sugar bread (Table 2) which could imply that moisture could be retained in the crumb. There could also be reduced water adsorption from the environment which could have resulted in the increased staling rate. It was also observed during the shelf life study that the packaging material of the bread samples stored under fluctuating temperature conditions had droplets of water during the day which could increase the moisture content and water activity of the bread. The less sugar in the tea bread samples further encouraged the growth of microorganisms. The absorption of water droplets from the packaging material of the bread could have also influenced the multiplication of these microbes.

4.2.3 Shelf life according to free fatty acid content

Table 16: Shelf life of 10% HQCF composite and 100% wheat bread stored at elevated and room temperature according to free fatty acid content

Types of bread	Storage conditions	Model	FFA limit (y) (mg KOH/g fat)	Shelf life (x)
100% wheat sugar	Room	$FFA = 1.73928 + 0.0527796 \times Days$	2.1507	5 days 18 hours
	Elevated	$FFA = \frac{1}{(0.547156 - 0.0483502 \times \sqrt{Days}}$	2.52113	5 days 14 hours
10% HQCF composite sugar	Room	$FFA = \frac{1}{0.535377 - 0.0223551 \times Days}$	2.70141	5 days 16 hours
	Elevated	$FFA = \frac{1}{0.55389 - 0.00396521 \times Days^2}$	2.98169	5 days 12 hours
Baker's sugar	Room	$FFA = 4.26284 + 0.551368 \times Days$	7.90563	5 days 12 hours
	Elevated	$FFA = \exp(1{,}49336 + 0.0988151 \times Days$	9.00704	5 days 6 hours
100% wheat tea	Room	$FFA = (1.23999 + 0.00868726 \times Days^2)^2$	3.30232	4 days 14 hours
	Elevated	$FFA = (1.23999 + 0.00868726 \times Days^2)^2$	2.85211	4 days 7 hours
10% HQCF composite tea	Room	$FFA = \exp(0.415713 + 0.0398708 \times Days^2$	10.8164	4 days 7 hours
	Elevated	$FFA = (1.16512 + 0.0436086 \times Days^2\ )^2$	10.8169	4 days 4 hours
Baker's tea	Room	$FFA = (2.099 + 0.0345699 \times Days^2)^2$	14.81169	4 days 11 hours
	Elevated	$FFA = \frac{1}{(0.227981 - 0.0234643 \times Days)}$	16.2254	4 days 4 hours

**Elevated temperature is indicative of fluctuating temperature condition

The Table 16 shows the shelf life models and calculated shelf life of 10% HQCF composite and 100% wheat sugar and tea bread stored at elevated and room temperature conditions according to free fatty acid content. The calculated shelf life of the 100% wheat sugar bread stored at room temperature according to FFA from the table was 5 days 18 hours at a FFA limit of 2.1507 mg KOH/g fat whilst the one stored at fluctuating temperature was 5 days 14 hours at a FFA limit of 2.52113 mg KOH/g fat. The storage condition affected the shelf life of the sugar bread slightly (a difference of 4 hours). The fluctuating temperature could have caused an increase in rancidity which affected the shelf life of the sugar bread samples stored at that condition. When bread is displayed in the sun the heat generated in the plastic bag increases the rate of rancidity of the fat used in baking the bread. According to Fadda *et al*. (2014) in a review of bread staling, the rancidity of fat used in baking bread could affect staling by causing the crystallization of starch in the bread through the interaction between the amylopectin and amylose/fat complex by releasing the moisture from the crumb surface. This will result in crumb firmness of the bread and faster rate of staling. However the staling rate and subsequent shelf life of the bread stored at fluctuating temperature conditions partly confirms the observation made by Fadda *et al*. (2014). The calculated shelf life of the 10% HQCF composite sugar bread stored at room temperature according to free fatty acid content was 5 days 16 hours according to Table 4.12 whilst the one stored at elevated temperature was 5 days 12 hours. The free fatty acid content of the bread stored at elevated temperature was higher as compared to the one stored at room temperature conditions. According to Bosmans *et al*. (2013) heat is also known to be a contributing factor to fat rancidity in bread which tends to affect a quicker staling rate and shelf life. This might have accounted for the relative short shelf life of the bread stored at elevated temperature conditions. In comparing the shelf life of the composite bread (between 5 days 12 hours and 5 days 16 hours) to that of the 100% wheat sugar (between 5 days 14 hours and 5 days 18 hours) bread shows an increase in shelf life of the latter than the former at both storage conditions. The calculated shelf life of the baker's control sugar bread stored at room and elevated temperature conditions according to free fatty acid content was 5 days 12 hours and 5 days 6 hours from Table 4.12 respectively. The free fatty acid content limit of the baker's sugar bread stored at room and elevated temperature conditions was 7.90563 mg/KOH/g fat and 9.00704 mg KOH/g fat respectively which was very high as compared to that of the composite (2.70141 and 2.98169 mg/KOH/g fat respectively) and 100% wheat sugar bread (2.1507 mg/KOH/g fat and 2.52113 mg/KOH/g fat respectively). This could be attributed to the different type of margarine used since all samples were given the same storage treatment. The baker used margarine with a brand name 'Menera' whilst the margarine used for this study was 'Cookbrand'. Fadda *et al*. (2014) in a review of bread staling process stated that even the type of fat and ratio of monoglycerides and triacylglycerides (TAGs) could affect the rate of staling, rancidity and subsequent shelf life of bread.

The Table 16 also shows the shelf life of 100% wheat tea bread, 10% HQCF composite tea bread and a baker's control tea bread according to free fatty acid content. The calculated shelf life of the composite tea bread stored at room temperature was 4 days 13 hours whilst the one stored at elevated temperature conditions was 4 days 10 hours from the table respectively. The shelf life of the composite tea bread was lower as compared to that of the composite sugar bread. The presence of the high amount of sugar in the sugar bread could have helped to extend the shelf life by acting as a preservative. The interaction between the sugar, starch, amylose and the fiber present in the bread could have caused an improvement in the water binding capacity of the bread thus reducing water loss during storage. This went a long way to reduce retrogradation and delay bread staling (Bosmans *et al*., 2013). The shelf life of all the sugar bread samples followed this trend of having a relatively longer shelf life (between 5 days 6 hours and 5 days 18 hours) than the tea bread samples (between 4 days 4 hours and 4 days 14 hours) according to free fatty acid content. All the tea bread samples had high free fatty acid content limit than the sugar bread samples. The high sugar present in the sugar bread could also have acted as humectant absorbing moisture from the environment and retaining the water binding capacity to delay bread staling rate. According to Fadda *et al*. (2014) when the balance between the fat and sugar is low, the fat molecules tend to be prone to rancidity than when the amount of sugar present is high. This result seems to confirm the findings in the survey conducted in the Eastern, Greater Accra and Ashanti Regions (Table 4.5) where most bakers emphasized that sugar bread lasted longer on the shelf than tea bread. Their perception of the sugar present in the sugar bread serving as a preservative is therefore somehow explained. According to Prescott & Klein (2002) sugar is known to retard the growth of microorganisms by drawing water away from their cell membranes and causing death or a delay in their proliferation. Another observation made from the shelf life data was that the baker's tea bread also followed the trend of having very high free fatty content limit than the sugar bread samples indicating a faster rate of fat rancidity. This fat rancidity could have caused the observed foul smell of the stale bread as shown also in Table 4.13 and Appendix 1 for the descriptive sensory data on the bread aroma.

4.3 Physical shelf life models for wheat and composite tea and sugar bread

Table 17: Shelf life of 10% HQCF composite and 100% wheat tea and sugar bread stored at room and elevated temperature according to sensory evaluation

Types of bread	Storage conditions	Model	Overall quality limit (y)	Shelf life (x)
100% wheat sugar	Room	$Overall\ quality = \frac{1}{(0.12203 + 0.0037847 \times Days^2)}$	3.91268	5 days 10 hours
	Elevated	$Overall\ quality = \exp(2.07522 - 0.0220107 \times Days^2$	3.41268	5 days 10 hours
10% HQCF composite sugar	Room	$Overall\ quality = \frac{1}{(0.131599 + 0.00441137 \times Days^2)}$	3.3126	5 days 10 hours
	Elevated	$Overall\ quality = 8.27639 - 0.784921 \times Days$	3.4128	5 days 8 hours
Baker's sugar	Room	$Overall\ quality = \frac{1}{(0.12197 + 0.00437727 \times Days^2)}$	3.51268	5 days 6 hours
	Elevated	$Overall\ quality = \exp(2.09859 - 0.28478 \times Days^2)$	1.03113	4 days 23 hours
100% wheat tea	Room	$Overall\ quality = \exp(2.10198 - 0.0302963 \times Days^2)$	1.02113	3 days 22 hours
	Elevated	$Overall\ quality = \exp(2.0958 - 0.0336589 \times Days^2)$	1.0374	3 days 19 hours
10% HQCF composite tea	Room	$Overall\ quality = 8.43889 - 0.929563 \times Days$	1.0203	3 days 18 hours
	Elevated	$Overall\ quality = \exp(2.0293 - 0.0370214 \times Days^2)$	1.0274	3 days 8 hours
Baker's tea	Room	$Overall\ quality = \exp(2.08147 - 0.0469465 \times Days^2$	1.22113	3 days 20 hours
	Elevated	$Overall\ quality = \exp(2.11869 - 0.0612895 \times Days^2)$	1.02113	3 days 16 hours

**Elevated temperature is indicative of fluctuating temperature condition

Table 17 shows the shelf life of 100% wheat tea bread, a 100% wheat baker's control tea bread and 10% HQCF composite tea bread according to sensory evaluation. The attributes used for the descriptive sensory evaluation by the panellists for the shelf life study were crumb texture and colour, crust colour, bread aroma and overall quality but only the overall quality was considered for the overall shelf life calculation. This is because the overall acceptability/quality gives an index of the sum of all the attributes tested (Kemp *et al*., 2009). The panellists were 'calibrated' by training for an hour per day for eight days (see Section 3.6.5: Training and screening of panellists) before being used for the shelf life study so it is assumed that they were able to detect changes in the tea bread samples after 3 days of storage according to the shelf life models generated from the sensory data. Even though the results from the chemical shelf life models (aw, FFA and moisture) showed a shelf life between 4 days 3 hours and 5 days 18 hours, the physical tests showed a shelf life between 3 days 8 hours and 5 days 10 hours. This result also confirms the observation made by consumers, retailers and bakers that tea bread could only last for at least three days (Tables 6, 7, 8, 9, 10, and 11). Another assumption from this results also suggest that humans or the panellists used as measuring instruments would not have preferred to consume the tea bread after three days of storage. It partly confirms a comment made by the respondents interviewed during the preliminary survey that it was difficult to consume stale bread (Table 11). The composite tea bread stored at elevated temperature recorded the lowest shelf life according to sensory evaluation of 3 days 8 hours. The presence of the HQCF and the high amount of moisture could have contributed to its staling faster than the 100% wheat tea bread. The crumb texture of the 10% HQCF was comparably harder than the 100% wheat bread and the baker's control tea bread. The rate of staling was observed to be faster in the composite bread than in the 100% wheat tea bread from the data from the crumb texture (Appendix). This can be explained by the results from Idowu *et al*. (2014) where the authors stated that the presence of composite flour could cause the crumb texture of composite bread to be harder than normal wheat bread. Interventions for extending the shelf life of composite bread suggested by Ohimain (2015) in his review on recent advances in the production of partially substituted and wheatless like chemical pre-treatment methods such as addition of bread additives or physical pre-treatment methods such as toasting could be applied.

4.4 Microbial shelf life models for HQCF composite and wheat tea and sugar bread

Table 18: Shelf life of 10% HQCF composite bread and 100% wheat tea and sugar bread stored at room and fluctuating temperature according to Total Plate Count

Types of bread	Storage conditions	Model	TPC limit (CFU/g) (y)	Shelf life (x)
100% wheat sugar	Room	$TPC = -0.259623 + 0.305577^{Days}$	2	5 days 12 hours
	Elevated	$TPC = \exp(0.680271 + 1.62288 \times \sqrt{Days})$	1541	5 days 9 hours
10% HQCF composite sugar	Room	$TPC = \exp(0.502987 + 1.57763 \times Days)$	2466	5 days 7 hours
	Elevated	$TPC = -2040.29 + 259.128Days^2$	1541	5 days 6 hours
Baker's sugar	Room	$TPC = ((33.0353 + 39.4536\sqrt{Days})^2$	164	4 days 19 hours
	Elevated	$TPC = \exp(1.35104 + 3.99065 \times \sqrt{Days})$	12329	4 days 16 hours
100% wheat tea	Room	$TPC = (26.4559 + 55.2225 \times Days)^2$	102740	3 days 18 hours
	Elevated	$TPC = \exp(3.00379 + 5.4009 \times \sqrt{Days})$	513699	3 days 14 hours
10% HQCF composite tea	Room	$TPC = -263450 + 42219 \times Days^2$	246575	3 days 16 hours
	Elevated	$TPC = \exp(-0.672695 + 6.26907 \times \sqrt{Days})$	1232887	3 days 9 hours
Baker's tea	Room	$TPC = \exp(3.2242 + 6.64502 \times \sqrt{Days})$	8125670000	3 days 5 hours
	Elevated	$TPC = \exp(-0.067936 + 7.5207 \times \sqrt{Days})$	10274670	2 days 20 hours

**Elevated temperature is indicative of fluctuating temperature condition

The microbial shelf life models for sugar bread according to Total Plate Count (TPC) Total aerobic microorganisms) for 100% wheat sugar bread, 10% HQCF composite and 100% wheat baker's control tea and sugar bread is displayed in Table 4.14. The total aerobic microorganisms a food sample contains gives an indication of both harmful, spoilage and beneficial microorganisms such as bacteria, yeasts, fungi, protozoa, etc. It usually gives an indication of the sanitary conditions, handling and safety of food such as bread (Prescott & Klein, 2002). It can be inferred from the shelf life models for the baker's control sugar and tea bread that the safety and the handling of the bread during, before and after processing was not the best. All the bread samples with the exception of the 100% wheat sugar bread exceeded the standard of TPC of 1,500 CFU set by American Public Health Association (2010) for ready-to-eat foods. The storage of ingredients used for the bread probably was not handled well and it could have served as a source of contamination. The source of water used for the dough mixing could be a vehicle of transmission of microorganisms. The improper personal hygiene practices such as improper hand washing of those who process the bread could also have been a factor of the high count of microbes in the bread. According to Prescott & Klein (2002) some microorganisms are able to form spores during unfavourable conditions such as when the dough is being baked and multiply once conditions are favourable such as after the bread has been baked. Sometimes the plastic polythene bags used for bread packaging are inoculated with a lot of microbes when bakers blow air into these bags to open them up. These plastic bags serving as primary packages for the bread indirectly transmit these microbes to the bread which tends to hasten the rate of bread spoilage. Sometimes during cooling of the bread after baking, bread stakeholders especially retailers and bakers leave bread uncovered and exposed to the atmosphere. Microorganisms are known to be ubiquitous in nature (Sahu & Bala, 2017) and they settle on the exposed bread. Retailers interviewed from the preliminary survey were observed to be displaying bread by the roadside so as to attract consumers in traffic. Dust could serve as a source of contamination for the bread which is usually uncovered (Plate 4). The above stated reasons could have accounted for the total aerobic microbial count which was above acceptable levels. The microbial limits recorded for all the bread samples were above the acceptable limit for microbial count for ready-to-eat foods. The microbial count for the tea bread samples was more than that of the sugar bread samples and this could be attributed to the low amount of sugar present in the tea bread samples.

4.4.2 Shelf life according to yeasts and mould count

Table 19: Shelf life of 10% HQCF composite and 100% wheat tea and sugar bread stored at room and fluctuating temperature conditions according to yeasts and mould count

Types of bread	Storage conditions	Model	Yeasts and mould count limit (CFU/g) (y)	Shelf life (x)
100% wheat sugar	Room	$YM = -7.25592 + 0.98176 \times Days^2)^2$	308	5 days 13 hours
	Elevated	$YM = (-8.63751 + 1.19444 \times Days^2)^2$	411	5 days 6 hours
10% HQCF composite sugar	Room	$YM = (-11.373 + 1.55379 \times Days^2)^2$	822	5 days 10 hours
	Elevated	$YM = (-15.1398 + 2.01169 \times Days^2)^2$	1233	5 days 6 hours
Baker's sugar	Room	$YM = -17413.7 + 2422.82 \times Days^2$	1541	4 days 20 hours
	Elevated	$YM = -1754.78 + 248.367 \times Days^2$	15411	4 days 14 hours
100% wheat tea	Room	$YM = -1278.32 + 78.749 \times Days^2$	1027.4	3 days 18 hours
	Elevated	$YM = -1470.45 + 209.287 \times Days^2$	1233	3 days 16 hours
10% HQCF composite tea	Room	$YM = (-6.30676 + 0.89323 \times Days^2)^2$	247	3 days 14 hours
	Elevated	$YM = -532.504 + 83.0102 \times Days^2$	514	3 days 13 hours
Baker's tea	Room	$YM = (15.8753 + 39.5044 \times Days)^2$	24658	3 days 5 hours
	Elevated	$YM = (7.2928 + 6.15213 \times Days^2)^2$	15412	3 days 3 hours

**Elevated temperature is indicative of fluctuating temperature condition

In calculating the overall shelf life of the bread samples, yeasts and mould count was considered over TPC since it gives a more specific indication of spoilage in bread during shelf life studies. The Table 4.15 shows the shelf life of the tea and sugar bread samples according to yeasts and mould count (Prescott & Klein, 2002). The shelf life of the tea bread samples ranged from 3 days 3 hours to 3 days 16 hours with the baker's tea bread stored at elevated temperature having the shortest shelf life of 3 days 3 hours. The high water activity level of the tea bread samples justified its high yeasts and mould count over the storage period and subsequent short shelf life. The shelf life of the sugar bread samples ranged from 4 days 14 hours to 5 days 20 hours with the baker's sugar bread stored at fluctuating conditions having the shortest among the sugar bread samples. The fluctuating storage conditions really affected the overall shelf life of the bread samples by creating a very conducive environment such as providing condensed moisture, fluctuating temperature and humidity for microbial growth and proliferation.

Table 20: Overall shelf life models of sugar and tea bread stored at elevated and room temperature conditions

Bread type	Storage condition	Model
Baker's sugar bread	Room	$Days = -4.81051 + 17.2011(Aw) - 1.21037(sensory) + 3.88045 \times 10^{-7}(microbial)$
Control sugar		$Days = 1.33657 + 12.6072(Aw) - 1.481(sensory) - 0.000178068(microbial)$
Composite sugar		$Days = 13.0463 - 2.90221(Aw) - 1.26542(sensory) + 0.0000214566(microbial)$
Baker's tea		$Days = 20.0608 + 29.5154(Aw) - 0.863076(sensory) + 0.0000105945(microbial)$
Control tea		$Days = -46.3975 + 59.3776(Aw) - 0.912733(sensory) + 0.0000262574(microbial)$
Composite tea		$Days = -11.1845 + 20.854(Aw) - 0.960121(sensory) + 0.000311919(microbial)$
Baker's sugar bread	Elevated	$Days = 30.3187 - 22.9383(Aw) - 0.00010379(microbial) - 1.118974(sensory)$
Control sugar		$Days = 6.87185 + 5.86152(Aw) - 1.42706(sensory) - 0.00020306(microbial)$
Composite sugar		$Days = 22.2381 - 15.0193(Aw) - 1.07367(sensory) + 0.0000962924(microbial)$
Baker's tea		$Days = -18.8038 + 25.7186(Aw) - 0.48785(sensory)$+0.000039124(microbial)
Control tea		$Days = 58.7482 + 71.3022(Aw) - 0.689187(sensory) + 0.000246171(microbial)$
Composite tea		$Days = 1718.81850.74(Aw) + 0.00508569(microbial) - 0.5446227(sensory)$

**Elevated temperature is indicative of fluctuating temperature condition

Table 21: Calculated shelf life in days and hours of bread stored at room and elevated temperature conditions

Bread type	Storage condition	Shelf life of bread: Days	Hours
Baker's sugar	Elevated	7	23
100% Wheat sugar		7	6
Composite sugar		6	8
Baker's tea		4	17
100% Wheat tea		6	17
Composite tea		5	6
Baker's tea	Room	5	1
100% Wheat tea		7	1
Composite tea		6	16
Baker's sugar		7	17
100% Wheat sugar		8	16
Composite sugar		7	19

**Elevated temperature is indicative of fluctuating temperature condition

4.4.1 Overall shelf life of wheat and composite tea and sugar bread

The chemical tests for the laboratory shelf life studies were free fatty acid content, water activity and moisture content. However, only water activity was considered in calculating the overall shelf life of the bread samples. This is because water activity is known as a better indicator of available water needed for microbial growth than moisture content. Water activity also affects the sensory properties of bread such as its crumb and crust texture (Kilcast & Subramaniam 2012). Water activity was chosen over free fatty acid content because the free fatty acid content only indicates the rancidity of the fat used in bread which only imparts on the smell of the bread but not the microbial or textural quality of the bread.

The calculated shelf life based on the different storage conditions (room and elevated) of 100% wheat tea and sugar bread according to a standard recipe and a baker's control, 10% HQCF composite tea and sugar bread stored at elevated and room temperature conditions is shown in Table 4.11. However, the overall average shelf life of 6 bread types in days is displayed in Table 4.12. The bread with the longest shelf life from the Table 4.11 is the 100% wheat sugar bread stored at room temperature conditions which had a shelf life of 8 days 16 hours. The bread which had the shortest shelf life was the 100% wheat baker's control tea bread stored at elevated temperature conditions. The bread had a shelf life of 4 days 17 hours. The table also shows that the sugar bread samples had a longer shelf life than the tea bread samples.

Bread stored at room temperature conditions also had a longer shelf life than those stored at elevated temperature conditions. The table 19 above shows the overall shelf life models of 100% wheat tea and sugar bread according to a standard recipe and a baker's control, 10% HQCF composite tea and sugar bread. The shelf life of the bread was modelled using sensory evaluation, yeasts and mould count and water activity to represent the physical, microbial and chemical parameters respectively.

The calculated shelf life of each of the 6 bread samples used for the shelf life study based on two storage conditions is also displayed in Table 20. The overall shelf life gives an estimation of the duration that the bread can be stored after which it cannot be consumed which is referred to as the 'expiry date'. However the shelf life of the bread calculated according to each of the chemical tests (moisture, FFA and moisture), physical test (descriptive sensory evaluation) and microbial tests (TPC and yeasts and mould count) only give an indication of the 'best before date' of the bread samples. Those shelf life models gave a shorter shelf life to each of the bread samples as compared to overall shelf life models. This implies that the bread samples could be consumed based on the moisture and water activity changes after the said duration though the bread was not totally wholesome. The multiple regression analysis done in calculating the overall shelf life of the bread samples took into consideration the chemical, physical and microbial parameter so there had to be a balance between all the three parameters. Table 4.11 shows clearly that the storage condition affected the shelf life of the bread samples. The storage condition used by most bread stakeholders especially retailers is not the best since it greatly affects the shelf stability of the bread. The 100% wheat sugar bread stored at room temperature had the longest shelf life of 8 days 16 hours. The results also showed that composite tea (6 days 16 hours) and sugar bread (7 days 19 hours) also had a considerably longer shelf life than the baker's control tea (5 days 1 hour) and sugar bread (7 days 17 hours).

The Table 21 shows that the all the sugar bread samples had an average shelf life of 8 days whilst the average shelf life between 5 and 7 days at an average temperature of 25-35 °C and an average relative humidity of 51-72%.

Table 21: Overall Shelf life of Bread in days

Bread type	Storage condition (Average Temperature/Relative humidity)	Shelf life of bread Days
Baker's sugar	25-35 °C/51-72%	8
100% Wheat sugar		8
Composite sugar		8
Baker's tea		5
100% Wheat tea		7
Composite tea		6

4.4.2 POTENTIAL MEANS OF ENHANCING BREAD SHELF LIFE

Shelf life of composite bread can be extended when other pre-treatment methods such as cold storage in the refrigerator and toasting are applied according to Ohimain (2015). Bread stakeholders such as consumers, retailers and bakers would be admonished to handle bread with proper care since it is a ready-to-eat product. Once bread leaves the oven, it should not be handled with bare hands as practiced by most bakers and retailers. Bread stakeholders should be advised to wear gloves during packaging and distribution of bread. The practice of keeping bread uncovered in the car boot during distribution by bread stakeholders should be avoided to prevent cross contamination of the bread (Table 4.7) In general, since the composite had shorter shelf life, bakers should be well educated on the need for proper handling and hygienic practices before and after baking to enhance shelf life of these bread types.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The preference and storage conditions of bread among the bread stakeholders (retailers, bakers and consumers) were investigated using a baseline survey in the Ashanti, Eastern and Greater Accra Regions of Ghana. The major findings from the baseline survey were that all the bread stakeholders stated that sugar bread was the most shelf stable among all the bread types. The most common storage place and condition used by bakers for leftover bread was at room temperature (about 27° C) on shelves whilst retailers used boxes for storage and sold bread under fluctuating conditions in and out of the sun. Consumers on the other hand stored leftover bread in the fridge or at room temperature on top of a table or fridge. The bread stakeholders also stated during the interview that the observed shelf life of composite bread was between 3 and 4 days.

Composite bread from 10% HQCF: 90% wheat bread (tea and sugar) and 100 % wheat bread (tea and sugar) from a standard recipe developed in the Department of Food Science and Technology, KNUST and 100% wheat (tea and sugar) from a baker's recipe used as a control were stored under room temperature conditions (25-31 ^{o}C) and at elevated but fluctuating temperature conditions (in and out of the sun: 26-35 ^{o}C) for 7 days. The changes in the moisture, water activity, sensory, free fatty acid content and microbial quality of the 100% wheat and composite bread was analyzed and used to predict the shelf life using simple and multiple regression modelling. Bread stored at room temperature had a longer shelf life between 6 and 8 days whereas bread stored at elevated temperature conditions had a shelf life between 5 and 7 days. It can also be concluded from the shelf life study that the sugar bread samples had a better storage life between 5 and 8 days than the tea bread samples which had a shelf life of 5 and 7 days because of the more sugar in the sugar bread which served as a preservative. The composite bread had a shelf life between 5 and 7 days whilst the 100% wheat bread had a shelf life between 7 and 8 days.

5.2 Recommendations

The following are recommended based on the findings from the shelf life study:

1. Bread stakeholders especially those who process and handle bread should be given hygiene training to ensure the microbial quality of bread is improved or maintained considerably.
2. The effect of various bread improvers on the shelf life of composite bread stored under different conditions by bread stakeholders should be investigated.

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APPENDIX 1

Table 2: Shelf life models of 100% wheat and 10% HQCF tea and sugar bread according to overall quality

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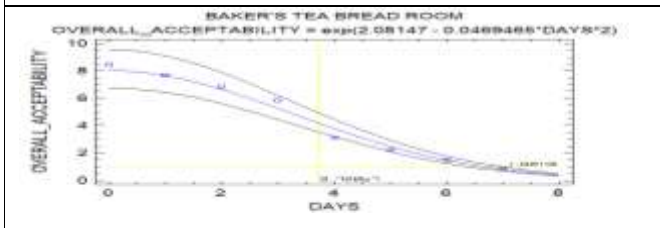	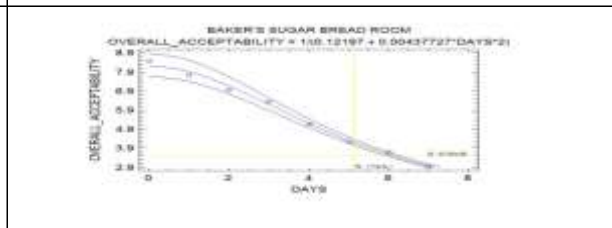
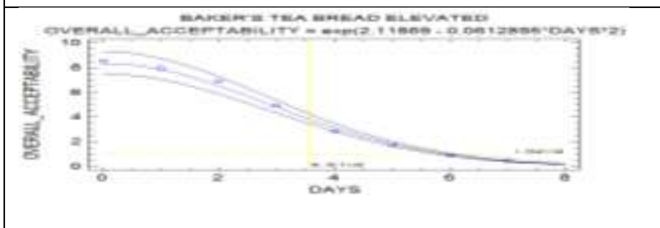	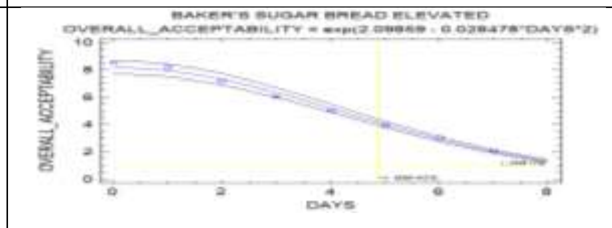
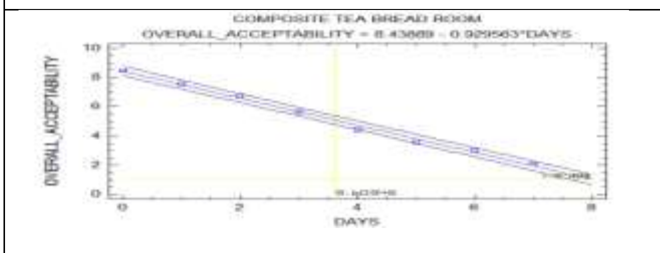	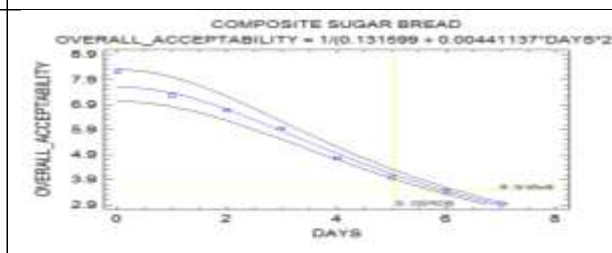
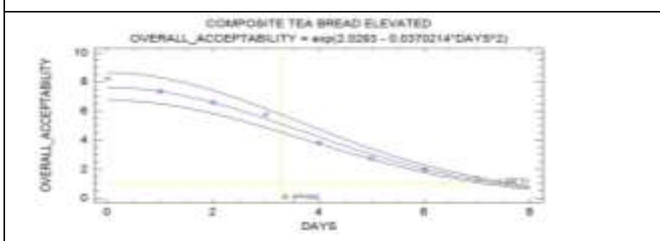	
	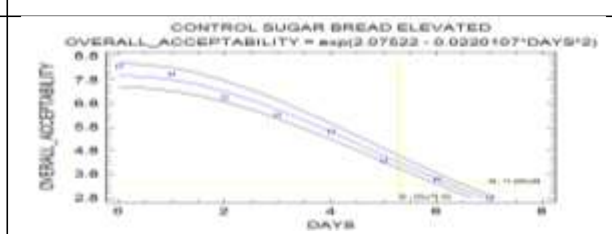
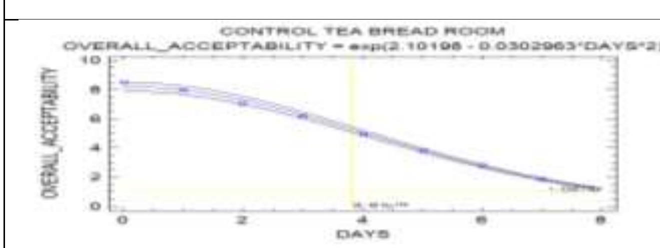	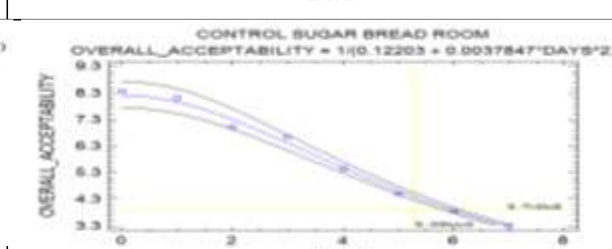

APPENDIX 2a

Table 3: Shelf life models of 100% wheat and 10% HQCF tea and sugar bread according to TPC

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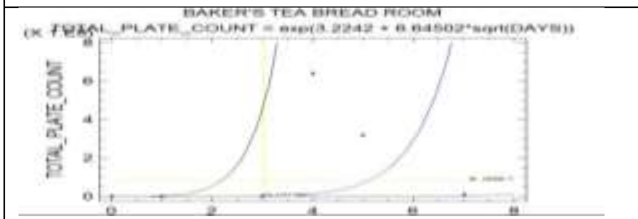
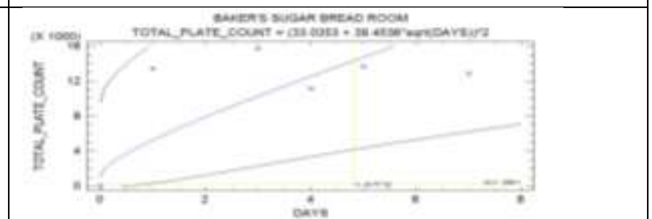
<tr><td>
</td><td>
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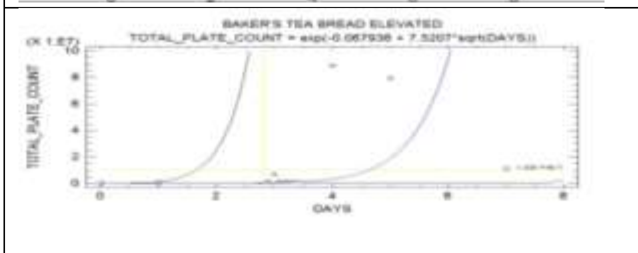
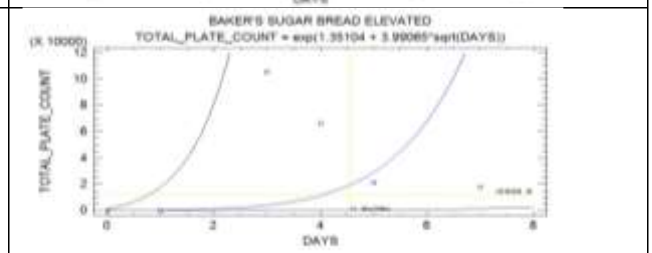
<tr><td>
</td><td>
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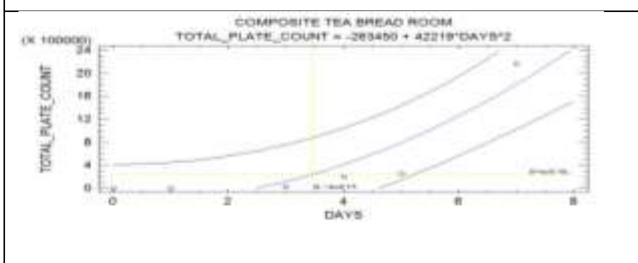
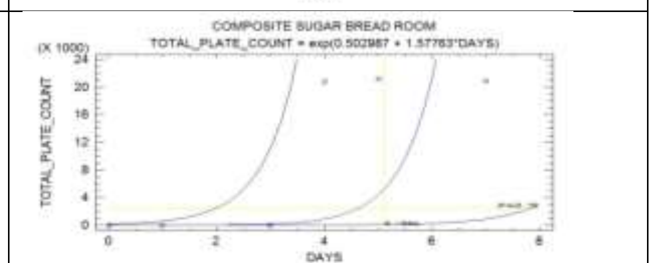
<tr><td>
</td><td>
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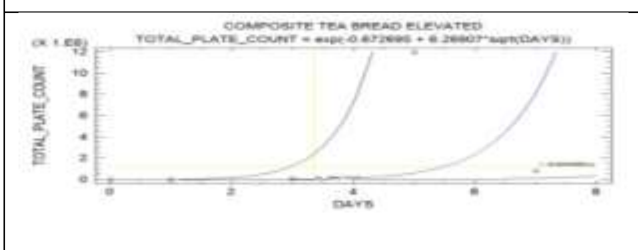
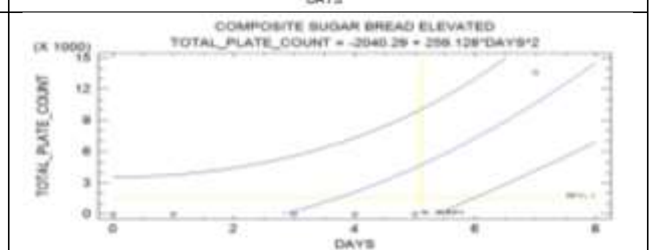
<tr><td>
</td><td>
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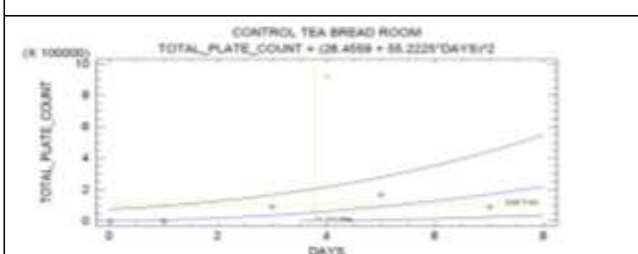
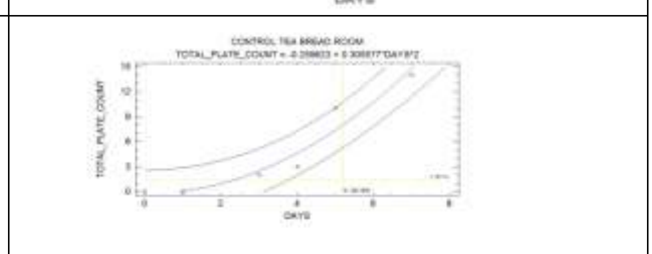
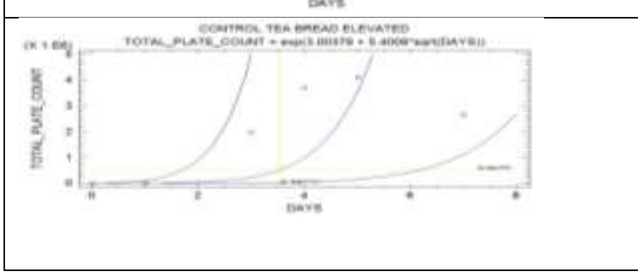
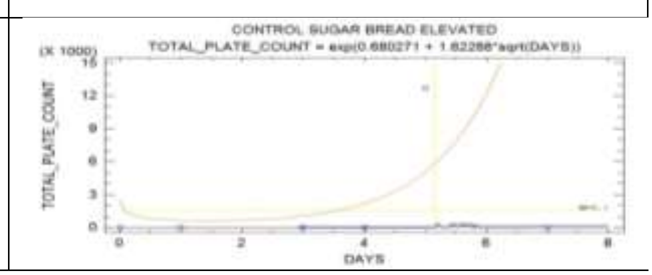
<tr><td>
</td><td></td></tr>
<tr><td>
</td><td>
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APPENDIX 2b

Table 4: Shelf life models of 100% wheat and 10% HQCF tea and sugar bread according to yeasts and mould

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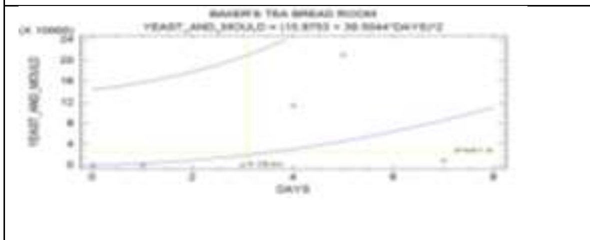	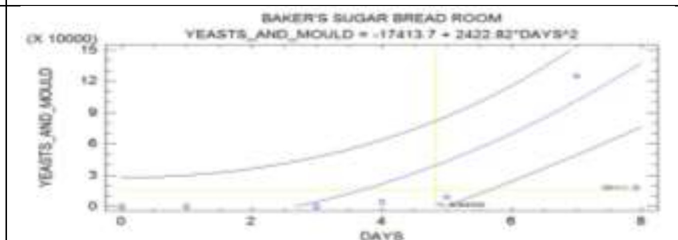
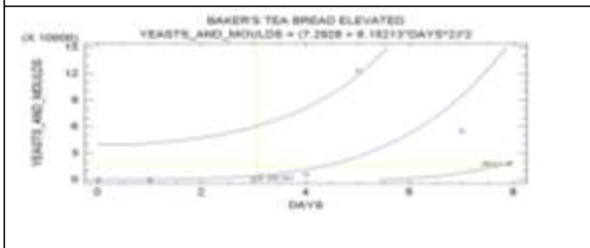	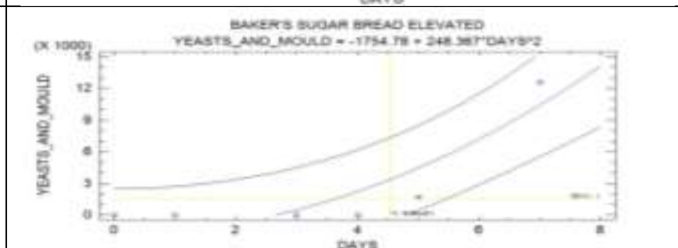
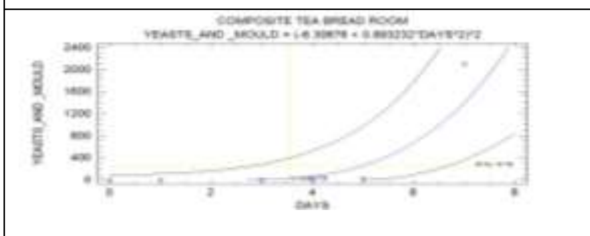	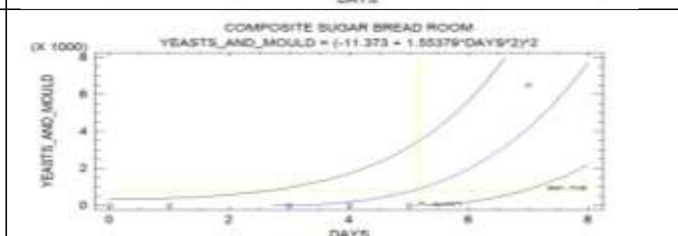
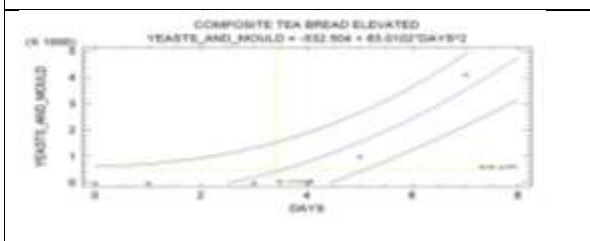	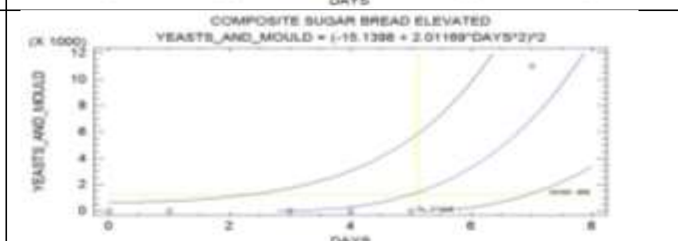
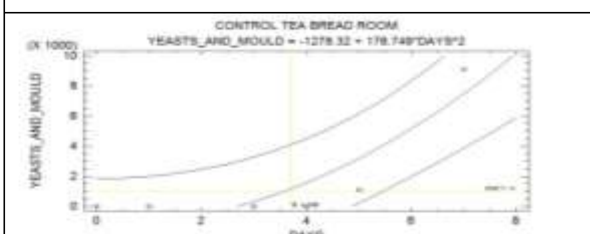	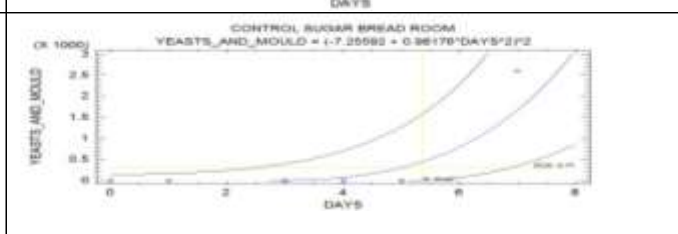
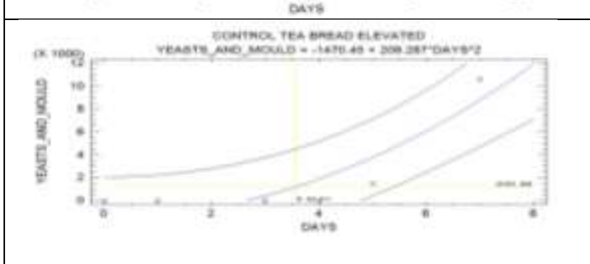	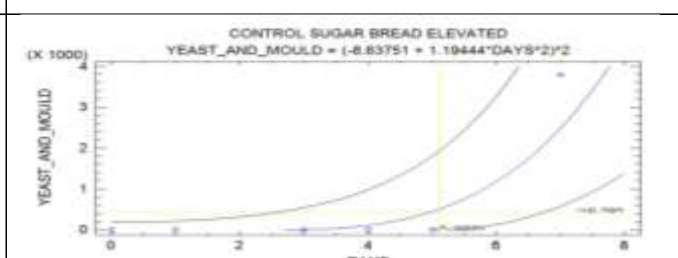

APPENDIX 3a

Table 5: Shelf life models of 100% wheat and 10% HQCF tea and sugar bread according to moisture content

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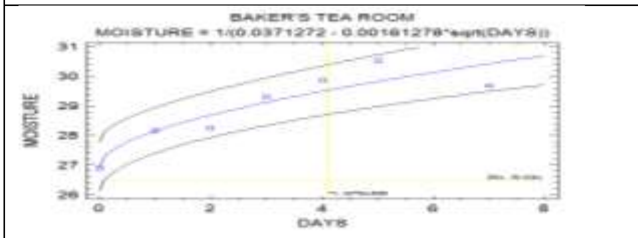

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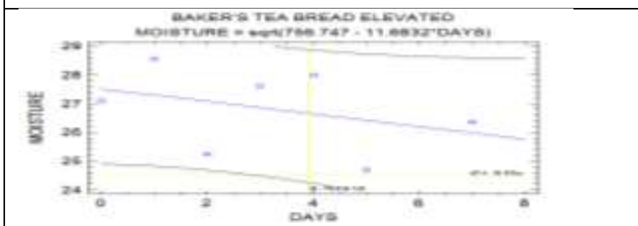

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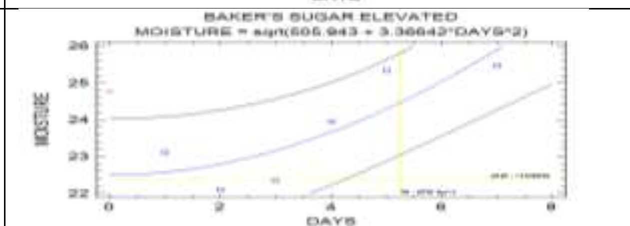

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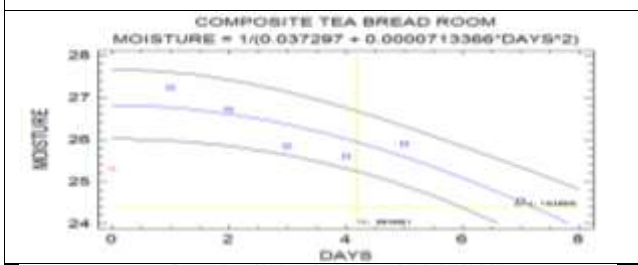

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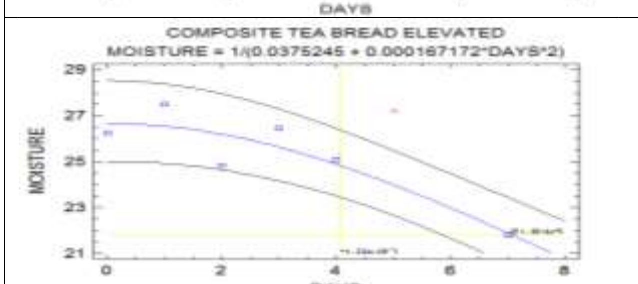

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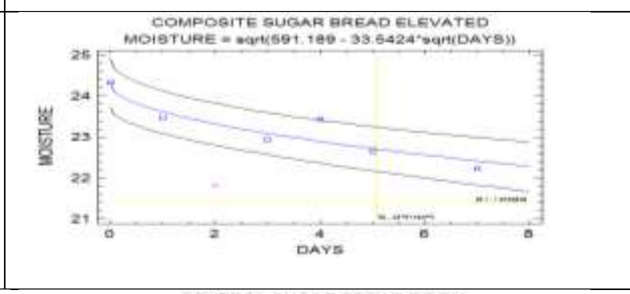

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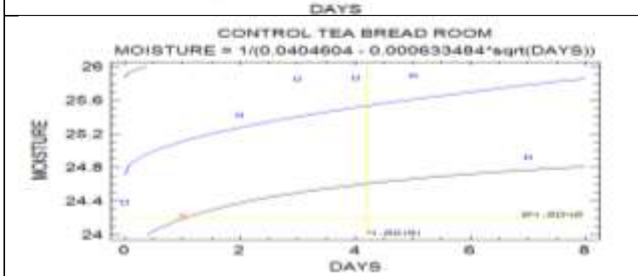

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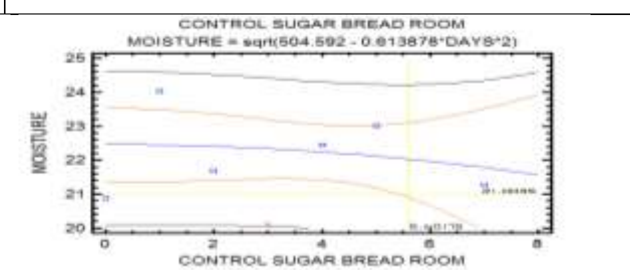

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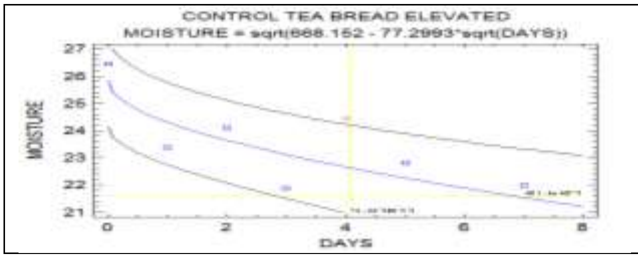
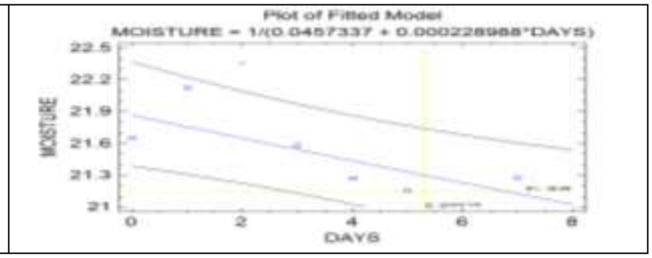
<tr><td>
</td><td>
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APPENDIX 3b

Table 6: Shelf life models of 100% wheat and 10% HQCF tea and sugar bread according to free fatty acid (FFA) content

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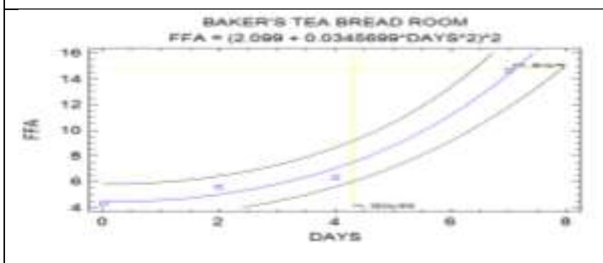
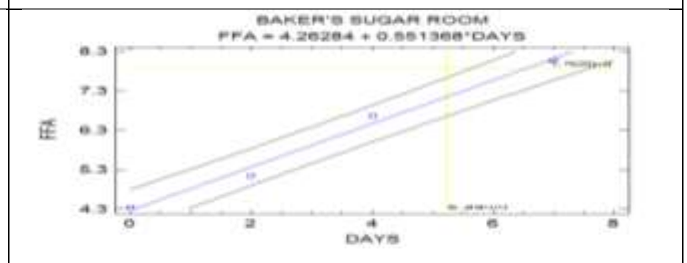
<tr><td>
</td><td>
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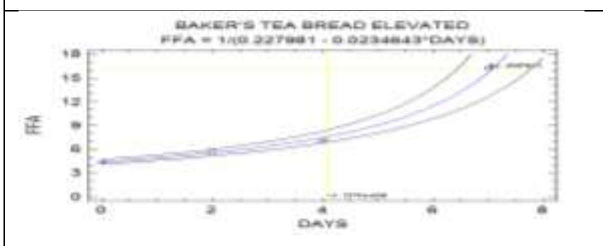
<tr><td>
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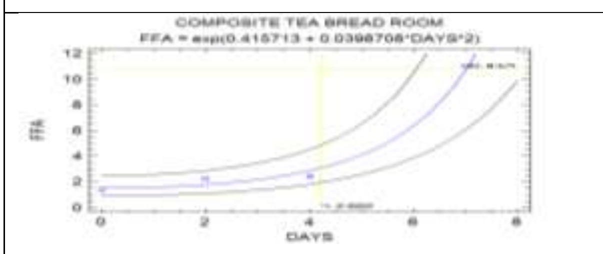
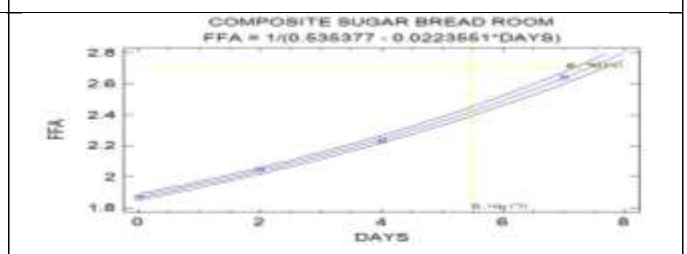
<tr><td>
</td><td>
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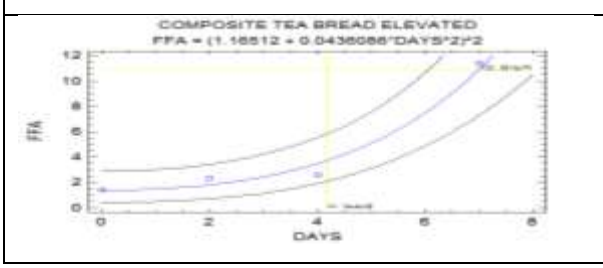
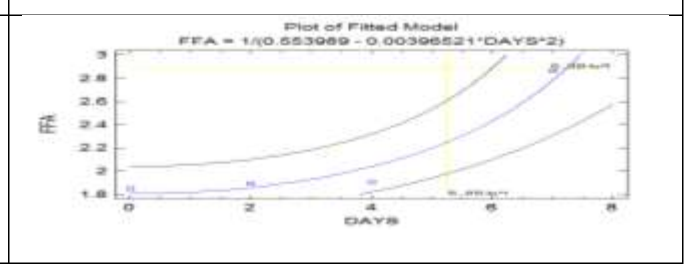
<tr><td>
</td><td>
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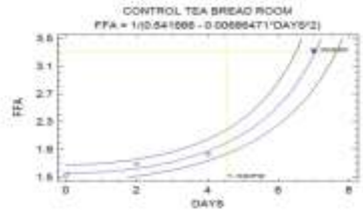
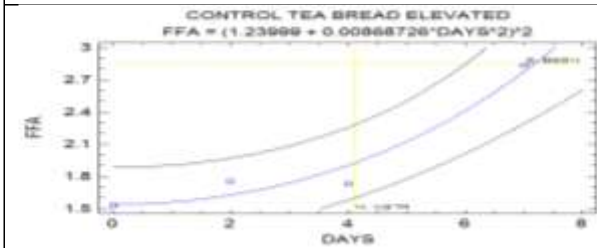
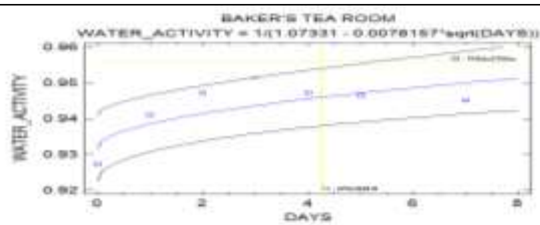
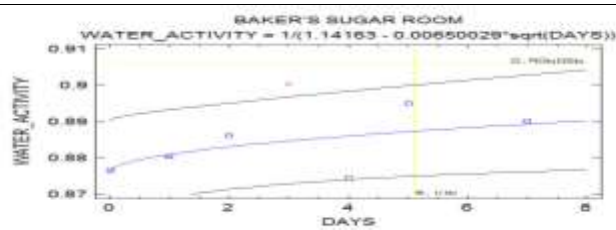
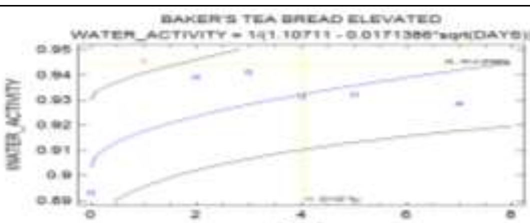
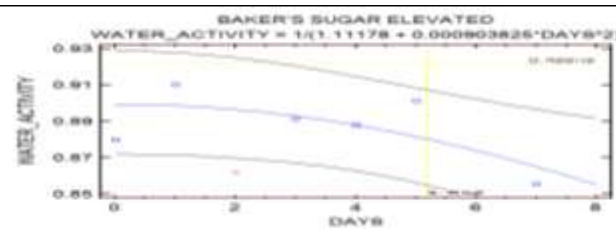
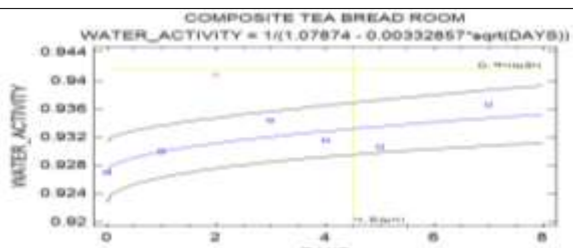
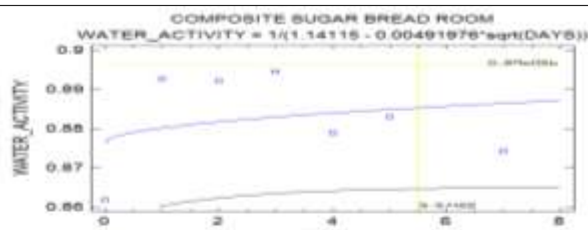
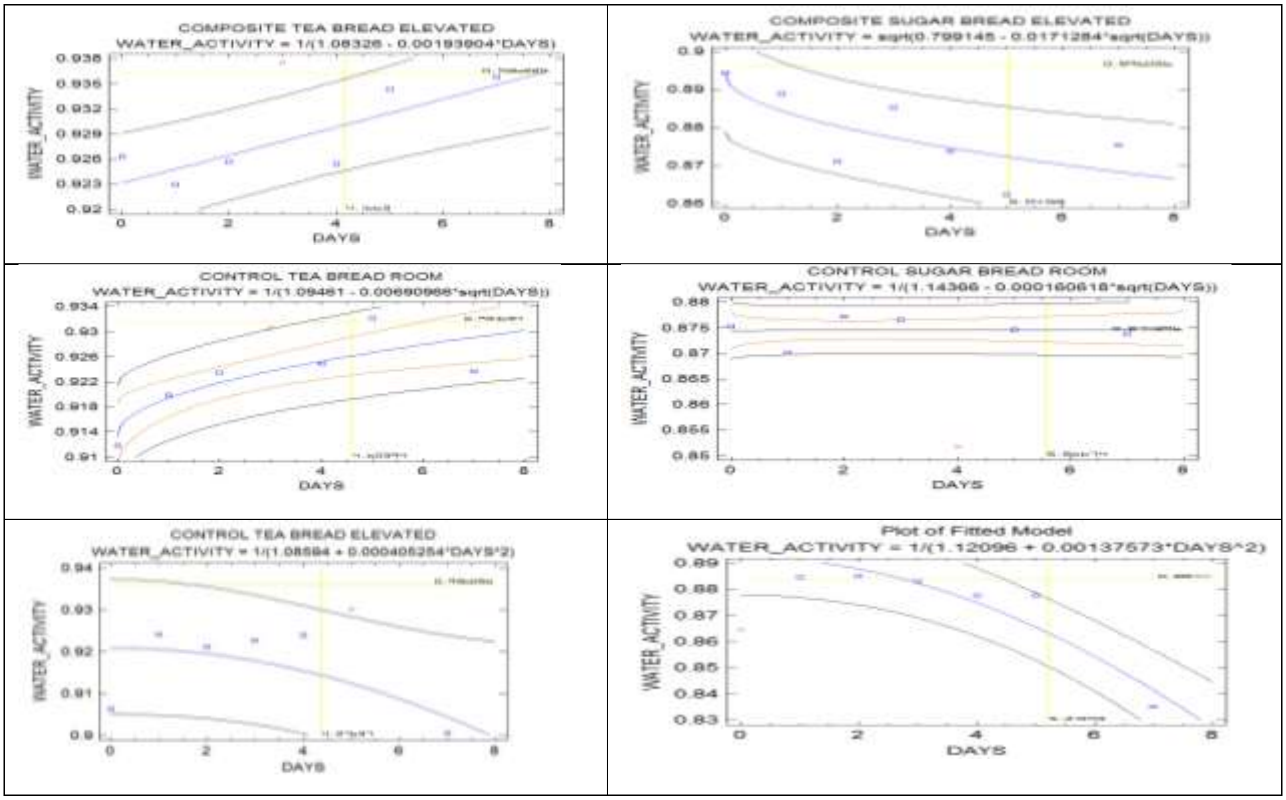
	
	

APPENDIX 3c

Table 7: Shelf life models of 100% wheat and 10% HQCF tea and sugar bread according to water activity

TEA BREAD	SUGAR BREAD
	
	
	


COMPOSITE TEA BREAD ELEVATED
WATER_ACTIVITY = 1/(1.08326 - 0.00193904*DAYS)
COMPOSITE SUGAR BREAD ELEVATED
WATER_ACTIVITY = sqrt(0.799145 - 0.0171284*sqrt(DAYS))
CONTROL TEA BREAD ROOM
WATER_ACTIVITY = 1/(1.09461 - 0.00690966*sqrt(DAYS))
CONTROL SUGAR BREAD ROOM
WATER_ACTIVITY = 1/(1.14366 - 0.000160618*sqrt(DAYS))
CONTROL TEA BREAD ELEVATED
WATER_ACTIVITY = 1/(1.08594 + 0.000405254*DAYS^2)
Plot of Fitted Model
WATER_ACTIVITY = 1/(1.12096 + 0.00137573*DAYS^2)
WATER_ACTIVITY
DAYS

APPENDIX 3d

Table 7: Comparison of baker's recipe with standard recipe for tea bread

INGREDIENT	Baker's recipe (g)	Standard recipe (g)
Sugar	256	218
Salt	33.6	29
Margarine	1056	461
Yeast	1.6	2
Nutmeg	2.16	9.6
Milk	25.6	0
Eggs	5	0
Wheat flour	4000	4000
Water (L)	2.5	2