PRODUCTION AND PRESERVATION OF *KUNUN-ZAKI* FROM MILLET AND GROUNDNUT BLEND

BY

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DECLARATION

I, Towobola MICHAEL, a M.Sc. student of the Department of Microbiology, Landmark University, Omu-Aran, hereby declare that this thesis entitled "Production and preservation of *kunun-zaki* from millet and groundnut blend", submitted by me is based on my original work. Any material(s) obtained from other sources or work done by any other persons or institutions have been duly acknowledged.

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CERTIFICATION

This is to certify that this thesis has been read and approved as meeting the requirements of the Department of Microbiology, Landmark University, Omu-Aran, Nigeria, for the award of Masters of Science (M.Sc.) Microbiology.

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DEDICATION

This work is dedicated to God Almighty, the most merciful, gracious and the only **TRUE** God.

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ABSTRACT

Kunun-zaki, is a traditional non-alcoholic fermented cereal beverage usually made from millet, sorghum or maize. Millet is majorly cultivated in the tropical and sub-tropical regions of Africa and serves as a major raw material for *kunun-zaki* production. Although *kunun-zaki* is commercially produced, it has major challenge of short shelf life of about 48 hours resulting to inconsistency in flavor, taste and acceptability during storage. This study assessed fortification of *kunun-zaki* with groundnut and improvement of shelf life by addition of selected essential oils.

Kunun-zaki was produced from millet and groundnut blend at varying combination of 90%-10%, 80%-20%, 70%-30%, 60%-40%, 50%-50% and 100% millet blends (control), at different steeping durations and from malted millets. Proximate composition and sensory properties of the products were determined using standard methods. Lemon and lime essential oil were hydro-distillated using fresh lemon and lime peel of citrus fruit; the essential oils and extract were used to preserve the *kunun-zaki* at 1% concentration each and their preservative properties were evaluated by microbial storage study on the shelf. Data were analysed using descriptive statistics and ANOVA.

The proximate result obtained showed that *kunun-zaki* from 50%-50% millet and groundnut blend has the highest protein and fat content of 5.50±0.15% and 0.87±0.06%, respectively, while, the control has the lowest protein and fat compositions of 1.50±0.07% and 0.39±0.2%, respectively. The sensory evaluation shows that there were significant differences ($p < 0.05$) observed in the *kunun-zaki* fortified with groundnut and the control in the appearance, taste, flavour and viscosity, but not in the overall acceptability. The lemon and lime essential oils preserved the *kunun-zaki* from

coliform bacteria for seven days as against the samples preserved with chemical preservatives and control sample which had coliform count of 5.5 $\log_{10}$ on the fourth and fifth day of production.

These results showed that groundnut fortification could improve the nutritional value of *kunun-zaki* and lemon and lime essential oils can be harnessed as natural additives to extend the shelf life of the beverage for seven days at ambient temperature.

LIST OF ABBREVIATIONS

ANFs – Anti nutritional factors

AOAC – Association of analytical chemistry

CFU – Colony forming unit

DMRT - Duncan Multiple Range Test.

EDTA – Ethylene diamine tetra acetic acid

FAN – Free amino nitrogen

FAO – Food and agricultural organization

FDA – Food and drug administration

FTU – Photometer turbidity unit

GRAS – Generally regarded as safe

HACCP – Hazard analysis critical control point

HCN – Hydrocyanic acid

IBD – Inflammatory bowel disease

LAB – Lactic acid bacteria

MRS – de Man Rogosa sharp agar

NA – Natural-active antimicrobial

PGA - Poly glutamic acid

SPSS – Statistical package for social sciences

TTA – Total titratable acid

TCA - Tri chloro acetic acid

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of study

Fermented foods have historically been developed in every country in the world because of their properties that potentially promote health. Food fermentation typically includes the conversion, sometimes under anaerobic conditions, of carbohydrates into carbon dioxide, organic acids or alcohol, utilizing bacteria or yeast. Several traditional fermented foods are produced in different countries of Africa (Kabak & Dobson, 2011). However, grains such as millet, maize and sorghum are the most common substrates for fermentation in Nigeria. Legumes include soya beans, groundnut seeds, African locust beans, and others. Lactic acid fermentation make up a large portion of Nigerian fermented foods (Adesulu-Dahunsi, Dahunsi, & Olayanju, 2020).

The production of many foods of African origin is usually affected by undue spoilage as a result of high moisture content. Many food preservation methods like; biological, physical and chemical methods have been explored as remedies to food spoilage (Amit, Uddin, Rahman, Islam, & Khan, 2017).

Food spoilage is a metabolic process that results in food being unwanted or undesirable for human consumption as a result of deviations in nutritional quality and sensory characteristics of food. Spoiled foods may be permissible to consume, i.e. if there are no pathogens or toxins present; such that it does not cause disease, but alterations in appearance, smell taste or texture cause them to be rejected (Adewumi & Arije, 2017). The word *'kunu'* is a *Hausa* word meaning beverage; and "*zaki*" means sweet. *'Kunun-zaki'* is a traditional non-alcoholic fermented sweet-beverage made from millet, sorghum or maize and is believed to be of remarkable nutritional, medicinal, economic

and social significance to its many consumers. In both rural and urban centers, *kunun-zaki* is considered to be an after-meal or refreshing drink (Abah, Ishiwu, Obiegbuna, & Oladejo, 2020). Although Nigeria produces and consumes different types of *kunu*, including: *kunun-gyada, kunun-shinkafa, kunun-akamu, kunun-tsamiya*. However, *kunun-zaki* is the most popular traditional beverage in Northern Nigeria and accepted as beverage of choice (Agarry, Nkama & Akoma, 2010).

Kunun-zaki is commonly consumed within hours of manufacturing because during processing and distribution, microorganisms can contaminate it at any time. The key sources of microbial contamination are soil, water and equipment or utensils used for the production (Adewumi & Arije, 2017).

According to Ageni, Ajibade, Yerima, & Appah, (2017) until recently, approaches to improving food safety and shelf life have focused on chemical storage or the use of physical method such as elevated temperatures (Pasteurization, Tyndallization). These types of methods has many disadvantages, such as the proven toxicity of many of the chemical preservative components, for example, nitrites. Another disadvantage is changes in the organoleptic and nutritional properties of foods. The use of chemical preservatives has been discouraged by recent market developments in the purchase and consumption of generally healthy, minimally processed, and non-additive foods (Ageni et al., 2017).

Tradition has relied on the use of plant metabolites (natural chemicals found in plants) for their therapeutic and preservative properties. These plants and their metabolites can be put into culinary use. Lemon and lime are examples of such plant. Hsouna and group in 2017, reportedly used lemon essential oil in the control of *Listeria monocytogenes* in

minced beef meat during storage. Hence, the potential in lime and lemon essential oil can be harnessed as preservative in beverages (*kunun-zaki*).

1.2 Statement of problem

Kunun-zaki beverages, though commercially produced, have major challenge of short shelf life of about 48 hours resulting to inconsistence flavor, taste and acceptability during storage. Consumers are becoming more conscious of artificial preservatives. Therefore there is a need to explore ways to reduce spoilage using locally available preservatives; that should lead to products that are safe, reliable and accessible.

1.3 Justification

There is a growing interest to improve preservation methods for fermented beverages. However there is at present a dearth of information on the preservation of *kunun-zaki* using lemon and lime essential oil. Thus, this study explored the application of lemon, lime oil (essential oil) and hydrogen peroxide (H_2O_2) as potential preservatives in *kunun-zaki*. Positive outcome of this exploration should enable its manufacturers and distributors to extend the shelf life of the beverage, and improve its safety to the consumers.

1.4 Aim

This study was aimed at enriching the nutritional value, sensory properties and shelf life of *kunun-zaki* from millet and ground nut blend.

1.5 Objectives

i. To determine the effect of millet: groundnut ratio on the proximate content, sensory properties, physicochemical compositions and microbial enumeration of *kunun-zaki*.

ii. To establish the effect of steeping time on the sensory properties, physicochemical compositions and microbial enumeration of the *kunun-zaki*.

iii. To evaluate malting effect on the microbial succession, sensory properties and physicochemical compositions of the *kunun-zaki*.

iv. To assess the effect of starter culture consortium (*Lactobacillus bulgaricus, Streptococcus thermophillus* and *Lactobacillus acidophilus)* on the sensory properties, microbial succession and physicochemical properties of *kunun-zaki*.

v. To determine the effectiveness of essential oils from lemon and lime in increasing the shelf life of *kunun-zaki.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Cereals

Cereals are of the family *Poaceae* that produce starchy seeds suitable for food; they are also known as grains. The nutritional content of grains are similar; they have high carbohydrate but low protein contents and are naturally poor in calcium and vitamin. Flours used to make breads and the refined flours are fortified by addition of the nutrients that they lack. Commonly cultivated grains are; wheat, rice, sorghum, rye, oats, millet, barley and maize (Koehler & Wieser, 2013).

These cereals are used for various traditional and technologically advanced processes worldwide. Cereal-based foods diverge widely in the structural, storage, and sensory characteristics of their grains. They differ in nutritional value due to basic differences in nutrient content and changes that occur during processing, which can be beneficial or detrimental. Cereals products, including starches, syrups, proteins and fiber isolates, as well as food ingredients, are also raw materials for the manufacturing of alcoholic beverages. Moreover, large quantities of cereal return to the food chain as livestock feed (Papageorgiou & Skendi, 2018). They remain the staple meal in most diets, even though they are not given high value as a nation becomes more affluent. The advantages of cereal foods as a source of complex carbohydrates and as foods that can replace some of the energy already supplied in the diet by fat were emphasized by dietary guidance (Laskowski, Górska-Warsewicz, Rejman, Czeczotko, & Zwolińska 2019). Cereals make a major contribution to the diet, they cannot sustain life on their own because vitamins A, D, C and iodine are missing. Cereal proteins, for example, do not contain

certain amino acids, particularly lysine. Refined and unrefined cereals products have advantages and disadvantages, some nutrients and fiber are lost during refinement, but the body is better able to make use of those nutrients. The importance of fortified cereals is to increase levels of specific vitamins (Spiro & Buttriss 2014).

Moreover, they have abundant bioactive components, with bioactivities as antioxidant, anti-inflammatory, satiety, cholesterol-lowering, antidiuretic, and others (Pessione & Cirrincione, 2016).

2.1.1 **Millet**

Millet (*Pennisetum glaucum*) is not as common as other grains for food, but the nutritional composition can be comparable to that of other cereals. Finger millet has between 5% and 8% protein content (Tiwari, Sharma, Sood, Jaiswal, Pachauri et al, 2020). Like other cereals, it has fiber, carbohydrates, ash and fat material. It is grown primarily in low-rainfall regions and is able to withstand adverse agro-climatic conditions. More than 80% of the produce is used for human consumption. Without significant quality changes, millet can be stored for long periods if the kernel is intact. Millet flour can be used in pizza, cookies and pasta products to replace wheat flour. The consistency of the grain, however deteriorate after millet is milled into food. In Africa there are five main forms of millets used (Jukanti, Gowda, Rai, Manga, & Bhatt, 2016).

In using millet to make local beverage, the dry grain is either dehulled or ground whole. The grain is soaked, wet milled, cooked or hot water is added and sieved into *kunun-zaki*, a local non-alcoholic drink. *Tuwo* is a meal (hard paste) prepared from millet flour, the dry millet grain is grounded or milled into flour from which snacks are prepared. The grains are soaked (steeped) in water for 12-24 hours, it is wet milled, sieved and the filtrate allowed to settle by standing for about eight hours, the surface

water is decanted and the slurry is used to prepare *Ogi*, *Akamu*, and *Kafa*, all of these are famous fermented porridge products in Nigeria (Aka, Konan, Fokou, Dje & Bonfoh, 2014). Millets, depending on the process, are soaked whole or in part, allowed to sprout, to make malted beverages such as *Pito* and *Burukutu*, the sprouted grains are dried and used. The bran and germ are used as livestock feed (Aka, Konan, Fokou, Dje, & Bonfoh, 2014).

2.2 Legumes

Legumes rank second among grains as human food, but they may serve people in a wider variety of other ways than grasses (Voisin, Guéguen, Huyghe, Jeuffroy, Magrini & Meynard, et al., 2014). Peas, large beans, garbanzo, common beans, navy beans, kidney beans, or butter beans, soybeans, bambara groundnut, African yam beans, groundnuts among others are legume seeds used as food for men. Legumes typically contain 17-25% protein (approximately double that of most cereals), with the exception of soybeans that contains about 40% protein and 40-70% carbohydrates. Legume seeds are also strong sources of minerals such as phosphorous and iron. Legume seeds are usually low in fat and oil, with the exception of soybeans and groundnuts, which contain 18% and 48% oil, respectively (Voisin et al., 2014).

Legumes are a good protein source; they have good lysine and tryptophan content, but poor in amino acids that contain sulphur such as methionine and cysteine. Legumes are consumed worldwide as a major protein source and are cheaper source of protein compared with animal protein products like meat, fish, poultry, and egg. Legumes are also good protein supplements in areas where derivatives of staple foods from cereals, roots and/or tubers are high in carbohydrates but low in protein (Schönfeldt, Pretorius & Hall 2016).

2.2.1 **Groundnut**

Groundnuts (*Arachis hypogaea*), also called peanut, is a legume, a staple food, cultivated majorly because of its edibility. The seeds have a delicious nutty flavour and can be eaten in their raw state or roasted (Bagheri, Kashaninejad, Ziaiifar, & Aalami 2019). Groundnut is commonly cultivated in the tropics and sub-tropics, it is a significant crop for both small and large scale commercial farmers. Peanut pods grow under the ground (geocarpy) rather than above the soil. Peanut belongs in the family *Leguminosae*, the family of beans, or peanut. Peanuts root nodules harbors nitrogen-fixing bacteria like most other legumes. This nitrogen-fixing potential means that peanuts does not need nitrogen containing fertilizer; it rather enhances soil fertility, which makes them important in crop rotations (Martínez-Hidalgo & Hirsch, 2017).

Peanuts are comparable to tree nuts like almond and walnuts in flavor, nutritional value, and are also eaten similarly in Western cuisines as a culinary nut. When the ovary wall of a fruit becomes hard at maturity it is botanically known as a "nut", the peanut is not a standard nut using this criterion, however, groundnuts are typically referred to as nuts for cookery purposes and in the general use of the English language (Sinha, Sidhu, Barta, Wu, & Cano, 2012). Groundnut seed contains 44%-56% oil, 22%-30% protein, it is also rich in minerals such as Ca, P, K and Mg (Savage & Keenan, 1994). It contains vitamin E, B-6, fiber, unsaturated fatty acids and protein. Studies has shown that groundnut has the potential to reduce age related impairments in cardio metabolic health and cognitive function that increases with ageing (Coates, Barbour, Buckley, Bryan, & Howe, 2016).

2.3 Citrus

Citrus belongs to the genus of flowering trees and shrubs in the family *Rutaceae* which comprises of about 140 genera and 1300 species (Hsouna et al., 2017). Plants in the genus produce citrus fruits, such as oranges, lemons, lime and grapefruits. *Citrus lemoni (*Lemon*)* is one of the genus's most common plants. According to the Food and Agricultural Organization (FAO), citrus is one of the most valuable crops in the world in terms of production, with 240,780 million metric tons produced in 2013 (FAO, 2016). Citrus plants are grown in many countries all over the world and among the major African citrus-producing countries is Tunisia. Citrus essential oils are made up of a variety of useful natural products that can be classified as mixtures of hydrocarbons, oxygenated compounds, and nonvolatile residues such as: sterols, alcohol, terpenes, sesquiterpenes, aldehydes and esters. Citrus plants are a major source of essential oils, which have been extensively researched for their possible applications in the food industry (Ju, Xie, Guo, Cheng, Qian & Yao, 2019).

2.4 Fermentation

Fermentation is a suitable method of biochemical alteration of the primary matrix of food produced by microorganisms and their enzymes (Nkhata, Ayua, Kamau, & Shingiro 2018). Fermentation Improves the bio accessibility and bioavailability of nutrients from various crops and extending the shelf life and organoleptic properties (Kumari & Platel, 2020). The major benefit of fermentation is the conversion of carbohydrate and other sugar into improved products such as the conversion of juice into wine, grains into beer, starch into alcohol and carbon dioxide, to leaven breads and the conversion of sugars in vegetables and fruits into preservative organic acids (Saranraj, Stella, & Reetha, 2012).

2.4.1 **Fermented beverages**

Fermented beverages are generally portable fermented drinks that are nourishing, thirst quenching and refreshing. The nutrients present in the fermented beverage provide the nourishment. These beverages have sugar content that provides the energy needed for nourishment (Vara, Karnena, & Dwarapureddi, 2019). Fermented foods are produced by traditional people using their native knowledge from locally available raw materials of plant or animal sources either naturally or by adding starter culture containing microorganisms. Fermentation modifies the substrates organoleptically and biochemically into edible products that are culturally and socially acceptable to the consumers (Tamang, 2010).

2.4.2 **Types of Fermented beverages**

There are three main forms of fermentation depending on the fermentation pattern: solid, liquid and semisolid fermentations. Products in solid fermentation include *Tempee, Banku, Zambu* and *Iru*. In liquid fermentation, the substrate is composed of liquid or submerged in liquid, for example, Soy sauce, *Kunun-banga, Ogi* And in semisolid fermentation, the substrate is moist, e.g., cheese.

There are two types of fermented foods, based on the use of microorganisms (Septembre-Malaterre, Remize, & Poucheret, 2018).

(1) Spontaneous or natural fermentation: Without the addition of starter cultures raw materials ferment spontaneously by microflora present on the raw ingredients or in the atmosphere such as in *Gundruk* and *Kunun-zaki* fermentation (Nout & Aidoo, 2011).

(2) Controlled fermentation: This is achieved by incorporating starter culture on purpose. There are two forms of controlled fermentation:

(a) Single culture fermentation: using a single strain of pure microbial culture and

(b) Diverse culture fermentation: using two or more cultural strains of microorganisms, or assorted innocula, e.g., yoghurt and cheese (Haruna et al., 2016).

Most native fermented foods are produced using solid raw materials (substrates), which enable the raw materials to ferment naturally or through inoculation with starter cultures (Nout & Aidoo, 2011).

Nonalcoholic fermented foods are classified into eight categories based on their substrates: (1) fermented fish (2) fermented soybean and non-soybean legumes; (3) fermented meats; (4) fermented vegetable; (5) fermented cereals; (6) fermented milk; (7) fermented root/tuber products; and (8) miscellaneous fermented products (Tamang, Watanabe & Holzapfel, 2016).

2.5 Microorganisms in Fermented Foods

Numerous microorganisms from the environment are found in fermented foods, these organisms include bacteria, yeast, mycelia, or filamentous molds. They are found in or on ingredients, plant or animal, utensils, and containers. The major groups of microorganisms associated with ethnic, fermented foods are bacteria, yeasts, and fungi (Jyoti & Kasipathy, 2010). During food fermentation, microorganisms change the chemical constituents of raw materials and increase the nutritional value of the products, enrich balanced diets with improved flavor and texture, preserve perishable foods, and synthesize bioactive compounds that promote health (Schutte, 2013).

2.5.1 Bacteria

Bacteria are dominant microorganisms which plays important roles in the production of many fermented foods, and lactic acid bacteria (LAB) constitute the majority found

in fermented foods. *Bacilli, micrococcaceae*, etc., are also involved in the fermentation of foods.

2.5.1.1 Lactic Acid Bacteria (LAB)

These are gram-positive, catalase-negative, and non-spore-forming; they are anaerobic to micraerophilic, fastidious, acid-tolerant; they are strictly fermentative bacteria with lactic acid as the major end-product of sugar fermentation. *Lactobacillus, Pediococcus, Enterococcus, Lactococcus, Leuconostoc, Oenococcus, Streptococcus*, and *Tetragenococcus* are some of the LAB genera isolated from fermented foods. *Corynebacterium, Vagococcus, and Weissella. Propionibacterium* and *Bifidobacterium species* are usually present in fermented milks and are also known to be LAB (Mendes-Ferreira & Mendes-Falia, 2020). Among the genera of LAB, *Lactobacillus* (both heterolactic and homolactic) and *Pediococcus* are the most dominant genus in fermented foods. LAB produce organic acids at the end of fermentation and lactic acid is the distinguishing end product which reduces the pH of the medium to a level where the growth of pathogenic, putrefactive, and toxicogenic bacteria are subdued. LAB are conferred the GRAS (generally recognized as safe) status in foods. Numerous species of LAB can also act as "biopreservatives" and some of them are used commercially. LAB modify the flavor of the original ingredients and improve the nutritional value of foods during fermentation (Zarour et al., 2017).

2.5.1.2 Yeasts

Yeast are single celled microorganisms which belongs to the fungus kingdom. Reproduction is asexual by mitosis through the process of budding. Yeast sizes are different depending on their species and environment; they vary in size from 3-4 µm in diameter, though some can grow to about 40 µm in size. The species *Saccharomyces*

cerevisiae converts carbohydrate to alcohol and carbon dioxide through fermentation process (Walker & Stewart, 2016). Many fermented foods and beverages contain yeasts; approximately 21 yeast genera and several yeast species have been identified from fermented foods and beverages (Tamang, Watanabe & Holzapfel, 2016). In almost every country in the world, yeast food fermentation is performed along with, or in addition with bacterial and fungal fermentation. During fermentation of any substrate, *Saccharomyces* ferments sugar to develop metabolites that inhibit mycotoxin-producing mold and has many enzymatic activities such as lipolytic, pectinolytic, glycosidasic, proteolytic and urease activities (Tamang et al., 2020).

2.6 Nutritional benefits of fermentation on foods and beverages

Microorganisms synthesize enzymes such as cellulases which are not synthesized by humans. Equally, peptinases soften the texture of food and then liberate sugar for breakdown in fermented foods. The LAB acidify starchy gruel through hydrolyzing starch into shorter chains of dextrose and glucose, thereby reducing the viscosity of the porridge and then increases energy density of the food for absorption (Peyer, 2017).

2.6.1 Enhanced nutritional values

Fermentation process improves the level of vitamins in products; Sorghum beer (*Burukutu*) contains high quantity of riboflavin and nicotinic acid which are important vitamins. The beer (*Burukutu*) is also rich in vitamin B_{12} which is very important for people with low meat intake and vegetarian. *Saccharomyces cerevisiae* is able to concentrate large amount of thiamine, biotin and nicotinic acid hence enriching the fermented product (Silva, Reto, Sol, Peito, Peres, et al., 2011). Fermentation processes improve on vitamin level in the fermented product, *Saccharomyces cerevisiae*

concentrate large amount of biotin, nicotinic acid and thiamine there by improve quality of the product (Sharma, Garg, Kumar, Bhatia, & Kulshrestha, 2020).

2.6.2 Reduction of antinutrients

Cereals contain anti-nutritional factors (ANFs). These components include phytate, phytic acid, polyphenols and tannins (Melini, Melini, Luziatelli, Ficca, & Ruzzi 2019). The presence of phytic acid plays an essential role in the nutritional quality of the food where it is found; this is because phytic acid has the ability to obstruct enzymatic activity and form chelates with metal ions, (i.e., with zinc, iron, calcium, and magnesium), thereby reducing their absorption, and thus their bioavailability (Samtiya, Aluko, & Dhewa, 2020). Phytic acid present also in legume seeds binds with minerals like iron and zinc thereby reducing their absorption when eaten. However, phytate can be broken down during fermentation, thereby making those minerals available in the fermentation product. An example is the Africa locust bean seed fermentation (*iru*). Anti-nutritive substances may be degraded by microorganisms that inhabit fermented foods, thereby making inconsumable products safe for consumption (Tamang, 2010).

2.6.3 Health benefit

The health benefit of fermented nonalcoholic food beverage is as a result of low cholesterol, high phenolics, high fiber and gluten-free contents. Fermented nonalcoholic food beverage products were used by people with cardiovascular diseases to reduce their level of cholesterol and for the vegetarian people to increase their vitamin B12, and thus avoid depression (Dongmo, Procopio, Sacher, & Becker 2016). Malted cereals are the most suitable substrates for the growth of LAB and are precursor for aromatic compounds (Dongmo et al., 2016, Gebremariam et al., 2015,). *L. acidophilus* and mould produce antibiotics and bacteriocins which are beneficial to the

intestinal floral, *L. plantarum* is a probiotic that has good result of usage in patients with symptoms of inflammatory bowel disease (IBD) (Maftei, 2019).

2.6.4 Shelf life extension and food protection

Fermenting microorganisms are employed to generate new products with improved sensory and nutritional qualities; they produce numerous metabolites that inhibit the growth of spoilage and/or pathogenic bacteria. These metabolites include organic acids such as lactic acid, propionic acid, acetic acid, etc. that decrease the initial pH value, creating an acidic environment in the food matrix; this acidic environment is unsuitable for spoilage and pathogenic microbes and therefore extend the shelf life of the fermented product (Nyanzi & Jooste, 2012). Antimicrobial peptides, bacteriocins, are produced by LAB and are partially related to the extended shelf life of fermented products (Shiferaw & Augustin, 2019).

2.7 Biochemistry of malting

During germination, grain components such as starch and protein experience qualitative improvements. Grains germination depends on the activities of enzymes that break down food stored in the seeds into functional product; and these enzymes activities can only take place when the seeds absorb water into their microphyle. Malting biotechnology uses natural germination events to provide favorable mashing content, so the key requirements for malt consistency are intact enzymes, starch supply and viability of the seeds (Gorzolka, Lissel, Kesslerb, Loch-Ahring, Niehaus, 2012). The diastase enzymes present in sorghum are the α-amylase and β-amylase which are the main source of biostatic activity, and as a major malting quality efficiency of malted products (Owuama, 2019).

During malting, breakdown of protein takes place as the grain structure changes to produce shoots and roots; the proteolytic activity in sorghum are augmented during malting by about 10% (Guzmán-Ortiz, Castro-Rosas, Gómez-Aldapa, Mora-Escobedo, Rojas-León et al., (2019). Though malting does not complete the development of new plant protein, malting results in a significant increase in free amino-nitrogen (FAN) levels over those present in the original grain (Hill & Stewart, 2019).

2.8 Microbiological quality of *kunun-zaki*

Different microorganisms are associated with the steeping, processing and storage of *kunun-zaki* fermentation (Akani & Nwankwo, 2018). The predominant microorganisms are the bacteria Lactobacilli, and the mould particularly *Aspergillus* and *Penicillum (*Ayo-Omogie & Okorie, 2016). The Yeast *Saccharomyces cerevisiaea* is isolated towards the end of 8hours fermentation. Yeast and numerous LAB are found in combination during the fermentation of cereal and are commonly the wide microbial community associated with varieties of traditional food and beverage fermentation. *Lactobacillus fermentun* appears in the intermediate and final stages of *kunun-zaki* fermentation; they impact the flavour and aroma typical of *kunun-zaki* (Agarry, Nkama, & Akoma, 2010).

Coliform standard is adopted for food and beverages; their evidence in food are pointer of sanitary and spoilage quality of food and beverages (Buchanan & Oni, 2012). The quality and protection of food is closely related to some significant microorganisms act as pathogenic and food poisoning organisms such as *Salmonella* and *Clostridium* (Forsythe, 2020).

2.9 Preservatives

Preservative are substances to slow down or to arrest microbial growth in order to prevent food spoilage and ensure safety (Rawat, 2015). They efficiently control growth or kill undesirable microorganisms in food and do not adversely affect the acceptance value of the food; thus, preservatives enhance food safety and extending shelf life (Nath, Chowdhury, Dora & Sarkar, 2014).

Preservatives are classified into two broad classes, namely;

(a) Natural preservatives; (vinegar, salt syrup, sugar, natural spices, honey and edible oil.

(b) Chemical or synthetic preservatives; (sorbate, nitrates, nitrites of sodium or potassium, sulfites, glutamates, benzoates and glycerides). In foods only one of the chemical classes of preservative is allowed for use in a food per time (Seetaramaiah, Smith, Murali, & Manavalan 2011).

2.9.1 Characteristics of a good preservative

- A good preservative must not be harmful to consumers health
- Must not putrefy into toxic substance when consumed
- It should have suitable or proper method of manufacturing
- It must have effective mode of action as a preservative
- Must not slow down digestive activities
- Must be easily identified and quantified for quality control of foods (Nath et al., 2014).

2.10 Preservation of beverages

Irrespective of all preservation efforts, contamination is still possible (Holck, Axelsson, McLeod, Rode, & Heir, 2017). However, there are other control measures to prevent microbial growth and activity of spoilage in fermented products which are; physical (aseptic packaging, filtration and heat) (Juvonen, Virkajärvi, Priha & Laitila, 2011). Preservation method depends on the degree of carbonation, acidity, the antimicrobial components of the end product, the desire of the consumer for preservative-free products.

Factors to be considered in selecting the type of preservation method are nutritional status and pH of the product. In whatever preservation method employed, success is achieved only when the hazard analysis critical control point (HACCP) is properly monitored and put in place, when the initial contamination of the product is only slight (Juvonen et al., 2011).

2.11 Biopreservation

Bio-preservation is a technique of extending the shelf life of food by using natural or controlled microbiota or antimicrobials (Singh, 2018). Bio-preservation is the use of "natural" compounds or elements, e.g. materials derived from microbial or plant metabolism, for the preservation of foods. One of the ancient methods to naturally improve the quality of food and to prevent food spoilers and pathogens in food is by fermentation. Apart from releasing compounds derived from microbial metabolism, microbial fermenters can also develop in food to create competition against the resident microbiota.

Hence, one can broadly define bio preservation as the use of naturally present or intentionally applied healthy plants, microorganisms and/or their antimicrobial substances to prolong shelf life and increase food protection. Lactic acid bacteria are the major microorganism in food biopreservation, because they are safe to consume and are generally regarded as safe (GRAS) owning to their long record of safe use in food, and are quickly becoming the dominating microbiota during spontaneous or started fermentation and remain during the storage of fermented foods. Beneficial bacteria are typically carefully selected in this process to control spoilage and render pathogens inactive, LAB and their metabolites are the organisms of special interest used for this purpose, they are able to show antimicrobial activities and are useful in effecting distinct texture and flavour to the food. Lactic acid bacteria produce major compounds such as bacteriocin, hydrogen peroxide and organic acids. Bacteriocin is a proteins that has antimicrobial activity and can be divided into four based on structure, size and post-translational modifications (Singh, 2018); Non-immunogenic, non-toxic, broad spectrum bactericidal activities and thermo-resistance characteristics. Nisin, a common LAB bacteriocins with wide use in food industries, is known to be good biopreservative agents (Müller-Auffermann, Grijalva, Jacob, & Hutzler, 2015) and is approved by Food and Drug Administration (FDA). Bacteriophages and endolysins, in addition to LAB metabolites, have a promising role in food processing, preservation, and safety. Biopreservation systems in foods are gaining popularity among consumers and industries. Bacteriocins isolated from LAB are regarded as safe additives (GRAS), useful to control the frequent development of pathogens and spoiling microorganisms in foods and feed. Due to increased antibiotic resistance and the demand for organic products with less chemicals, there is a need to explore new

alternative preservatives, in order to reduce the abusive use of preservative drugs (Parada, Caron, Medeiros, & SoccolL 2007).

2.11.1 **Bacteriocins**

Bacteriocins are low-molecular-weight antimicrobial peptides produced by bacteria; nisin is the most studied and *Lactococcus lactis subsp. lactis* strains are responsible for its production. It is the only bacteriocin that has been approved for specific food applications. Nisin (E234) has the ability to produce pores in cell membranes and is majorly effective against Gram-positive bacteria (Sözbilen, 2019). It has also been shown to be effective against spore-formers at concentrations of 5–10 µg/ml. AS-48 Enterococcal bacteriocin (enterocin) has wide antibacterial activity against Gram-positive bacteria, including spore-formers and many food pathogens. AS-48 (2.5 µg/ml) killed *Alicyclobacillus acidoterrestris* vegetative cells and spores in commercial fruit juice stored at 37^0C for 14 days (Khan, Flint, & Yu, 2010). Drawback of bacteriocins is that they have only slight antimicrobial activities, hence are best used in combination with other preservation methods (Khan, Flint, & Yu, 2010).

2.11.2 **Lysozyme**

Lysozyme is a cell wall lytic antimicrobial enzyme that can be used to suppress microbial growth in food and beverages. Lysozyme was used in a novel way to prolong the shelf life of unpasteurized beer, and it had a powerful inhibitory effect against LAB. The sensory test showed that lysozyme had no detrimental effects on the beer taste (Silva et al., 2010). Although the use of lysozyme in beverages is not allowed in

Europe, it is GRAS, with a maximum limit of 500 mg/kg for berries and ciders (Younes, 2018).

2.11.3 Spices, herbs and their derived essential oils and extracts

They contain significant amounts of natural active antimicrobials (NAs) (Tajkarimi, Ibrahim, & Cliver 2010). The active ingredients help plants defend themselves against pathogens and radiation. More than 1340 plant species have been identified as possible sources of antimicrobial compounds. These antimicrobial compounds in plants are frequently found in the essential oil fractions. The constituents of essential oils include a diverse range of chemicals, but phenolics are primarily responsible for antimicrobial activity (Tajkarimi et al. 2010). The phenols in plants can be grouped into phenolic acid and simple phenol, e.g. Gallic and vanilla (Aneja, Dhiman, Aggarwal, & Aneja 2014).

Plant phenolic compounds are known to have the ability to inhibit the growth of spoilage microbes and bacterial pathogens associated with beverages. Multiple mechanisms are involved in the inhibition of microbial growth (Lacombe, Wu, Tyler, & Edwards 2010). Phenolic compounds extracted from cloves, cinnamon and thyme, have a broad antimicrobial range (Zhang et al., 2019). They are mostly active at 0.05–0.1% concentrations (Tajkarimi et al., 2010).

Although plant-based antimicrobials are unlikely to work miracles, their use in conjunction with other mild preservation methods can have a synergistic impact, allowing for chemical-free preservation while also enhancing the nutritional and sensorial qualities of beverages (Bagchi, 2014).

2.12 Chemical preservatives

Chemical preservatives are artificial or synthetic substance that inhibit microbial activities and helps to preserve food for a longer period of time without affecting its natural properties. Chemical preservatives contain antimicrobial agents and antioxidants. Antimicrobial agents are used to inhibit the activities of microorganisms. Example of antimicrobial chemical agents are nitrites, calcium propionate, benzoates, sodium benzoates, EDTA and sorbate. Antioxidants are the agents which are used to prevent the oxidation caused in the food material (Seetaramaiah, Smith, Murali, & Manavalan, 2011). Example of chemical preservatives are as follows;

(a) Sulphites (such as sulphur dioxide)
(b) Sorbates (potassium sorbate, sodium sorbate)
(c) Nitrites (sodium nitrite)
(d) Benzoate (benzoic acid, sodium benzoate)

2.12.1 Harmful effects of chemical Preservatives

Chemical preservatives are blamed for residual toxicity, carcinogenicity, and teratogenicity (Mologноni, Daguer, Motta, Merlo & Lindner, 2019). As a result of these factors, customers have become more cautious when selecting goods containing chemical preservatives. Certain combinations of food additives, on the other hand, result in the formation of harmful and hazardous compounds, such as the carcinogenic compound benzene, which is formed when benzoic acid reacts with ascorbic acid (Del Olmo, Calzada, & Nuñez 2017). When nitrates are consumed, they are converted to nitrites, which react with hemoglobin to form methemoglobin, causing suffocation, loss of consciousness, and death, particularly in infants. Another consequence is the development of carcinogens such as nitrosamines when stomach proteins react with

nitrites (Bryan, Alexander, Coughlin, Milkowski, & Boffetta, 2012). Sulfite-containing food preservatives cause severe allergic reactions and asthma worsening (Vally & Misso, 2012). When toxic paraben chemicals are combined with methylisothiazolinone, it has a detrimental effect on fetal brain development when consumed by pregnant women (Petric, Ružić, & Žuntar, 2021). When ascorbic acid and sodium benzoate are present in drinks, benzene is formed; this is increased in drinks when they are stored at high temperatures for long periods of time. Though the frequency and levels of benzene formation in the past did not pose a public health danger, the beverage industry has developed strategies to avoid or reduce its occurrence. As a result of the introduction of modern processing methods, the use of benzoates has decreased in recent years.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample collection

Millet, groundnut, ginger, cloves and black pepper were purchased from a local market in Bida, Bida Local Government Area of Niger State, Nigeria. Lemon and lime fruits were purchased in Omu-Aran market, Irepodun local Government Area of Kwara State, Nigeria.

3.2 Sterilization of materials

All materials were sterilized as described by Fawole & Oso (2001). The work bench was disinfected using 70% ethanol. All media were also sterilized using autoclave at 121 ^{o}C for 15 minutes.

3.3 Preparation of media

Nutrient agar, Potatoes dextrose agar (PDA), de Man Rogosa Sharpe agar (MRS) and MacConkey agar were prepared as described by the manufacturer's instructions and were all sterilized in the autoclave at 121 ^{o}C for 15 minutes.

3.4 *Kunun-zaki* production from millet and groundnut blends

Grain of millet (8150 g) and 6600 g of groundnut were sorted to remove dirt and pebbles. Blends of the millet and groundnut were prepared by mixing in ratios of 9:1, 8:2, 7:3, 6:4, 5:5 and 10:0 accordingly. The blends were washed and steeped in 1 L of sterile distilled water in plastic buckets with covered lid for 24 hours for primary

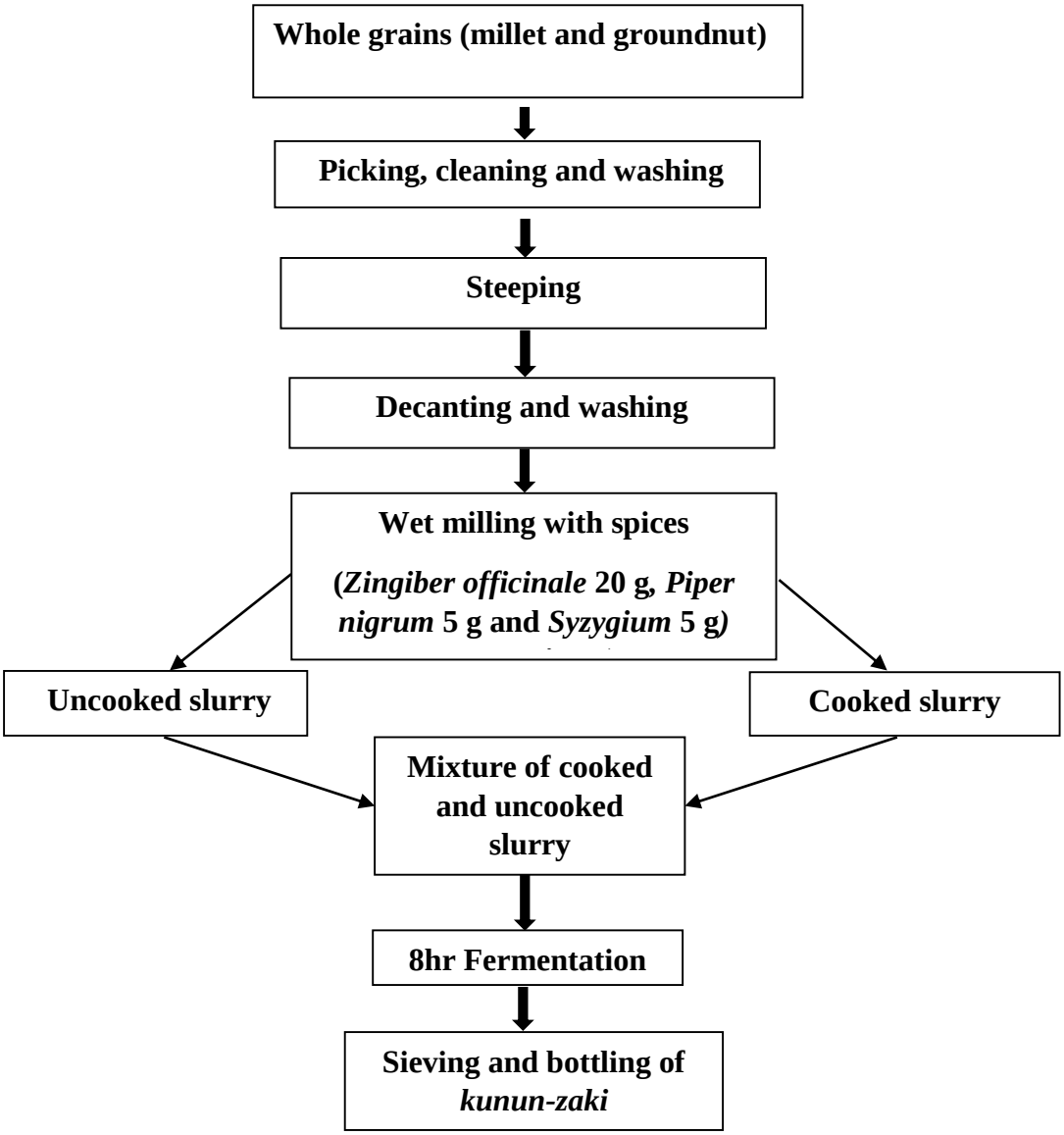

Figure 1: Flow chart for *kunun-zaki* production

fermentation and the steeping water were decanted. Then, the grains were washed and wet-milled using Marlex; Excella, mixer grinder with 3 stainless steel jars, Daman, India. Reg. trademark no: 396210. Spices were added; (ginger 20 g, clove 5 g, African black pepper 5 g) and milled together. The slurry was divided into two parts (4:1). The larger part (80% of the bulk) was heated by adding 2.5-3 liters of boiling water at 100 °C and allowed to cool to a temperature of about 45-50 °C; the unheated slurry was then added to the cooked portion and mixed thoroughly. The pooled slurry was left to ferment for 8 hours (secondary fermentation). The slurry was then sieved, bottled and refrigerated for analysis (modification of Akoma et al., 2010, Olaoye, Ubbor, & Uduma, 2016).).

3.5 Proximate analysis of *kunun-zaki* from millet and groundnut blend at different combination

Samples were analyzed for proximate composition using the standard methods of AOAC, 2015. Moisture, ash, protein, carbohydrate and fat were the major components determined. All determinations were carried out in duplicates.

3.5.1 Crude Protein

A 10 mL sample of *kunun-zaki* was transferred into a digestion tube and one tablet of Kjeidahl reagent was added. 5 mL of concentrated H_2SO_4 was added to the sample. This was digested in a digester at 350 ^oC for 4 hours. The sample was distilled using Kjeidahl apparatus with boric acid as indicator, and the sample was then titrated against 0.1 N HCl. Two samples were thus replicated (duplicate samples).

The titer value obtained was used to calculate the percentage nitrogen as:

$$\%N = \frac{\text{Titer of sample} - \text{Blank sample} \times 0.1N \times 14.01 \times 100}{\text{Weight of the sample}}$$

Where:

%Crude protein = %N x 6.25

0.1N = Normality of acid

14.01 = Equivalent weight

6.25 = factor used to multiply nitrogen to get crude protein

3.5.2 Crude fat content

Five mililitter samples of the *kunun-zaki* was transferred into separating funnels, 10 mL of petroleum ether was added to each sample and the mixture was agitated for 30 minutes. The mixture was left to settle so that the ether and water layers were distinctly separated, the water fraction was first collected off. The ether fraction was then collected into a pre weighed beaker. The solvent was allowed to evaporate to dryness by low heat and the percentage fat was calculated.

$$\% \text{ Fat } = \frac{\text{wt of fat}}{\text{wt of sample}} \times 100$$

3.5.3 Crude Fiber

Ten mL sample of *kunun-zaki* was measured into a beaker and 80 mL of Tri chloro acetic acid (T.C.A) was added. The mixture was boiled for about one hour and allowed to cool. It was filtered and the residue collected. The residue was transferred into a pre-weighed crucible and then weighed. The crucible containing the sample residue was dried in the oven. The crucible was reweighed and the sample was taken to the muffle furnace for ashing. It was cooled in a dessicator and the final weight taken. Then percentage fiber was calculated from the formula:

%Crude fiber:

$$\frac{(\text{Dry residue wt}+\text{wt of crusible})-(\text{wt of crusible}+\text{ash wt})}{\text{Weight of the sample}} \times 100$$

3.5.4 Ash content

Porcelain crucible was ignited in a hot flask, transferred into a dessicator to cool, and was then weighed (W1). 10ml sample of the *kunun-zaki* was measured into the crucible and weighed (W2). The crucible containing the sample was gently heated (to remove water) on a burner in a fume cupboard until smoke ceased; it was transferred to a muffle furnace, heated at 550ºC for 3 hour to burn off all organic matter. The crucible was removed and cooled in a dessicator and the percentage ash was calculated.

$$\%\ \text{Ash} = \frac{\text{Weight of ash}}{\text{Weight of the sample}} \times 100$$

3.5.5 Moisture content

The method of AOAC (2015) hot-air oven was used to determine the moisture content. 2 ml of the sample *kunun-zaki* was measured into a clean pre-weighed porcelain crucible, heated at 105ºC for 1 hour to dryness and then cooled in a dessicator. The sample was weighed and the moisture content was calculated as follows:

$$\%\ \text{Moisture content} = \frac{\text{Weight of dried sample}}{\text{Weight of the sample}} \times 100$$

3.5.6 Carbohydrate

The percentage carbohydrate in the sample was calculated by the percentage weight difference; that is, by subtracting the sum of (% moisture content, % crude protein, %

crude fat, % crude fiber and % ash) from 100% (AOAC, 2015). It was calculated using the formula below:

% Carbohydrate = 100 – (moisture content + crude protein + crude fat +crude fiber + ash).

3.6 Sensory Evaluation

Sensory evaluation was performed on the *kunun-zaki* produced within 24 hours of production. Thirty-two trained panelist scored the sample for appearance and colour, taste, flavour, viscosity and overall acceptability, using a 9-point hedonic scale: (9 for "*like extremely*", 8 for "*like greatly*", 7 for "*like moderately"*, 6 for "*like slightly*", 5 for "*neither like nor dislike*", 4 for "*dislike slightly*", 3 for "*dislike moderately*", 2 for "*dislike greatly*" and 1 for "*dislike extremely*"). Each panelist was provided with enough privacy to avoid biased assessment (Rolim, Hu, & Gänzle, 2019).

3.7 Measurement of Physicochemical properties and mineral contents of *kunun-zaki*

The pH value was measured by inserting a pH probe directly into the sample of *kunun-zaki* and recorded using a pH electrode (PP-15, Sartorius, Goettingen. Germany).

The total titratable acidity (TTA) was determined using the method described by Association of Analytical Chemists (AOAC, 2015). Ten mililitter (10mL) of the *kunun-zaki* sample was titrated against 0.1 N NaOH, using 3 drops of phenolphthalein as indicator, and expressed in degrees. One degree (1°) of acidity corresponds to 1 mL of 1 M NaOH required to neutralize the organic acids present in 100 mL of sample.

Other physicochemical parameters, namely, alkalinity (mg/L), turbidity (FTU), colour (mg/L), total hardness (mg/L) and concentrations of potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) and iron (Fe) in mg/L of the *kunun-zaki* produced were determined using Multi-parameter Photometer- Palintest (ELE International). A 0.5 ml of the sample was thoroughly mixed with 9.5 ml of distilled in Palintest test tube Palintest photometer. The photometer displayed the concentrations of the physicochemical parameters and minerals which were then recorded (modified method of Adekola, Bashir, & Kasimu, 2015).

3.8 Microbial Enumeration

3.8.1 Enumeration of total aerobic bacteria

Serial dilution was carried out, the total aerobic bacteria in *kunun-zaki* samples were enumerated by pipetting 1 mL from the 10^5 dilutions unto sterile Petri dish and molten nutrient agar (HIMEDIA) was pour plated (pour plate technique). The Petri dishes were allowed to set and incubated inverted aerobically at 37 ^oC for 24 hour. The observed colonies were counted, calculated and expressed in logarithm to base ten colony forming unit per millimeter (log_{10} cfu/mL) (Aboh & Oladosu, 2014).

3.8.2 Enumeration of lactic acid bacteria (LAB)

Enumeration of LAB was done, using de Man Rogosa Sharpe agar (MRS) for the isolation of lactic acid bacteria (HIMEDIA). The plates were incubated inverted anaerobically at 35 ^oC for 48 hours. Characteristics colonies were enumerated, calculated and presented in logarithm to base ten, colony forming unit per millimeter (log_{10} cfu/ml).

3.8.3 Enumeration of total coliforms

Total coliform count was performed using MacConkey agar (Oxoid UK, CM 0007) 1 mL of the sample dilution (10^5) was pour plated using molten MacConkey agar at 45^{o}C. The plates were allowed to set and were incubated aerobically at 37 ^{o}C for 24 hours and characteristic pinkish red colonies were enumerated and presented in logarithm to base ten, colony forming unit per millimeter ($\log_{10}$ cfu/mL).

3.8.4 Enumeration of yeasts

The total fungi was counted in the samples by pour plating 1mL of the dilution (10^5) using potatoes dextrose agar (Oxoid UK, CM0139) in duplicate to which chloramphenicol was added (250 mg/500 ml). The plates were allowed to solidify and were incubated inverted aerobically at 25 ^{o}C for 5 days. Characteristic fungi colonies were enumerated and expressed in logarithm to base ten, colony forming unit (Log cfu/ml).

3.9 *Kunun-zaki* production from millet and groundnut blends steeped at different durations

A mixture of 320 g of millet grains and 80 g of groundnut (4:1) was steeped in 1L of sterile distilled water in a plastic bucket with covered lid at different durations of 6, 12, 18 and 24 hours. The steeped grains were washed and wet milled after the addition of spices using a domestic grinder (Marlex Excella grinder, Daman, India). The slurry was used for *kunun-zaki* production as previously described (modification of Olaoye et al., 2016, Akoma et al., 2010).

3.9.1 Sensory Evaluation of *kunun-zaki* produced from millet and groundnut blends steeped at different durations

Sensory attributes of the *kunun-zaki* samples were evaluated by 32 members of trained panel on a 9-point hedonic scale from 9 (like extremely) to 1 (dislike extremely) (Rolim et al., 2019).

3.9.2 Physicochemical properties of *kunun-zaki* produced from millet and groundnut blends steeped at different duration

The pH value was measured with a pH meter (PP-15, Sartorius and Goettingen. Germany), the total titratable acidity (TTA) was determined according to AOAC, 2015 and the alkalinity, turbidity, colour, total hardness were determined with Palintest photometer (ELE International) (modified method of Adekola et al., 2015).

3.9.3 Mineral contents of *kunun-zaki* produced from millet and groundnut blends steeped at different duration

The mineral contents of *kunun-zaki* produced from millet and groundnut blends steeped at different duration were determined with Palintest photometer (ELE International) (modified method of Adekola et al., 2015).

3.9.4 Microbial Enumeration *kunun-zaki* produced from millet and groundnut blends steeped at different duration

Kunun-zaki samples were serially diluted and appropriate dilution factor was pour plated on nutrient agar (HIMEDIA), MRS agar (HIMEDIA), MacConkey agar (Oxoid UK, CM0139) and PDA potatoes dextrose agar (Oxoid UK, CM0139) to observe the colonies of total aerobic bacteria, lactic acid bacteria, total coliform bacteria, and yeasts

counts respectively. The observed colonies were counted, calculated and expressed in logarithm to base ten colony forming unit per millimeter (log_{10} cfu/mL) (Aboh & Oladosu, 2014).

3.10 *Kunun-zaki* production from malted and unmalted millets and groundnut blends steeped for 18 hours

The grains (5000 g of millet) were weighed into a bucket, thoroughly washed and steeped in water for 24 hours in order to hydrate the grains to germinate. The grains were then strained and spread on a germinating bed where a depth of 1-4 inches was maintained. The grains were allowed to germinate within four days. The moisture content was monitored daily by sprinkling water to ensure enough moisture for germination. Then, kilning was done by drying the germinated grains in the sun at 25-30 ^{o}C for 24 hours to stop the growth of the grains at the ideal time. The malted millet, unmalted millet and groundnut were mixed in ratios of 8:0:2, 6:2:2, 4:4:2, 2:6:2 and 0:8:2 and used for *kunun-zaki* production as previously described (modification of Olaoye et al., 2016, Akoma et al., 2010).

3.10.1 Sensory Evaluation of *kunun-zaki* produced from malted and unmalted millets and groundnut blends steeped for 18 hours

The organoleptic attributes, appearance and colour, taste, flavour, viscosity and overall acceptability of the *kunun-zaki* samples were evaluated by 32 members of a trained panel on a 9-point hedonic scale from 9 (like extremely) to 1 (dislike extremely) (Rolim et al., 2019).

3.10.2 Physicochemical properties of *kunun-zaki* produced from malted and unmalted millets and groundnut blends steeped for 18 hours

The pH, total titratable acidity, alkalinity, turbidity, colour, total hardness and minerals were determined as earlier described) (AOAC, 2015; Adekola et a l., 2015).

3.10.3 Mineral contents of *kunun-zaki* produced from malted and unmalted millets and groundnut blends steeped for 18 hours

The mineral contents of *kunun-zaki* produced from malted and unmalted millets and groundnut blends steeped at 18 hours duration were determined with Palintest photometer (ELE International) (modified method of Adekola et al., 2015).

3.10.4 Microbial Enumeration *kunun-zaki* produced from malted and unmalted millets and groundnut blends steeped for 18 hours

Kunun-zaki samples were observed for the colonies of total aerobic bacteria, lactic acid bacteria, total coliform bacteria, and yeasts and moulds, using nutrient agar (HIMEDIA), MRS agar (HIMEDIA), MacConkey agar (Oxoid UK, CM0139) and PDA potatoes dextrose agar (Oxoid UK, CM0139), respectively as described previously. The observed colonies were counted, calculated and expressed in logarithm to base ten colony forming unit per millimeter ($\log_{10}$ cfu/mL) (Aboh & Oladosu, 2014).

3.11 *Kunun-zaki* production with starter cultures

Kunun-zaki was produced from 20% malted millet, 60% unmalted millet and 20% groundnut blends steeped for 18 hours with added starter cultures. Freeze-dried bacterial consortium of *Lactobaccilus bulgaricus, Streptococcus thermophiles* and

Lactobacillus acidophilus was obtained, (Yogourmet freeze-dried yogurt starter CP 598 QC, Canada) was used for *kunun-zaki* production. A 5 g of the freeze-dried starter culture was inoculated aseptically into 20 mL of the *kunun-zaki* sample and left to stand for 30 minutes and then added to 980 mL of *kunun-zaki* at the onset of secondary fermentation. The mixture was incubated according to manufacturer's instructions, for 6-8 hour at ambient temperature (modification of Olaoye et al., 2016, Akoma et al., 2010).

3.11.1 Sensory Evaluation of *kunun-zaki* produced with starter cultures

Sensory attributes of the *kunun-zaki* samples were determined as described before (Rolim et al., 2019).

3.11.2 Physicochemical properties of *kunun-zaki* produced with starter cultures

The pH value, the total titratable acidity (TTA), alkalinity, turbidity, colour, total hardness and minerals were determined as stated earlier (AOAC, 2015; Adekola et al., 2015).

3.11.3 Mineral contents of *kunun-zaki* produced with starter cultures

The mineral contents of *kunun-zaki* produced with starter culture and spontaneously fermented were determined with Palintest photometer (ELE International) (modified method of Adekola et al., 2015).

3.11.4 Microbial Enumeration *kunun-zaki* produced with starter cultures

The counts of total aerobic bacteria, lactic acid bacteria, total coliform bacteria, and yeasts in the *kunun-zaki* were determined as earlier explained (Aboh & Oladosu, 2014).

3.12 Extraction of lemon and lime essential oils

Lemons and Lime fruits were transported to Landmark University Microbiology laboratory, washed and air dried. The fruits were peeled using a grater to obtain as much peel as possible. Two hundred gram samples of the lemon and lime peels were weighed into separate round bottom flasks and 100 mL of sterile distilled water was added, the peels were distillated using simple distillation apparatus. The distillate was received in a distillate receiver and the poured into a separatory funnel, it was in two layers (oil and water) and the oil was separated from the water layer by the use of a separating funnel as illustrated in (Figures 2 and 3) (Al-Jabri & Hossain 2018).

The fruits extracts were obtained by weighing 500g of the peel into a clean blender cup, 200 mL of sterile distilled water was added and homogenized in a food blender (Marlex; Excella, mixer grinder with 3 stainless steel jars, Daman, India. Reg. trademark no: 396210), the homogenate was sieved using a sterile muslin cloth, the filtrate was distillated and water evaporated. The extract left in the flask was collected in a sterile screw cap bottle and placed in the refrigerator until required (modification of Anticona, Blesa, Frigola & Esteve, 2020).

Plate 1: Simple distillation apparatus used for extracting essential oil from lemon and lime

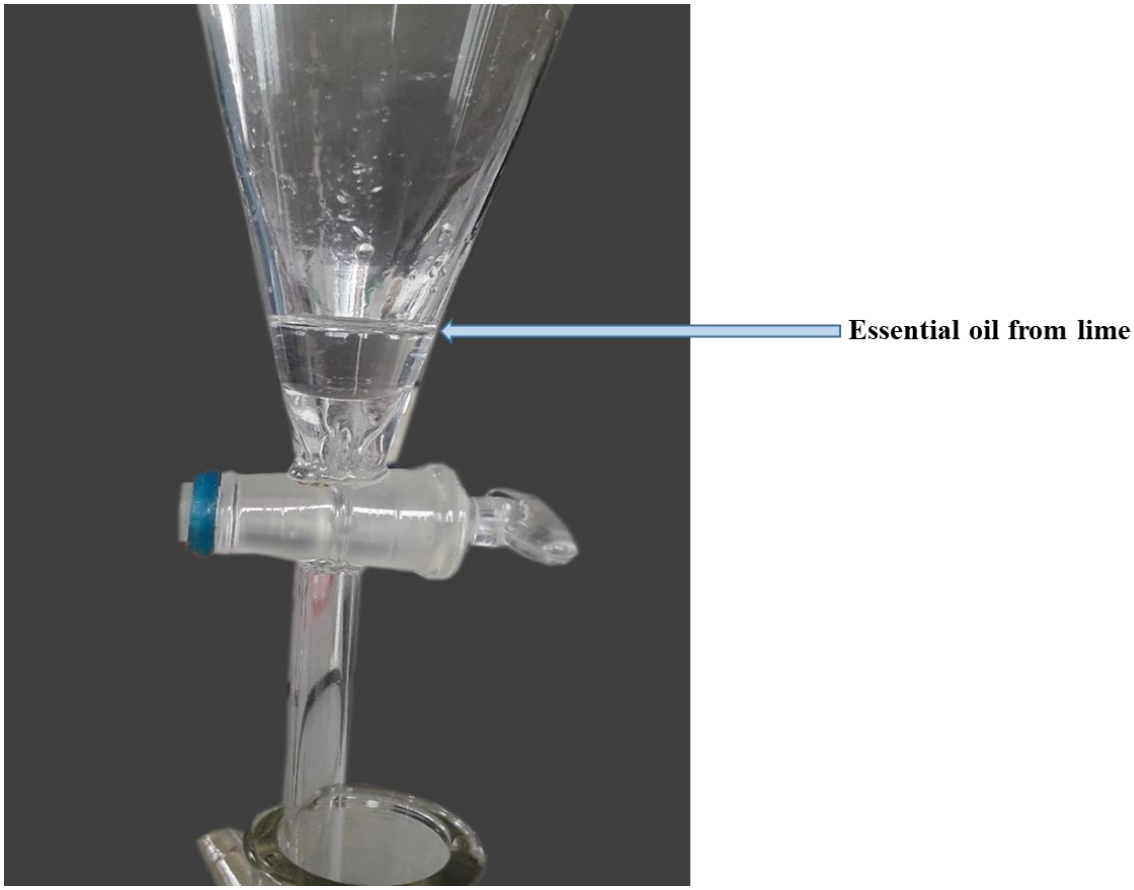

Plate 2: Setup of separatory funnel to separate the essential oil from water distillate

3.13 *Kunun-zaki* production with natural and chemical preservatives

Kunun-zaki was produced from 20% malted millet, 60% unmalted millet and 20% groundnut blends steeped for 18 hours and fermented spontaneously as earlier described. After production, 200 mL of *kunun-zaki* was dispensed into different sterile flasks. One percent each of lemon essential oil, lime essential oil, lemon extract, lime extract, hydrogen peroxide (3.332 mg/ml), citric acid, (9.876 mg/ml) and sodium benzoate (9.942 mg/ml). The *kunun-zaki* without preservative served as the control (Adewumi & Arije, 2017).

3.13.1 Physicochemical properties of *kunun-zaki* produced with natural and chemical preservatives

The pH value, the total titratable acidity (TTA) of the *kunun-zaki* samples were determined daily for seven days as earlier described (AOAC, 2015).

3.13.2 Mineral elements of *kunun-zaki* produced with natural and chemical preservatives

The mineral elements of kunun-zaki produced with natural and chemical preservatives were carried out with Palintest photometer (ELE International) (modified method of Adekola et al., 2015).

3.13.3 Microbial Enumeration of *kunun-zaki* produced with natural and chemical preservatives

The counts of total aerobic bacteria, lactic acid bacteria, total coliform bacteria, and yeasts and moulds in the *kunun-zaki* were determined daily for seven days as earlier explained (Aboh & Oladosu, 2014).

3.14 Statistical Analysis

Data generated from the analyses were subjected to statistical analysis using one way ANOVA with Duncan multiple range test, on SPSS statistical software version 15 at 95% confidence level.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

The proximate composition of *kunun-zaki* produced from millet and groundnut blend is presented in table 1. The Moisture, ash, crude fat, crude protein and crude fibre, carbohydrate and energy were in the range of 85.14-84.38%, 0.34-0.24%, 0.87-0.39%, 5.5-1.5%, 0.06-0.02%, 14.65-9.05% and 68.71-60.66 (kcal/g), respectively. Crude protein and crude fat generally increased along with increasing groundnut inclusion, while crude fiber and carbohydrate generally increased along increasing inclusion of millet. There was slight increase in crude fiber as the percentage millet increased. Energy is highest with highest millet inclusion. Other variables do not show definite patterns.

Sensory properties of *kunun-zaki* from millet and groundnut blend showed significant differences ($p<0.05$) in the appearance, taste, flavour and viscosity of the samples (Table 2). However, there were no significant differences ($p<0.05$) in the overall acceptability among the samples.

The physicochemical parameter shows that the *kunun-zaki* from different blends of millet and groundnut has the alkalinity, turbidity, colour and total hardness increasing with decrease in groundnut addition (Table 3). The control (100% millet) having the highest concentrations (224.0 mg/L, 137.5 FTU, 787.5 mg/L and 122.0 mg/L, respectively) while the sample with 50:50 millet/groundnut ratio has the least concentrations (103.0 mg/L, 63.5 FTU, 230.0 mg/L and 37.5 mg/L, respectively). The pH value was high in the sample with 50:50 millet/groundnut ratio. However, the total

titratable acidity was high in the sample with millet/groundnut ratio and lowest in the control sample.

Table 4 shows the mineral composition of *kunun-zaki* from different blends of millet and groundnut. The control (100% millet) has K, Ca, Mg, P and Fe (mg/L) content of 3.70, 70.20, 37.65, 70.41 and 3.85 respectively, which represent the highest values for each of the elements. While sample KE has the lowest values for calcium, magnesium, phosphorous and iron; 63.50, 18.00±0.0, 33.65 and 2.08 respectively.

As the fermentation duration increased there were increase in the total aerobic bacteria counts of *kunun-zaki* increased from 5.1 to 7.3 (log_{10}), the LAB counts also increased from 5.2 to 7.4 (log_{10}) and the total yeast count are in the range of 5.1 to 5.7. (Figures 4, 5, 6 and 7). There were no growth on the Mac Conkey plates after 48 hour incubation at 37 ^{o}C in all the three stages of production (steep, fermentation and in the product) which was indicative of no coliform bacteria in the product. It is observed that the 70:30 millet/groundnut ratio tend to support relatively high total aerobic bacterial count, total LAB, total yeast/mould count. This suggests that that blend best supports microbial growth.

Table 5 shows the sensory evaluation of *kunun-zaki* steeped for different time durations during production from 80:20 millet: groundnut blend. Sample NZK (18 hour steep) was most significant ($p \leq 0.05$) in appearance, taste, flavour, viscosity and over all acceptability with 6.94, 6.67, 6.71, 6.77 and 7.13 mean values respectively. In terms of appearance, taste, flavour, viscosity and overall acceptability ZNK (24 hours steep) had the lowest values of 5.81, 4.24, 4.26, 5.19 and 5.00 respectively. Hence, statistically different from other samples, these organoleptic parameters tended to rise to a peak at 18 hours steep of *kunun-zaki* production, after which ratings declined.

Table 1: Proximate composition of *kunun-zaki* from millet/groundnut blend at different ratios

Millet to Groundnut ratio	Moisture (%)	Ash (%)	Crude fat (%)	Crude Protein (%)	Crude fiber (%)	CHO (%)	Energy Value (kcal/g)
100%MT	85.14 ±0.36^{b}	0.28±0.007^{a}	0.39±0.00^{a}	1.50±0.07^{a}	0.06±0.00^{d}	14.65±0.46^{d}	68.71±0.71^{d}
90MT:10GT	84.44±0.08^{a}	0.25±0.03^{a}	0.53±0.02^{b}	3.05±0.07^{b}	0.05±0.01^{c}	12.22±0.17^{c}	60.66±1.63^{a}
80MT: 20GT	84.59±0.43^{ab}	0.30±0.02^{a}	0.63±0.04^{c}	3.80±0.01^{c}	0.03±0.00^{b}	11.12±0.47^{b}	63.10±0.35^{b}
70MT : 30GT	84.38±0.21^{a}	0.32±0.01^{a}	0.72±0.1^{d}	4.81±0.13^{d}	0.03±0.00^{b}	9.30±0.01^{a}	64.22±0.15^{bc}
60MT : 40GT	84.64±0.01^{ab}	0.34±0.09^{a}	0.74±0.02^{d}	5.05±0.07^{e}	0.03±0.00^{b}	9.58±0.13^{a}	65.26±0.40^{c}
50MT : 50GT	84.73±0.08^{ab}	0.24±0.12^{a}	0.87±0.06^{e}	5.50±0.15^{f}	0.02±0.00^{a}	9.05±0.08^{a}	64.13±0.34^{ab}

MT (Millet); GT (Groundnut); Data are means of two independent experiments ± standard deviation (n=2)

Legend: 100%MT; 100% millet, 90MT:10GT; 90% millet and 10% groundnut, 80MT: 20GT; 80% millet and 20% groundnut, 70MT:30GT; 70%millet and 30% groundnut, 60MT:40GT; 60% millet and 40% groundnut, 50MT:50GT; 50% millet and 50% groundnut.

Table 2: Sensory evaluation of *kunun-zaki* from different blend combinations of millet and groundnut

Millet to Groundnut ratio	Appearance/ Color	Taste	Flavour	Viscosity	Overall acceptability
100%MT	6.81±1.1 ^{a,b}	6.64±2.0^{b}	6.62±1.2^{b}	6.77±1.5^{b}	6.9±1.1 ^{a}
90MT:10GT	6.48±2.0^{a,b}	5.64±1.8^{a,b}	6.13±1.9^{a,b}	5.32±2.3^{a}	5.90±1.9^{a}
80MT: 20GT	6.55±1.8^{a,b}	5.48±2.6^{a,b}	5.48±2.1^{a}	6.22±2.0^{a,b}	6.29±1.9^{a}
70MT : 30GT	5.87±2.1^{a}	5.64±2.2^{a,b}	5.74±2.0^{a,b}	6.10±1.8^{a,b}	6.45±2.0^{a}
60MT : 40GT	6.12±2.4^{a,b}	5.00±2.5^{a}	5.68±2.0^{a,b}	6.10±2.5^{a,b}	6.23±2.2^{a}
50MT : 50GT	7.03±2.0^{b}	5.81±2.3^{a,b}	6.39±1.8^{a,b}	6.74±2.1^{b}	6.45±2.2^{a}

MT (Millet); GT (Groundnut); Data are means of two independent experiments ± standard deviation (n=2); Means in each row with different superscript represent a significant difference ($p \leq 0.05$) by Duncan Multiple Range Test (DMRT).

Legend: 100%MT; 100% millet, 90MT:10GT; 90% millet and 10% groundnut, 80MT: 20GT; 80% millet and 20% groundnut, 70MT:30GT; 70%millet and 30% groundnut, 60MT:40GT; 60% millet and 40% groundnut, 50MT:50GT; 50% millet and 50%

Table 3: Physicochemical parameters of *kunun-zaki* from different blends of millet and groundnut

Millet to Groundnut ratio	Alkalinity (mg/l)	Turbidity (FTU)	Colour (mg/l)	Total Hardness (mg/l)	pH	Total titratable acidity (TTA mg/ml)
100%MT	225.0±21.2^{a}	137.50±3.5^{c}	787.50±9.2^{d}	122.00±4.2^{c}	5.2±0.7^{bc}	0.26±0.00^{a}
90MT:10GT	175.0±14.1^{c}	117.50±10.6^{bc}	740.0±42.4^{cd}	112.5±3.5^{c}	4.8±0.0^{a}	0.68±0.00^{c}
80MT: 20GT	150.0±7.1^{bc}	112.5±23.4^{bc}	675.0±7.1^{c}	77.5±3.5^{b}	5.1±0.1^{b}	0.72±0.06^{c}
70MT : 30GT	102.5±3.5^{a}	102.5±3.5^{b}	415.0±21.2^{b}	70.0±7.0^{b}	5.2±0.1^{bc}	0.76±0.01^{c}
60MT : 40GT	135.0±7.1^{b}	98.5±0.7^{b}	415.0±21.2^{b}	32.5±3.5^{a}	5.1±0.0^{b}	0.55±0.1^{b}
50MT : 50GT	103.0±1.4^{a}	63.5±2.1^{a}	230.0±56.6^{a}	37.5±3.5^{a}	5.3±0.0^{c}	0.50±0.03^{b}

MT (Millet); GT (Groundnut); Data are means of two independent experiments ± standard deviation (n=2)

Legend: 100%MT; 100% millet, 90MT:10GT; 90% millet and 10% groundnut, 80MT: 20GT; 80% millet and 20% groundnut, 70MT:30GT; 70%millet and 30% groundnut, 60MT:40GT; 60% millet and 40% groundnut, 50MT:50GT; 50% millet and 50% groundnut

Table 4: Mineral elements of *kunun-zaki* from millet and groundnut at different ratio

Millet to Groundnut ratio	Potassium (mg/L)	Calcium (mg/L)	Magnesium (mg/L)	Phosphorous (mg/L)	Iron (mg/L)
100%MT	3.70±0.1^{c}	70.20±4.0^{b}	37.65±2.2^{d}	70.40±1.8^{d}	3.85±0.1^{d}
90MT:10GT	3.10±0.0^{b}	68.00±1.0^{b}	22.50±0.5^{b}	66.30±1.6^{d}	2.50±0.0^{b}
80MT: 20GT	3.70±0.0^{c}	67.50±1.5^{b}	40.50±1.5^{d}	58.25±1.7^{c}	3.80±1.0^{d}
70MT : 30GT	2.65±0.1^{a}	64.50±0.5^{a}	31.00±1.0c	39.45±2.1^{b}	3.75±0.1^{d}
60MT : 40GT	2.70±0.0^{a}	68.50±0.5^{b}	23.00±1.0^{b}	33.90±1.5^{a}	3.00±0.1^{c}
50MT : 50GT	2.95±0.1^{b}	63.50±0.5^{a}	18.00±0.0^{a}	33.65±1.3^{a}	2.08±0.0^{a}

MT (Millet); GT (Groundnut); Data are means of two independent experiments ± standard deviation (n=2)

Legend: 100%MT; 100% millet, 90MT:10GT; 90% millet and 10% groundnut, 80MT: 20GT; 80% millet and 20% groundnut, 70MT:30GT; 70%millet and 30% groundnut, 60MT:40GT; 60% millet and 40% groundnut, 50MT:50GT; 50% millet and 50% groundnut

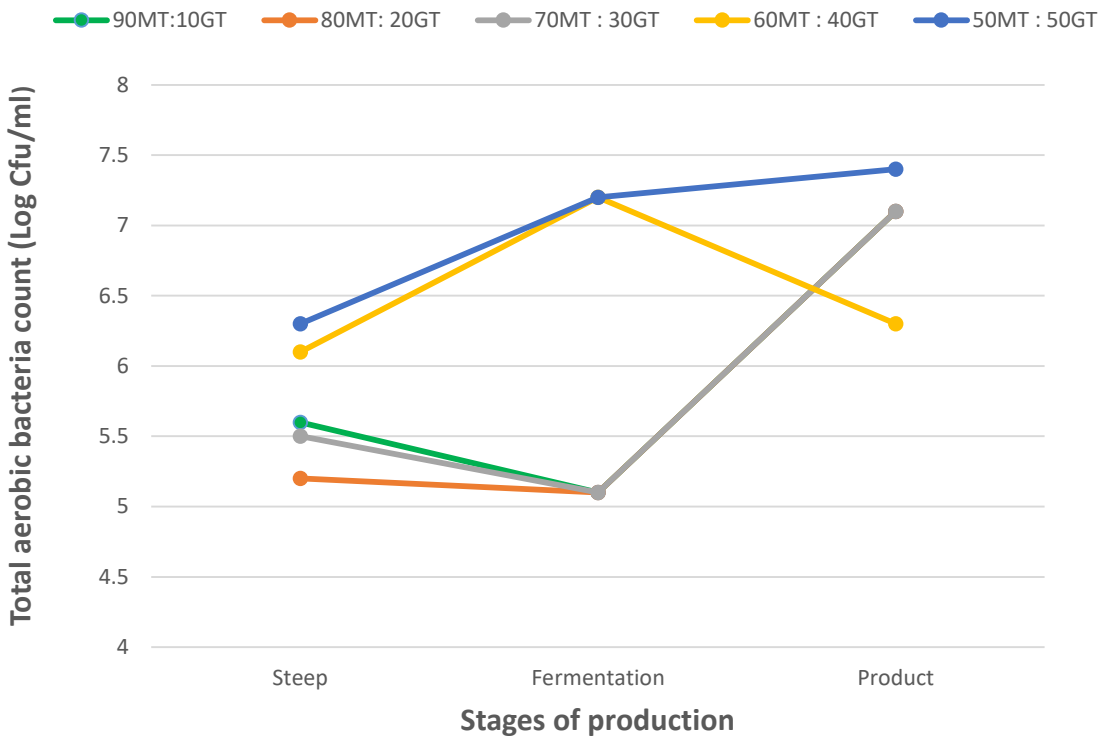

Figure 2: Total aerobic bacteria count of *kunun-zaki* from different blends of millet and groundnut blend during production

Legend: 100%MT; 100% millet, 90MT:10GT; 90% millet and 10% groundnut, 80MT: 20GT; 80% millet and 20% groundnut, 70MT:30GT; 70%millet and 30% groundnut, 60MT:40GT; 60% millet and 40% groundnut, 50MT:50GT; 50% millet and 50% groundnut

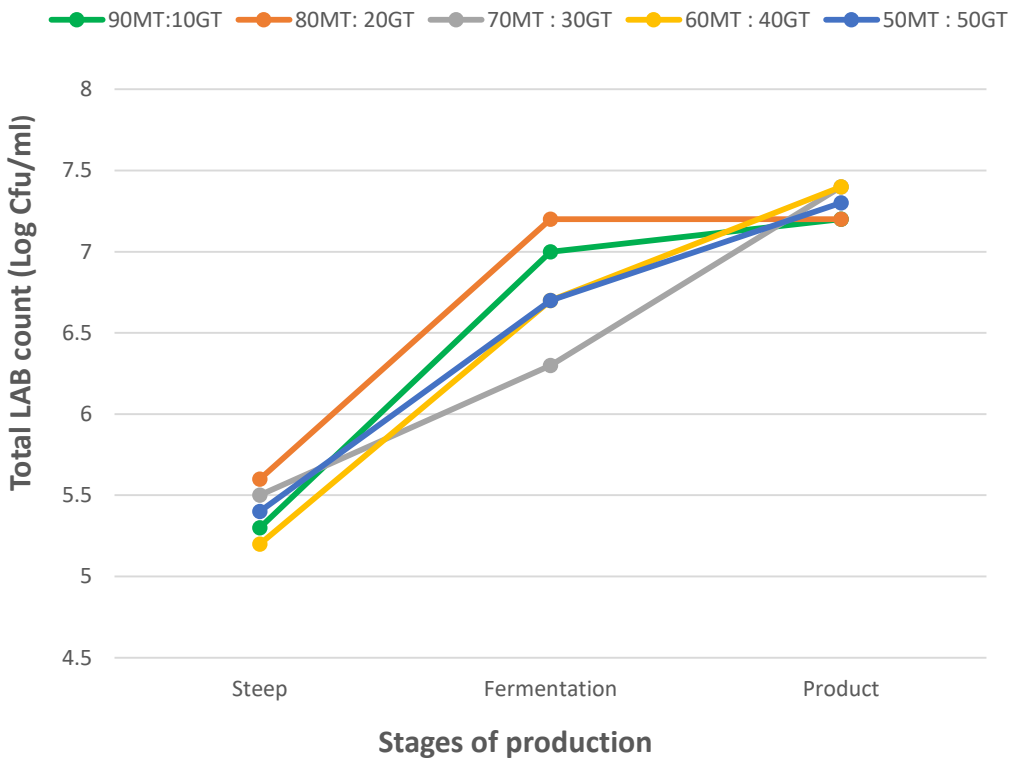

Figure 3: Total LAB count of *kunun-zaki* from different blends of millet and groundnut blend during production

Legend: 100%MT; 100% millet, 90MT:10GT; 90% millet and 10% groundnut, 80MT: 20GT; 80% millet and 20% groundnut, 70MT:30GT; 70%millet and 30% groundnut, 60MT:40GT; 60% millet and 40% groundnut, 50MT:50GT; 50% millet and 50% groundnut

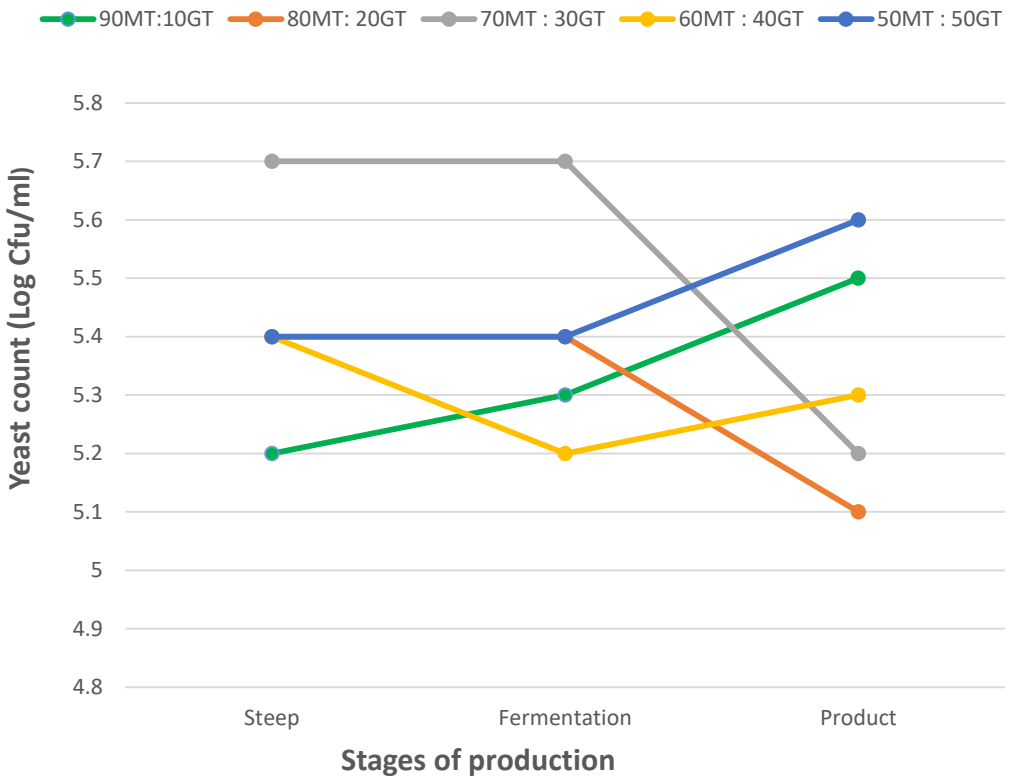

Figure 4: Total yeast count of *kunun-zaki* from different blends of millet and groundnut blend during production

Legend: 100%MT; 100% millet, 90MT:10GT; 90% millet and 10% groundnut, 80MT: 20GT; 80% millet and 20% groundnut, 70MT:30GT; 70%millet and 30% groundnut, 60MT:40GT; 60% millet and 40% groundnut, 50MT:50GT; 50% millet and 50% groundnut

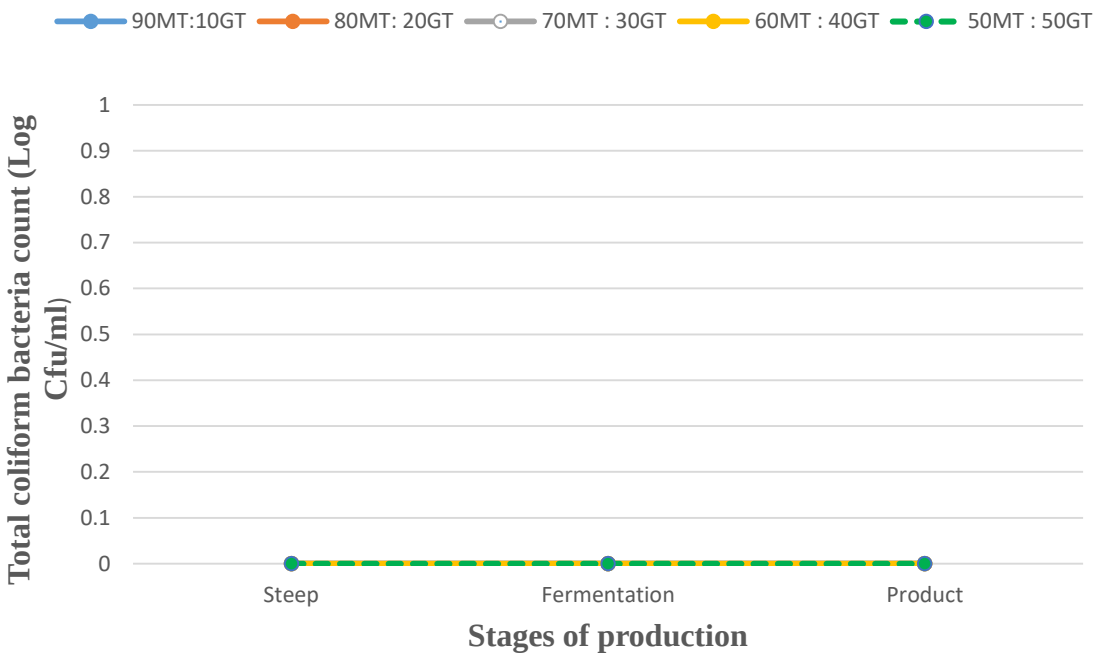

Figure 5: Total coliform bacteria count of *kunun-zaki* from different blends of millet and groundnut blend during production

Legend: 100%MT; 100% millet, 90MT:10GT; 90% millet and 10% groundnut, 80MT: 20GT; 80% millet and 20% groundnut, 70MT:30GT; 70%millet and 30% groundnut, 60MT:40GT; 60% millet and 40% groundnut, 50MT:50GT; 50% millet and 50% groundnut

The physicochemical parameters of *kunun-zaki* produced from millet (80%) and groundnut (20%) blend steeped at different duration showed that the pH for all the samples ranged from 5.1 to 6.1 (Table 6). Sample KNZ had alkalinity (mg/l) and turbidity values of 350.0 and 395.0 respectively these values represents the highest value for each of the parameters while sample NZK had 245.0, 177.5 and 695.0 respectively which also indicates the lowest values for alkalinity, turbidity and colour. The trend indicates that as steeping time of the beverage increases alkalinity, turbidity, colour, total hardness and pH decreased.

The mineral element of *kunun-zaki* from millet (80%) and groundnut (20%) at different steeping duration showed that sample ZNK (6 hour steep) had the highest K (mg/l), Mg (mg/l) and P (mg/l) content of 4.05, 47.50 and 1.55.while sample KZN had the lowest contents of 3.70, 36.0 and 1.28 respectively, sample NZK had the highest iron content of 4.75. These figures are presented in Table 7. The trend shows that as the duration of steeping of the beverage increased the potassium content decreased and was peak at 24 hours, phosphorus and iron increased.

Table 5: Sensory parameters of *kunun-zaki* from 80% millet and 20% groundnut Steeped at different duration

Sample Code	Hours of steeping	Appearance/ colour	Taste	Flavour	Viscosity	Overall acceptability
KNZ	6	6.07 ± 1.8^{b}	6.03 ± 1.9^{b}	6.16 ± 2.1^{b}	5.71 ± 2.2^{b}	6.26 ± 2.1^{b}
KZN	12	$6.58\pm2.0^{a,b}$	6.13 ± 2.5^{b}	6.07 ± 2.0^{b}	$6.26\pm2.1^{a,b}$	6.42 ± 1.9^{b}
NZK	18	$6.94\pm1.5^{a,b}$	6.68 ± 1.8^{b}	6.71 ± 1.4^{b}	$6.77\pm1.6^{a,b}$	7.13 ± 1.5^{b}
ZNK	24	5.81 ± 2.5^{a}	4.74 ± 2.2^{a}	4.26 ± 2.4^{a}	5.19 ± 2.8^{a}	5.00 ± 2.2^{a}

Data are means of two independent experiments± standard deviation (n=2); Means in each row with different superscript represent a significant difference ($p \leq 0.05$) by Duncan Multiple Range Test (DMRT).

Legend: KNZ: 6 hours steep, KZN: 12 hours steep, NZK: 18 hours steep and ZNK: 24 hours steep

Table 6: Physicochemical parameters of *kunun-zaki* from 80%millet and 20% groundnut steeped at different duration

Sample Code	Hours Of Steepest	Alkalinity (mg/l)	Turbidity (FTU)	Colour (mg/l)	Total hardness (mg/l)	pH
KNZ	6	350.0 ± 0.0^{c}	395.0 ± 5.0^{c}	735.0 ± 5.0^{b}	225.0 ± 5.0^{c}	6.1 ± 0.1^{c}
KZN	12	252.5 ± 2.5^{a}	185.0 ± 0.0^{b}	765.0 ± 5.0^{c}	170.0 ± 0.0^{b}	5.9 ± 0.0^{b}
NZK	18	245.0 ± 0.0^{a}	177.5 ± 2.5^{a}	695.0 ± 5.0^{a}	125.0 ± 0.0^{a}	5.8 ± 0.1^{b}
ZNK	24	315.0 ± 5.0^{b}	227.5 ± 2.5^{b}	725.0 ± 5.0^{b}	265.0 ± 10.0^{d}	5.1 ± 0.1^{a}

Data are means of two independent experiments± standard deviation (n=2).

Legend: KNZ 6 = 6 hour steep, KZN 12 = 12 hour steep, NZK 18 = 18 hour steep and ZNK 24 = 24 hour steep

Table 7: Mineral elements of *kunun-zaki* from 80% millet and 20% groundnut steeped at different duration

Sample code	Hours of steeping	Potassium (mg/l)	Calcium (mg/l)	Magnesium (mg/l)	Phosphorous (mg/l)	Iron (mg/l)
KNZ	6	3.85 ± 0.1^{b}	63.00 ± 3.0^{bc}	39.50 ± 0.5^{b}	1.38 ± 0.0^{ab}	3.90 ± 0.1^{b}
KZN	12	3.70 ± 0.0^{ab}	70.00 ± 0.0^{c}	36.00 ± 1.0^{a}	1.28 ± 0.0^{a}	3.30 ± 0.0^{a}
NZK	18	3.70 ± 0.1^{a}	61.00 ± 3.0^{b}	43.00 ± 0.0^{c}	1.45 ± 0.0^{bc}	4.75 ± 0.1^{c}
ZNK	24	4.05 ± 0.1^{c}	38.00 ± 0.0^{a}	47.50 ± 0.5^{d}	1.55 ± 0.1^{c}	4.10 ± 0.0^{b}

Data are means of two independent experiments± standard deviation (n=2).

Legend: KNZ 6 = 6 hour steep, KZN 12 = 12 hour steep, NZK 18 = 18 hour steep and ZNK 24 = 24 hour steep

Millet and groundnut were steeped at different duration (6hour, 12hour, 18hour and 12hour) to produce *kunun-zaki*. Samples were platted for microbial enumeration at the three stages of production (steep, fermentation and in the product). The microbial count at 10^5 dilution showed that the total aerobic bacteria count ranged from 6.7 to 7.4 (log cfu/ml), total LAB count ranged from 6.7 to 7.4 (log cfu/ml), and the total yeast count ranged from 5.1 to 6.2 (log cfu/ml). There were no coliform count in all the plates after 48hr incubation at 37 ^oC in all the three stages of production, the count results are shown in Figures 8, 9, 10 and 11.

It is also observed that steeping time affected the microbial counts. In general, as steeping time increased aerobic bacterial count increased but yeast/mould counts decreased. LAB count showed a definite increased trend. And there were no coliform counts.

During the fermentation stage, counts of each of aerobic bacteria, LAB and yeast/mould increased with the duration of fermentation and again, coliform count was not observed throughout fermentation. Aerobic bacterial count was almost constant for the first 18 hours, then dropped significantly between the 18^{th} and 24^{th} hours. LAB was relatively constant in the 24 hours of the product. Yeast/mould count increased in the first 12 hours but decreased thereafter.

The microbial count of *kunun-zaki* produced from malted millet, unmalted millet and groundnut blend at different ratio was performed and the counts expressed in log cfu/ml. The total aerobic bacteria count at 10^5 dilution ranged from 7.1to 7.4, total LAB count

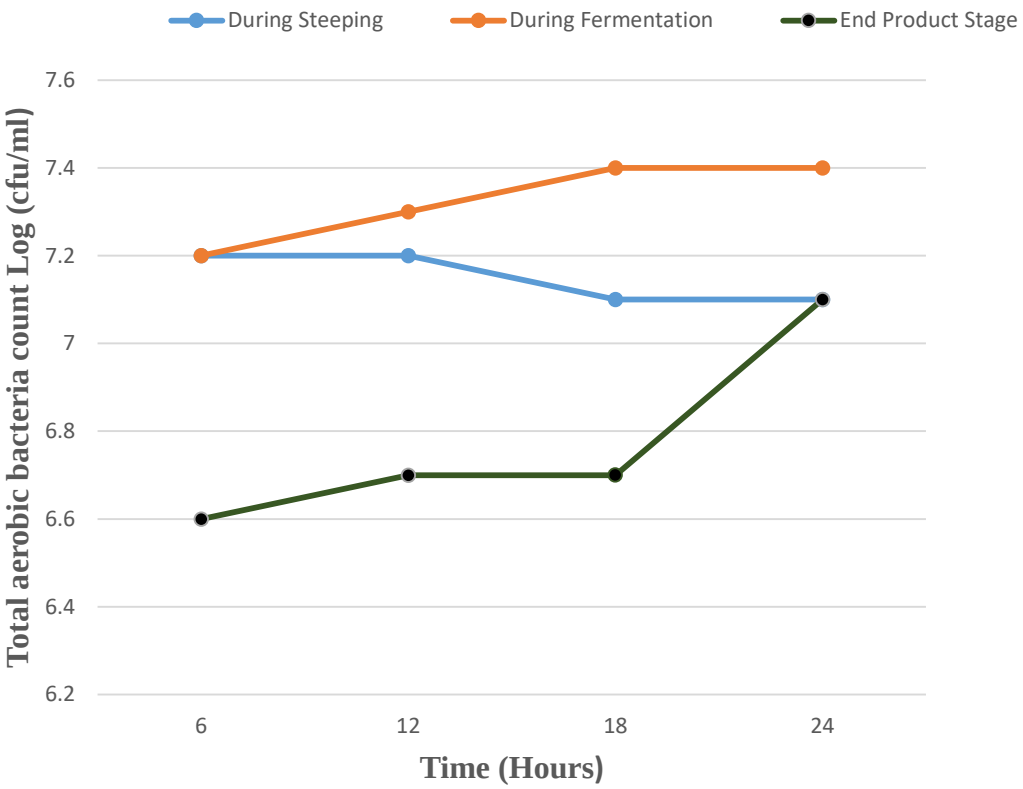

Figure 6: Total Aerobic bacteria count of *kunun-zaki* from millet and groundnut blend at different steep time

Legend: KNZ 6 = 6 hour steep, KZN 12 = 12 hour steep, NZK 18 = 18 hour steep and ZNK 24 = 24 hour steep

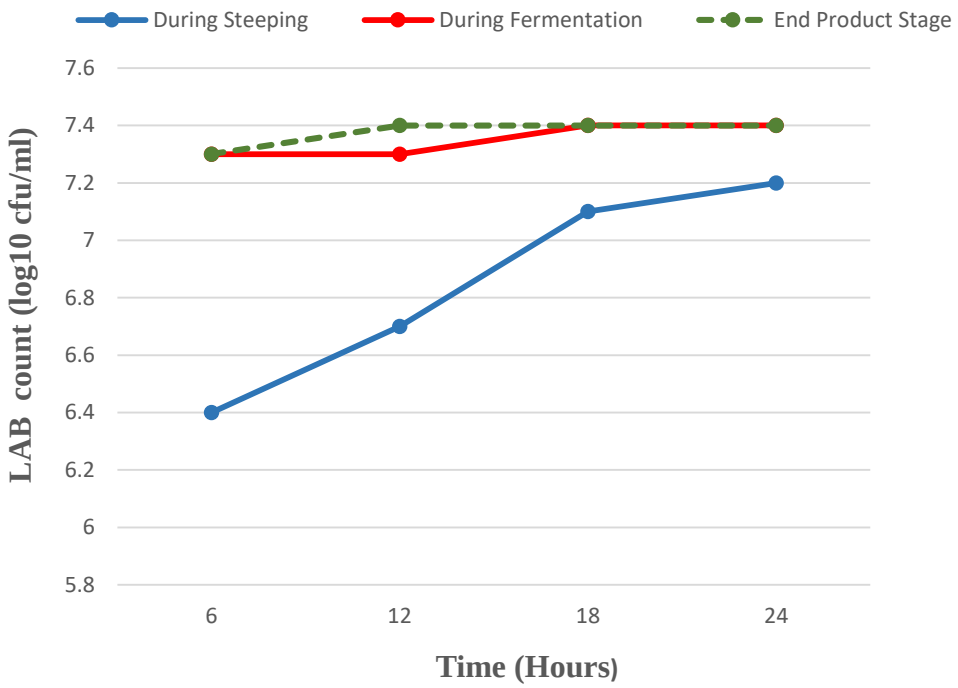

Figure 7: Total LAB count of *kunun-zaki* from millet and groundnut blend at different steep time

Legend: KNZ 6 = 6 hour steep, KZN 12 = 12 hour steep, NZK 18 = 18 hour steep and ZNK 24 = 24 hour steep

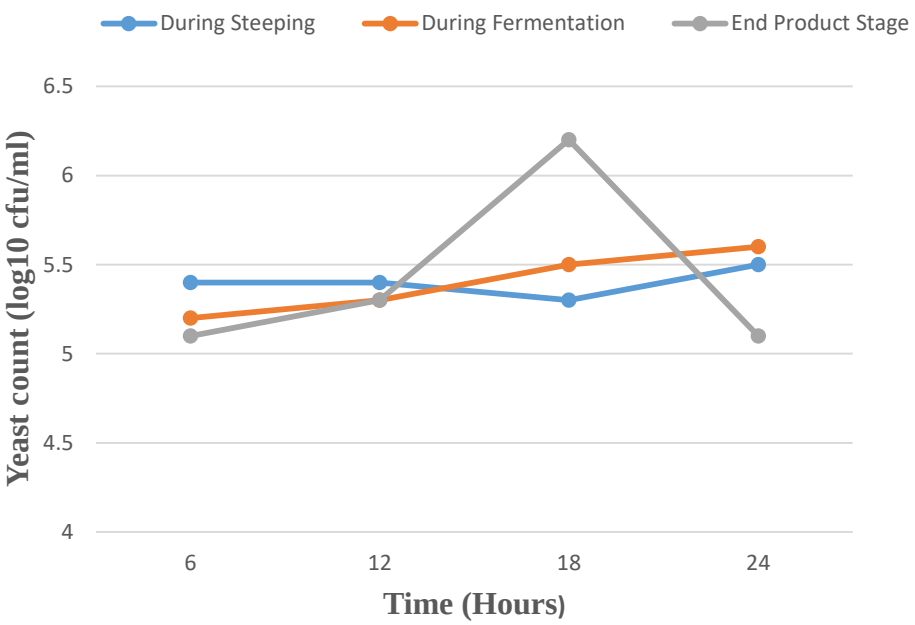

Figure 8: Total yeast count of *kunun-zaki* from millet and groundnut blend at different steep time

Legend: KNZ 6 = 6 hour steep, KZN 12 = 12 hour steep, NZK 18 = 18 hour steep and ZNK 24 = 24 hour steep

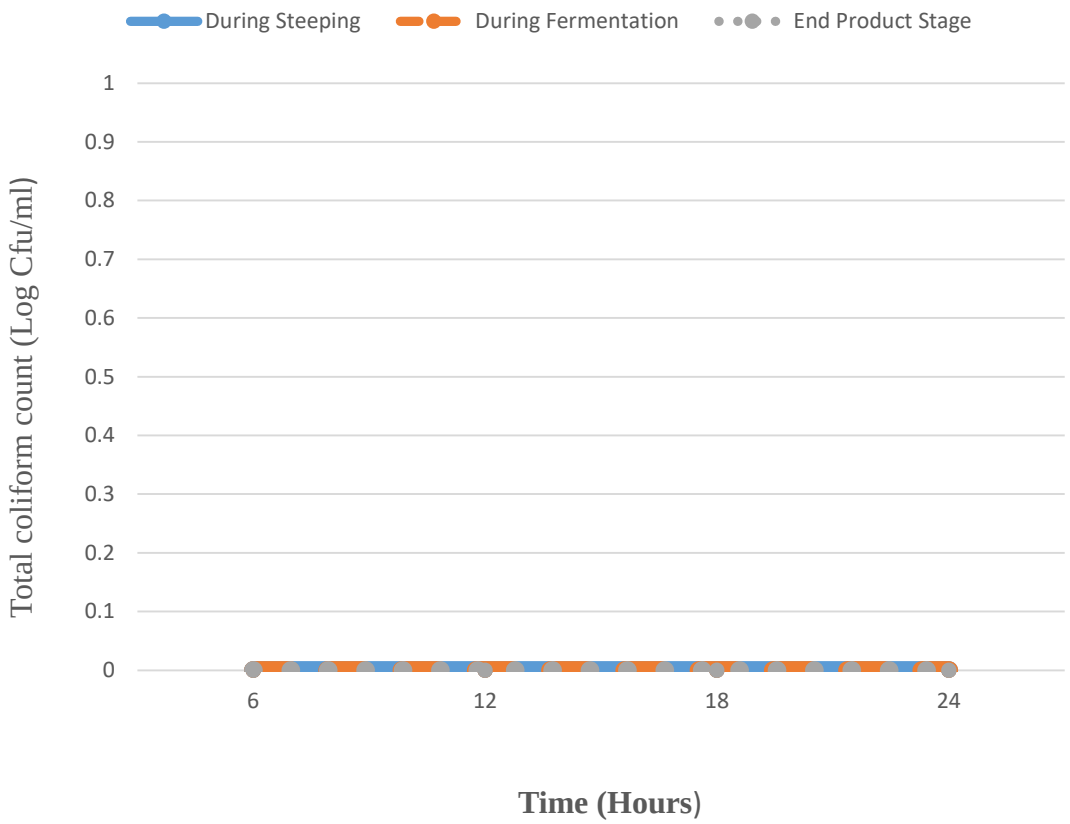

Figure 9: Total coliform count of *kunun-zaki* from millet and groundnut blend at different steep time

Legend: KNZ 6 = 6 hour steep, KZN 12 = 12 hour steep, NZK 18 = 18 hour steep and ZNK 24 = 24 hour steep

ranged from 6.8 to 7.4 and the total yeast count ranged from 0.0 to 5.9 (log cfu/ml). There were no coliform counts in all the plates in the three stages of production (steeping, fermentation and in the product) after 48hr incubation at 37ºC. Count results are presented in Figures (12, 13, 14 and 15).

The trend indicated that, during the preliminary steeping, as the malted component of the beverage increased the total aerobic bacterial count increased, LAB increased, total yeast/mould count did not portray discernible trend and coliform count was not detectable. During fermentation stage as the malted component increased aerobic bacterial count showed no discernible pattern, LAB count tended to increase, total yeast count tended to increase but coliform were not detectable. In the produced beverage as component malted millet increased aerobic bacterial count and LAB tended to increase. But yeast count tended to decrease, while coliforms were not detected.

The sensory evaluation of *kunun-zaki* from malted and unmalted millet with groundnut blend showed that sample KEM (20% malted millet +60% unmalted millet + 20% groundnut) was most significant at confidence level of $P<0.05$ in taste, colour, flavour and over all acceptability (post Hoc test) with mean of 7.45 ± 1.1^{c} , shown in Table 8. No discernible consistent trend is visible about the effect of inclusion of malted millet in the blend on the sensory evaluations.

The physicochemical parameters showed that sample KAM (80% unmalted millet + 20% unmalted groundnut steep at 18hours) had the highest alkalinity and turbidity of 225.0 and 200.0 while sample KCM (60% malted millet + 20% unmalted millet + 20% unmalted groundnut steep at 18 hours) had the lowest value of; 135.0, 130.0 respectively.

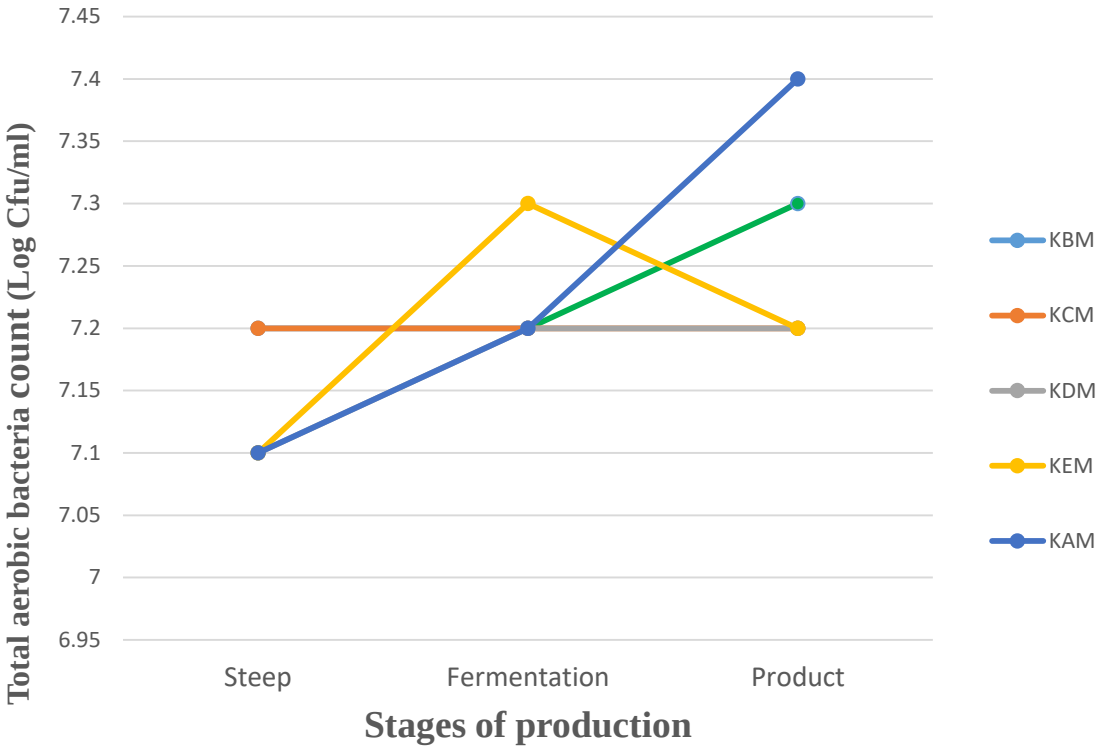

Figure 10: Total aerobic bacteria count of *kunun-zaki* produced from malted and unmalted millet and groundnut combination

Legend: KAM: 80% unmalted millet + 0% malted millet + 20% groundnut, KBM: 0% unmalted millet + 80% malted millet + 20% groundnut; KCM: 20% unmalted millet + 60% malted millet + 20% groundnut; KDM: 40% unmalted millet + 40% malted millet + 20% groundnut; KEM: 60% unmalted millet + 20% malted millet + 20% groundnut

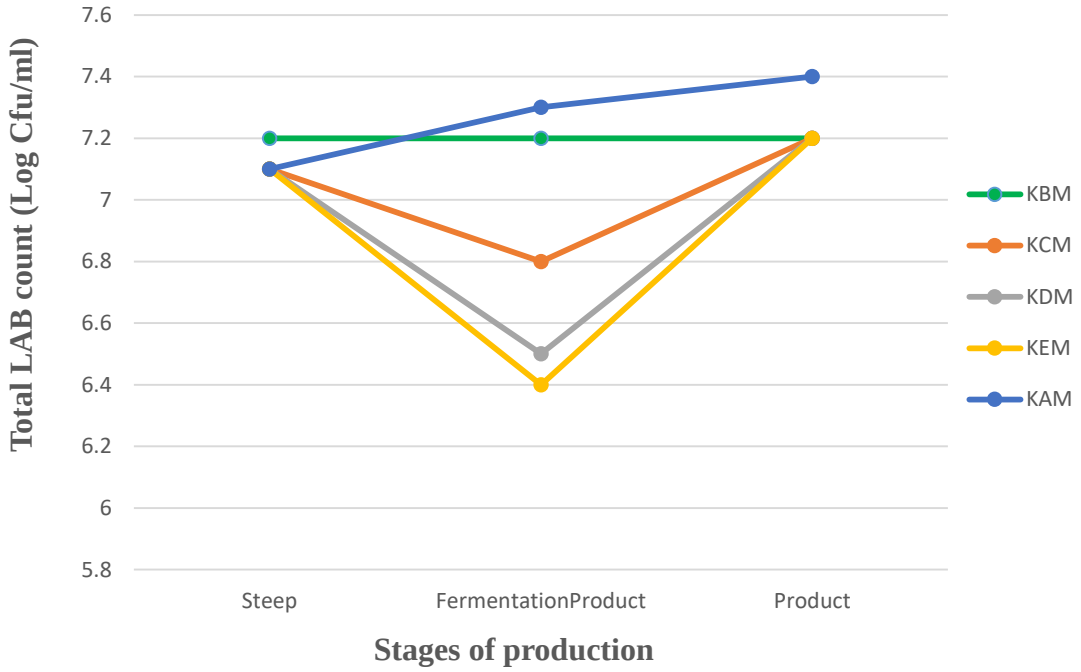

Figure 11: Total LAB count of *kunun-zaki* produced from malted and unmalted millet and groundnut combination

Legend: KAM: 80% unmalted millet + 0% malted millet + 20% groundnut, KBM: 0% unmalted millet + 80% malted millet + 20% groundnut; KCM: 20% unmalted millet + 60% malted millet + 20% groundnut; KDM: 40% unmalted millet + 40% malted millet + 20% groundnut; KEM: 60% unmalted millet + 20% malted millet + 20% groundnut

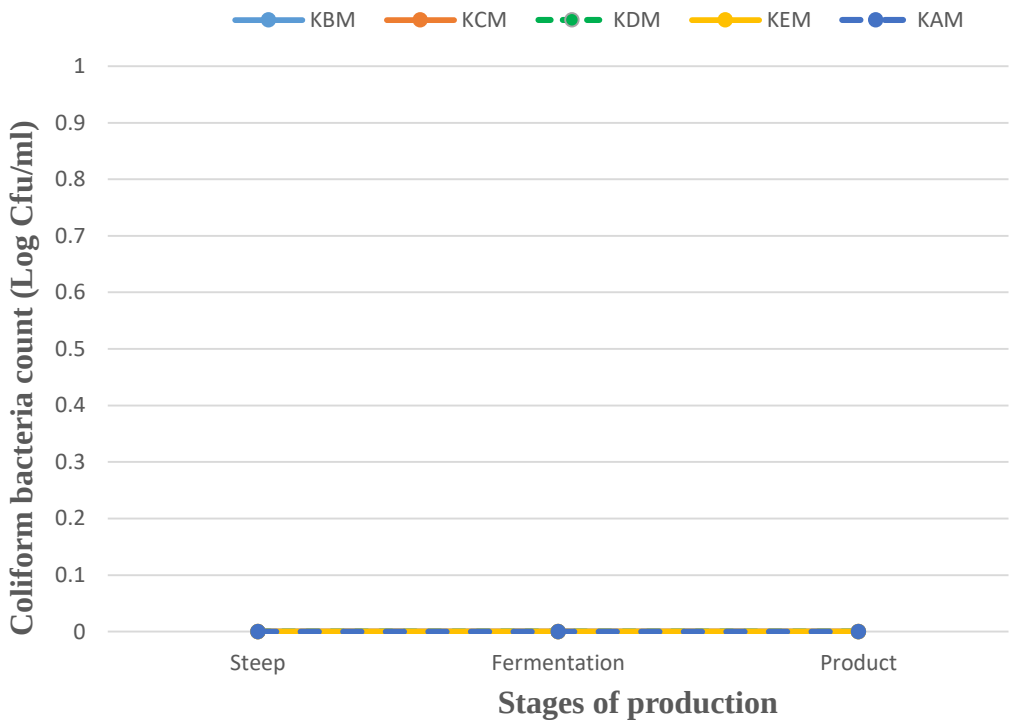

Figure 12: Total coliform count of *kunun-zaki* produced from malted and unmalted millet and groundnut combination

Legend: KAM: 80% unmalted millet + 0% malted millet + 20% groundnut, KBM: 0% unmalted millet + 80% malted millet + 20% groundnut; KCM: 20% unmalted millet + 60% malted millet + 20% groundnut; KDM: 40% unmalted millet + 40% malted millet + 20% groundnut; KEM: 60% unmalted millet + 20% malted millet + 20% groundnut

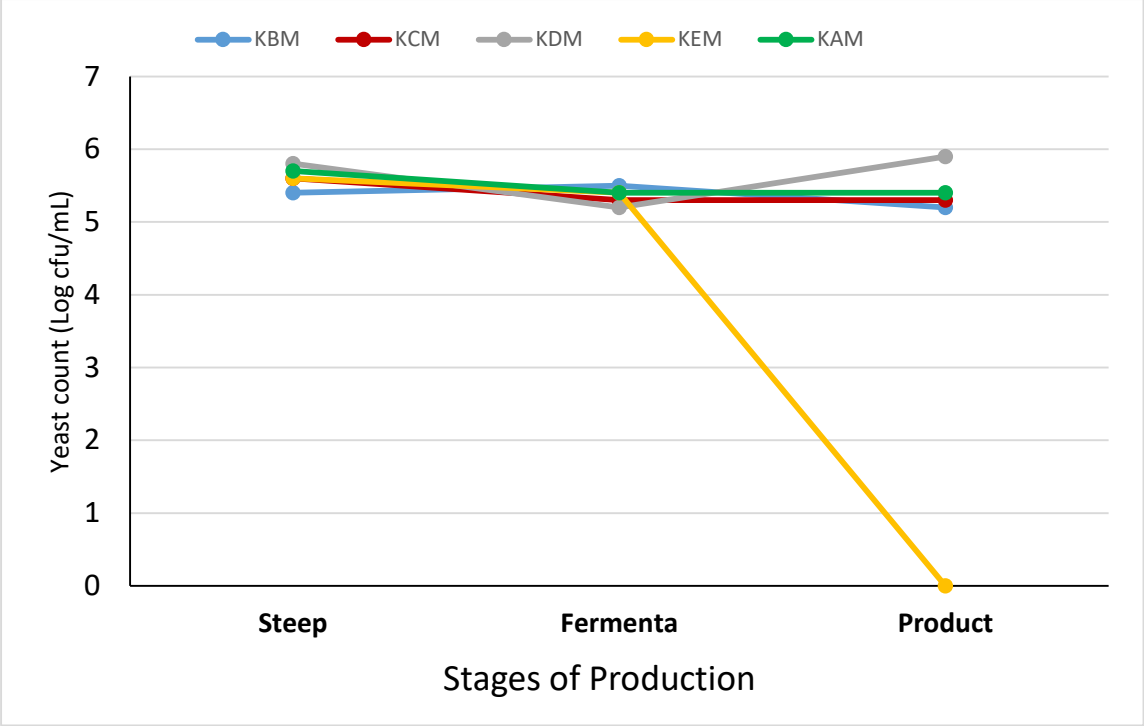

Figure 13: Total yeast count of *kunun-zaki* produced from malted and unmalted millet and groundnut combination

Legend: KAM: 80% unmalted millet + 0% malted millet + 20% groundnut, KBM: 0% unmalted millet + 80% malted millet + 20% groundnut; KCM: 20% unmalted millet + 60% malted millet + 20% groundnut; KDM: 40% unmalted millet + 40% malted millet + 20% groundnut; KEM: 60% unmalted millet + 20% malted millet + 20% groundnut

Table 8: Sensory evaluation of *kunun-zaki* produced from malted and unmalted millet and groundnut combination

Sample Code	Unmalted millet: Malted millet: Unmalted Groundnut (%)	Appearance/ colour	Taste	Flavour	Viscosity	Overall Acceptability
KAM	80:0:20	6.32 ± 1.6^{a}	4.81 ± 1.9^{a}	4.68 ± 2.2^{a}	5.55 ± 1.7^{a}	5.55 ± 1.8^{b}
KBM	0:80:20	7.03 ± 1.6^{b}	5.68 ± 2.2^{b}	5.58 ± 2.1^{b}	6.61 ± 1.9^{b}	6.10 ± 2.1^{b}
KCM	20:60:20	$5.10\pm2.3^{b,c}$	3.16 ± 2.0^{b}	3.36 ± 2.1^{b}	$4.55\pm2.3^{b,c}$	3.65 ± 2.2^{b}
KDM	40:40:20	$6.81\pm1.4^{b,c}$	$4.90\pm.2.3^{b}$	5.07 ± 2.1^{b}	$5.71\pm2.5^{c,d}$	5.29 ± 2.1^{a}
KEM	60:20:20	7.52 ± 1.0^{c}	7.32 ± 1.4^{c}	7.13 ± 1.5^{c}	7.10 ± 1.4^{d}	7.45 ± 1.1^{c}

Data are means of two independent experiments± standard deviation (n=2); Means in each row with different superscript represent a significant difference ($p \leq 0.05$) by Duncan Multiple Range Test (DMRT).

Legend: KAM: 80% unmalted millet + 0% malted millet + 20% groundnut, KBM: 0% unmalted millet + 80% malted millet + 20% groundnut; KCM: 20% unmalted millet + 60% malted millet + 20% groundnut; KDM: 40% unmalted millet + 40% malted millet + 20% groundnut; KEM: 60% unmalted millet + 20% malted millet + 20% groundnut

Table 9: Physicochemical parameters of *kunun-zaki* produced from blends of malted and unmalted millet and groundnut combination

Sample code	Unmalted millet: Malted millet: Unmalted Groundnut (%)	Alkalinity (mg/l)	Turbidity (FTU)	Colour (mg/l)	Total hardness (mg/l)	pH
KAM	80:0:20	225.0±0.0^{d}	200.0±0.0^{b}	440.0±10.0^{b}	167.5±2.5^{c}	6.9±0.0^{b}
KBM	0:80:20	220.0±0.0^{d}	192.5±2.5^{b}	605.0±5.0^{c}	167.5±2.5^{c}	6.8±0.0^{a}
KCM	20:60:20	135.0±0.0^{a}	130.0±10.0^{a}	350.0±10.0^{a}	100.0±0.0^{a}	6.9±0.0^{b}
KDM	40:40:20	177.5±2.5^{b}	140.0±0.0^{a}	765.0±5.0^{d}	105.0±0.0^{a}	6.8±0.0^{a}
KEM	60:20:20	192.5±2.5^{c}	200.0±0.0^{b}	370.0±20.0^{a}	132.5±2.5^{b}	6.8±0.0^{a}

Data are means of two independent experiments ± standard deviation (n=2).

Legend: KAM: 80% unmalted millet + 0% malted millet + 20% groundnut, KBM: 0% unmalted millet + 80% malted millet + 20% groundnut; KCM: 20% unmalted millet + 60% malted millet + 20% groundnut; KDM: 40% unmalted millet + 40% malted millet + 20% groundnut; KEM: 60% unmalted millet + 20% malted millet + 20% groundnut

Table 10: Mineral elements of *kunun-zaki* produced from malted and unmalted millet and groundnut combination

Sample Code	Unmalted millet: Malted millet: Unmalted Groundnut (%)	Potassium (K) (mg/l)	Calcium (Ca) (mg/l)	Magnesium (Mg) (mg/l)	Phosphorous (P) (mg/l)	Iron (Fe) (mg/l)
KAM	80:0:20	3.7 ± 0.1^{d}	59.0 ± 1.0^{a}	40.0 ± 0.0^{e}	44.6 ± 0.5^{a}	3.5 ± 0.0^{d}
KBM	0:80:20	3.8 ± 0.0^{d}	59.0 ± 1.0^{a}	39.0 ± 0.0^{d}	58.8 ± 0.8^{b}	3.8 ± 0.1^{e}
KCM	20:60:20	3.2 ± 0.0^{b}	71.5 ± 0.5^{b}	25.0 ± 0.0^{b}	80.5 ± 4.3^{c}	2.7 ± 0.0^{c}
KDM	40:40:20	2.9 ± 0.0^{a}	62.0 ± 1.0^{a}	17.0 ± 0.0^{a}	46.9 ± 4.1^{a}	2.0 ± 0.1^{a}
KEM	60:20:20	3.5 ± 0.0^{c}	107.5 ± 2.5^{d}	28.5 ± 4.1^{c}	82.9 ± 4.1^{c}	2.6 ± 0.0^{b}

Data are means of two independent experiments ± standard deviation (n=2)

Legend: KAM = 80% unmalted millet + 20% unmalted groundnut steep at 18 hours; KBM = 80% malted millet + 20% unmalted groundnut steep at 18 hours; KCM = 60% malted millet + 20% unmalted millet + 20% unmalted groundnut steep at 18 hours; KDM = 40% malted millet + 40% unmalted millet + 20% unmalted groundnut steep at 18 hours; KEM = 20% malted millet + 60% unmalted millet + 20% unmalted groundnut steep at 18 hours.

The result is presented in Table 9. No consistent trend is observable about the effect of inclusion of malted millet on the physicochemical parameter.

The mineral element result of *kunun-zaki* from malted and unmalted millet with groundnut indicated that sample KBM (20% malted millet +60% unmalted millet + 20% groundnut)) had the highest K and Mg content of 3.8 and 39.0 (mg/l) respectively as shown in Table 10. No discernible consisted trend was observed between the level of inclusion of malted millet and the levels of other mineral elements in the beverage.

The sensory quality of sample KAN (*kunun-zaki* spontaneously fermented) shows that it was most significant ($P<0.05$) in taste, flavour, colour and overall acceptability with mean of 7.72 ± 1.1^{a} of nine point scale as shown in Table 11. Addition of starter cultures did not significantly affect the colour appearance score. High addition (200%) of starter culture significantly reduced taste score. High addition of starter culture significantly reduced flavor score. Addition of starter did not affect the viscosity score. High addition of starter significantly reduced the overall acceptability.

Kunun-zaki produced from malted and unmalted millet and groundnut, some with the addition of starter culture and others spontaneously fermented, samples were plated, microbial counts were made and the counts expressed in $\log_{10}$ cfu/ml. The total aerobic bacteria count at 10^5 dilution ranged from 6.1 to 6.7 (log cfu/ml), total LAB count was ranged from 5.2 to 6.9 (log cfu/ml), and the total yeast count ranged from 5.2 to

Table 11: Sensory properties of *kunun-zaki* produced with starter culture and spontaneously fermented using 20%malted millet, 20% unmalted

Sample Code	% Starter culture added	Alkalinity (mg/L)	Turbidity (FTU)	Colour (mg/L)	Total hardness (mg/L)	pH
KAN	0	125.0±0.0^{b}	94.0±4.0^{a}	515.0±5.0^{b}	50.0±0.0^{a}	6.8±0.1^{a}
KBN	50	115.0±0.0^{a}	88.0±0.0^{a}	490.0±0.0^{a}	45.0±0.0^{a}	6.8±0.0^{a}
KCN	100	145.0±0.0^{c}	107.5±2.5^{b}	602.5±2.5^{c}	70.0±0.0^{a}	6.8±0.1^{a}
KDN	200	127.5±2.5^{b}	90.0±2.0^{a}	480.0±10.0^{a}	50.0±0.0^{a}	6.8±0.1^{a}

Data are means of two independent experiments± standard deviation (n=2); Means in each row with different superscript represent a significant difference ($p \leq 0.05$) by Duncan Multiple Range Test (DMRT).

Legend: KAN 0; *kunun-zaki* without starter culture, KBN 50; *kunun-zaki* with 50% starter culture, KCN 100; *kunun-zaki* with 100% starter culture and KDN 200; *kunun-zaki* with 200% starter culture.

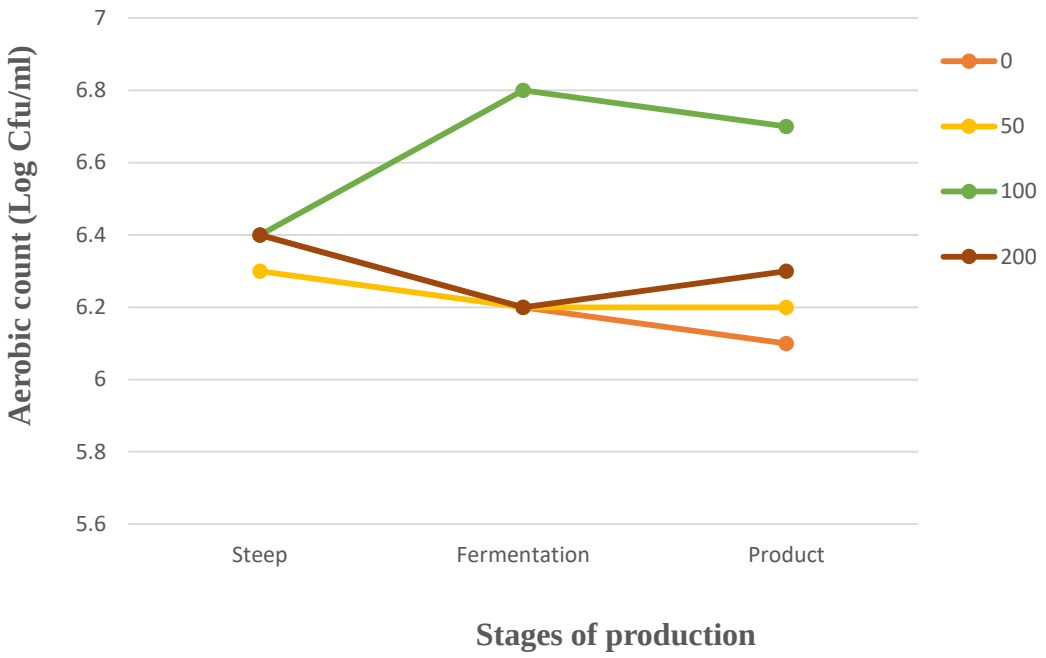

Figure 14: Total aerobic bacteria count of *kunun-zaki* produced with starter culture and spontaneously fermented using 20% malted millet, 60% unmalted millet and 20% groundnut, 18 hours steep

Legend: KAN 0; *kunun-zaki* without starter culture, KBN 50; *kunun-zaki* with 50% starter culture, KCN 100; *kunun-zaki* with 100% starter culture and KDN 200; *kunun-zaki* with 200% starter culture.

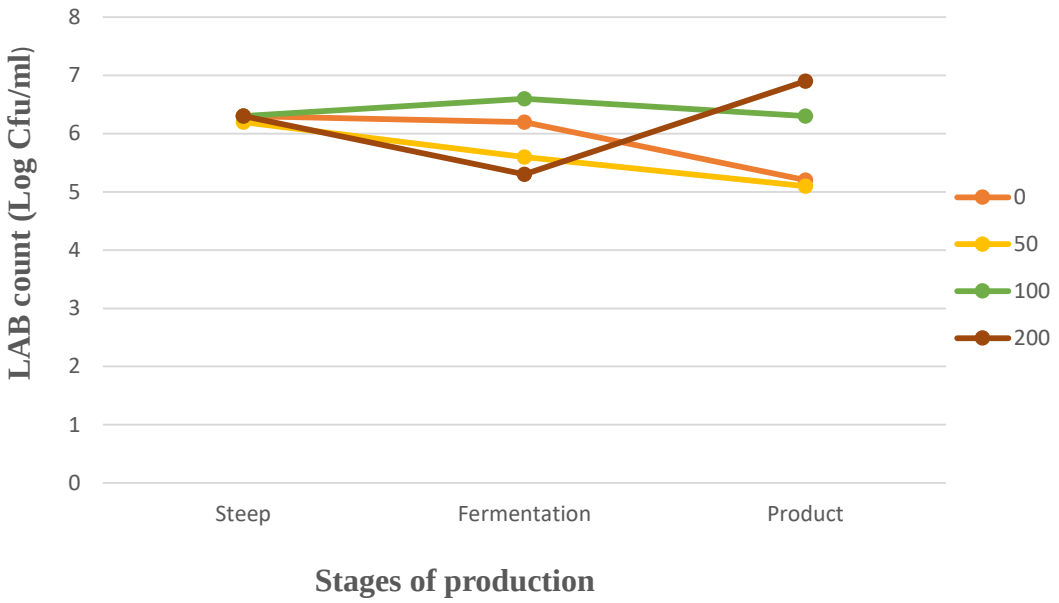

Figure 15: Total LAB count of *kunun-zaki* produced with starter culture and spontaneously fermented using 20%malted millet, 20% unmalted groundnut and 60% unmalted millet, 18 hour steeped

Legend: KAN 0; *kunun-zaki* without starter culture, KBN 50; *kunun-zaki* with 50% starter culture, KCN 100; *kunun-zaki* with 100% starter culture and KDN 200; *kunun-zaki* with 200% starter culture.

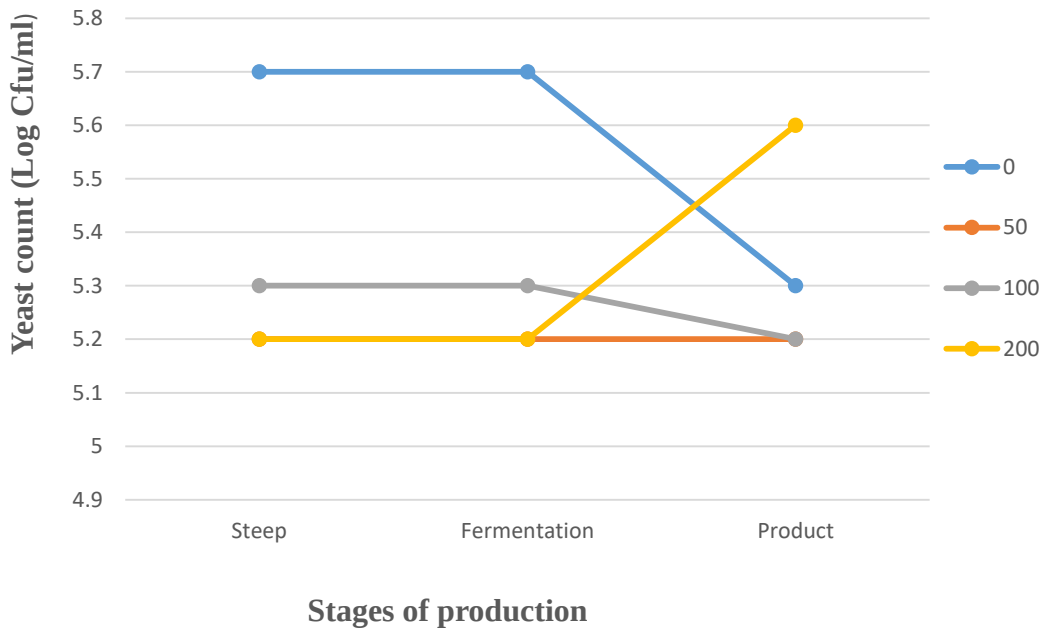

Figure 16: Total yeast count of *kunun-zaki* produced with starter culture and spontaneously fermented using 20%malted millet, 20% unmalted groundnut and 60% unmalted millet, 18 hour steeped

Legend: KAN 0; *kunun-zaki* without starter culture, KBN 50; *kunun-zaki* with 50% starter culture, KCN 100; *kunun-zaki* with 100% starter culture and KDN 200; *kunun-zaki* with 200% starter culture.

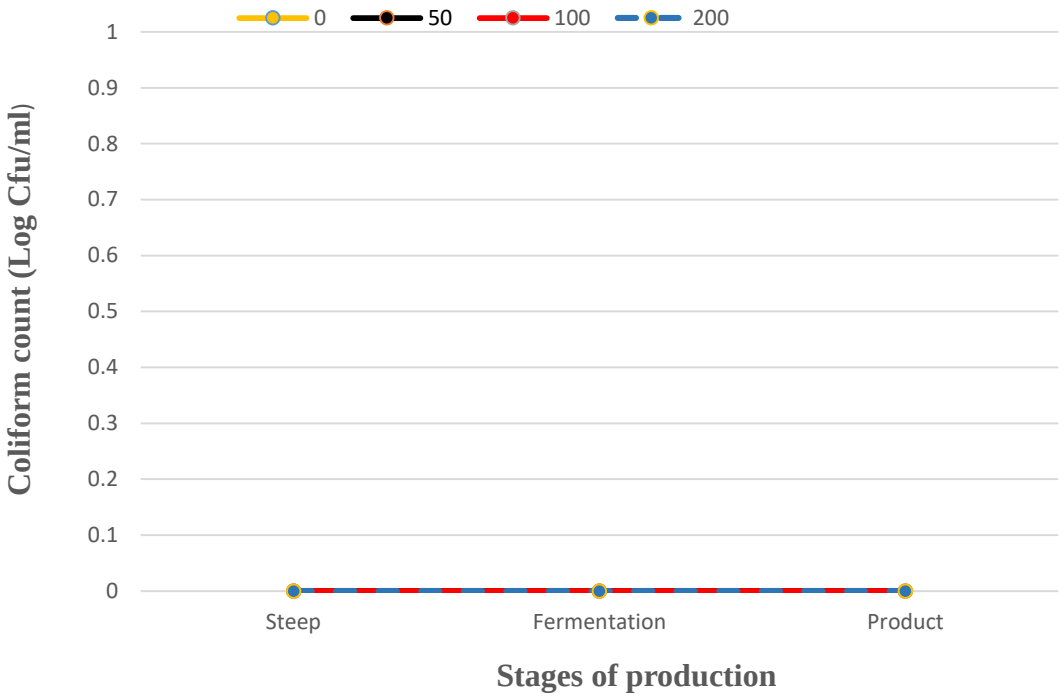

Figure 17: Total coliform count of *kunun-zaki* produced with starter culture and spontaneously fermented using 20%malted millet, 20% unmalted groundnut and 60% unmalted millet, 18 hour steeped

Legend: KAN 0; *kunun-zaki* without starter culture, KBN 50; *kunun-zaki* with 50% starter culture, KCN 100; *kunun-zaki* with 100% starter culture and KDN 200; *kunun-zaki* with 200% starter culture.

5.7 (log cfu/ml). There were no coliform counts in all the plates in the three stages of production (steeping, fermentation and in the product) after 48 hour incubation at 37 ^{o}C. Count results are presented in Figures 16 to 19. Level of inclusion of malted millet in the beverage did not show any discernible trend with any of the microbial counts, nor at any of the three stages of the production (steeping, fermentation and finished products).

The addition of starter culture did not cause any discernible trend in any physicochemical property of the beverage (Table 12).

Mineral element result shows that sample KCN (*kunun-zaki* fermented with 100% starter culture) had the highest potassium, magnesium, phosphorous and iron content of 3.0, 18.5, 55.9 and 2.1 (mg/l) respectively, as compared with sample KAN (spontaneously fermented *kunun-zaki*) with lowest values for potassium, magnesium and iron content of 2.9, 15.0 and 1.7 (mg/l) respectively. (Table 13).

The pictorial representation of *kunun-zaki* from millet (80%) and groundnut blend (20%), steeped vat 18 hour and fermented spontaneously with natural and chemical preservatives added to assess the effectiveness of essential oils from lemon and lime in increasing the shelf life of *kunun-zaki* (Plate 4).

The total aerobic bacteria counts in *kunun-zaki* preserved with 1% lemon essential oil (sample KLE), 1% lime essential oil (sample KLI), 1% lemon extract (sample KLX) and 1% lime extract (sample KLT) were 6.2, 6.2, 6.1,and 6.2 respectively on day one (Figure 21). There were decline in the bacteria counts with 5.2, 5.1, zero count and 5.3 respectively on day seven. As against the control, sample KNP (*kunun-zaki* without preservative) with 7.3 count on day one and 7.3 on day seven.

Plate 3: *Kunun-zaki* with preservatives dispensed in conical flasks prior to analysis

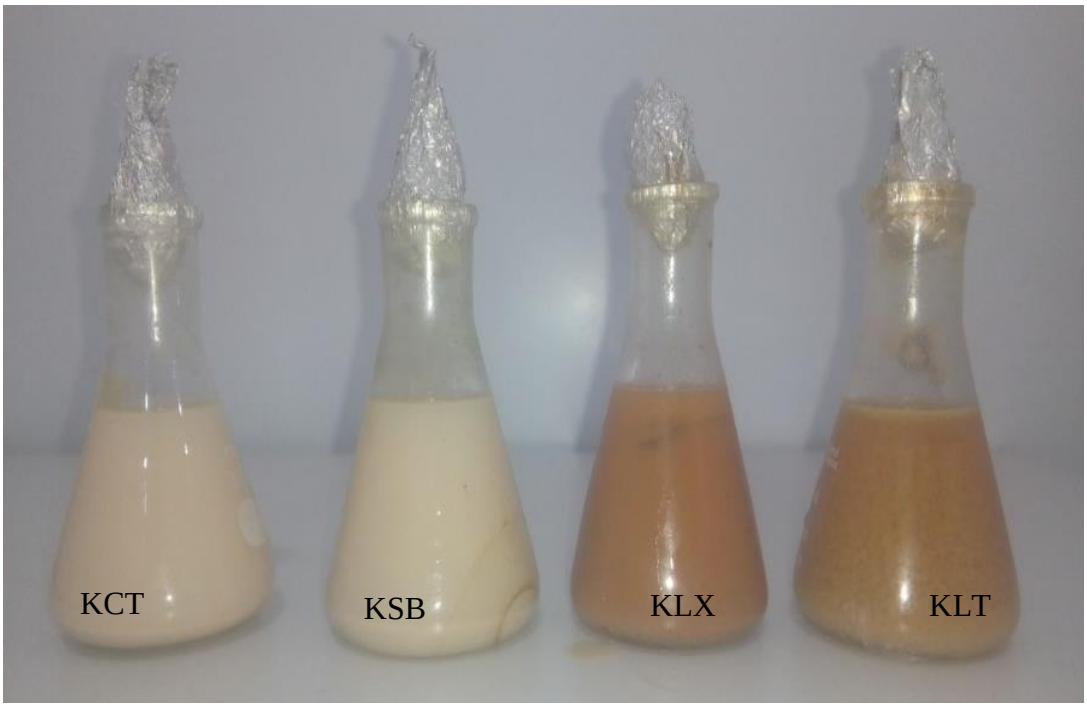

Plate 4: *Kunun-zaki* with preservatives dispensed in conical flasks prior to analysis

KNP: without preservative; KLE: 1% lemon essential oil; KLI: 1% lime essential oil; KHO: 1% hydrogen; KCT: 1% citric acid; KSB: 1% sodium benzoate; KLX: 1% lemon extract; KLT: 1% lime extraction

Table 12: Physicochemical parameters of *kunun-zaki* produced with starter culture and spontaneously fermented using 20% malted millet, 20% unmalted groundnut and 60% unmalted millet, 18 hour steeped

Sample Code	% Starter culture added	Alkalinity (mg/L)	Turbidity (FTU)	Colour (mg/L)	Total hardness (mg/L)	pH
KAN	0	125.0 ± 0.0^{b}	94.0 ± 4.0^{a}	515.0 ± 5.0^{b}	50.0 ± 0.0^{a}	6.8 ± 0.1^{a}
KBN	50	115.0 ± 0.0^{a}	88.0 ± 0.0^{a}	490.0 ± 0.0^{a}	45.0 ± 0.0^{a}	6.8 ± 0.0^{a}
KCN	100	145.0 ± 0.0^{c}	107.5 ± 2.5^{b}	602.5 ± 2.5^{c}	70.0 ± 0.0^{a}	6.8 ± 0.1^{a}
KDN	200	127.5 ± 2.5^{b}	90.0 ± 2.0^{a}	480.0 ± 10.0^{a}	50.0 ± 0.0^{a}	6.8 ± 0.1^{a}

Data are means of two independent experiments ± standard deviation (n=2)

Legend: KAN 0; *kunun-zaki* without starter culture, KBN 50; *kunun-zaki* with 50% starter culture, KCN 100; *kunun-zaki* with 100% starter culture and KDN 200; *kunun-zaki* with 200% starter culture.

Table 13: Mineral elements of *kunun-zaki* produced with starter culture and spontaneously fermented using 20%malted millet, 20% unmalted groundnut and 60% unmalted millet, 18 hour steeped

Sample Code	% Starter culture added	Potassium (mg/l)	Calcium (mg/l)	Magnesium (mg/l)	Phosphorous (mg/l)	Iron (mg/l)
KAN	0	2.9±1.0^{b}	47.0±1.4^{c}	15.0±0.0^{b}	49.5±0.7^{b}	1.7±0.1^{b}
KBN	50	2.7±0.0^{a}	21.0±1.4^{b}	13.0±0.0^{a}	43.9±0.7^{a}	1.5±0.0^{a}
KCN	100	3.0±0.0^{c}	16.0±0.0^{a}	18.5±0.5^{c}	55.9±0.7^{c}	2.1±0.0^{c}
KDN	200	2.7±0.0^{a}	16.0±0.0^{a}	12.5±0.5^{a}	43.2±0.2^{a}	1.7±0.1^{ab}

Mineral element mean ± standard deviation of replicate samples.

Legend: KAN 0; *kunun-zaki* without starter culture, KBN 50; *kunun-zaki* with 50% starter culture, KCN 100; *kunun-zaki* with 100% starter culture and KDN 200; *kunun-zaki* with 200% starter culture.

Bacteria counts were higher in the control sample compares with other samples; sample KHO *(kunun-zaki* + 3.332 mg/ml hydrogen peroxide), KCT (*kunun-zaki* + 9.876 mg/ml citric acid) and sample KNB (*kunun-zaki* + 9.941 mg/ml sodium benzoate) which had counts of 7.1, 6.1 and 6.4 on day one of preservation and 5.3, 6.6, and 6.1 respectively on day seven of preservation. The samples preserved with essential oils and the extracts showed a significant decline in the microbial population against the control samples.

Figure 22 shows the LAB succession in the preserved *kunun-zaki* samples as samples KLE, KLI, KLX, and KLT had 6.5, 6.5, 6.1 and 6.2 counts respectively on day one of preservation and 6.2, 6.2, 5.5 and 5.4 on day seven respectively. Control sample (KNP) had 7.1 on day one and 6.7 on day seven of preservation.

Total/yeast counts is presented in figure 23 which indicates that samples KLE, KLI, KLX and KLT had 5.1, 5.2, 5.1 and 5.2 counts respectively on day one and 4.1 count in all four samples on day seven. The control (KNP) had count of 5.6 on day one and 5.4 on day seven of preservation.

There were no coliform counts in all the preserved samples from one to day three but on day four, there was 5.5 count in sample KHO (*kunun-zaki* 3.332 mg/ml hydrogen peroxide). In the samples preserved with the essential oils there were no coliform growth until day seven with coliform counts of 5.7, 5.8, 5.2 and 5.2 in samples KLE, KLI, KLX and KLT, respectively. While the control, sample KNP (*kunun-zaki* without preservative) had 7.2 on day seven which indicates the highest coliform count on the seventh day of preservation.

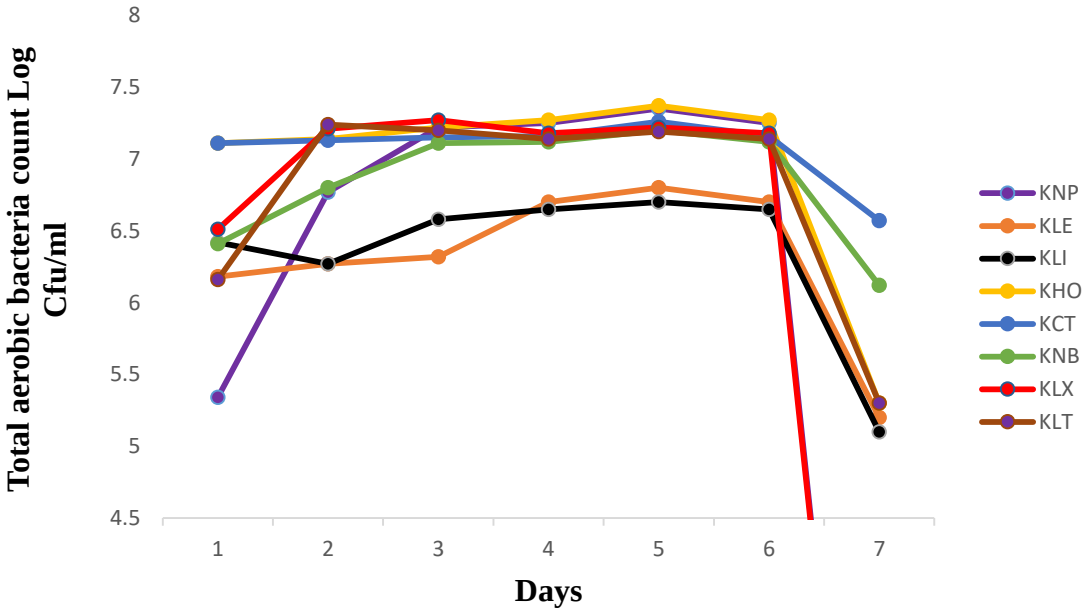

Figure 18: Total aerobic bacteria count of *kunun-zaki* during storage

KNP: without preservative; KLE: 1% lemon essential oil; KLI: 1% lime essential oil; KHO: 1% hydrogen; KCT: 1% citric acid; KSB: 1% sodium benzoate; KLX: 1% lemon extract; KLT: 1% lime extraction

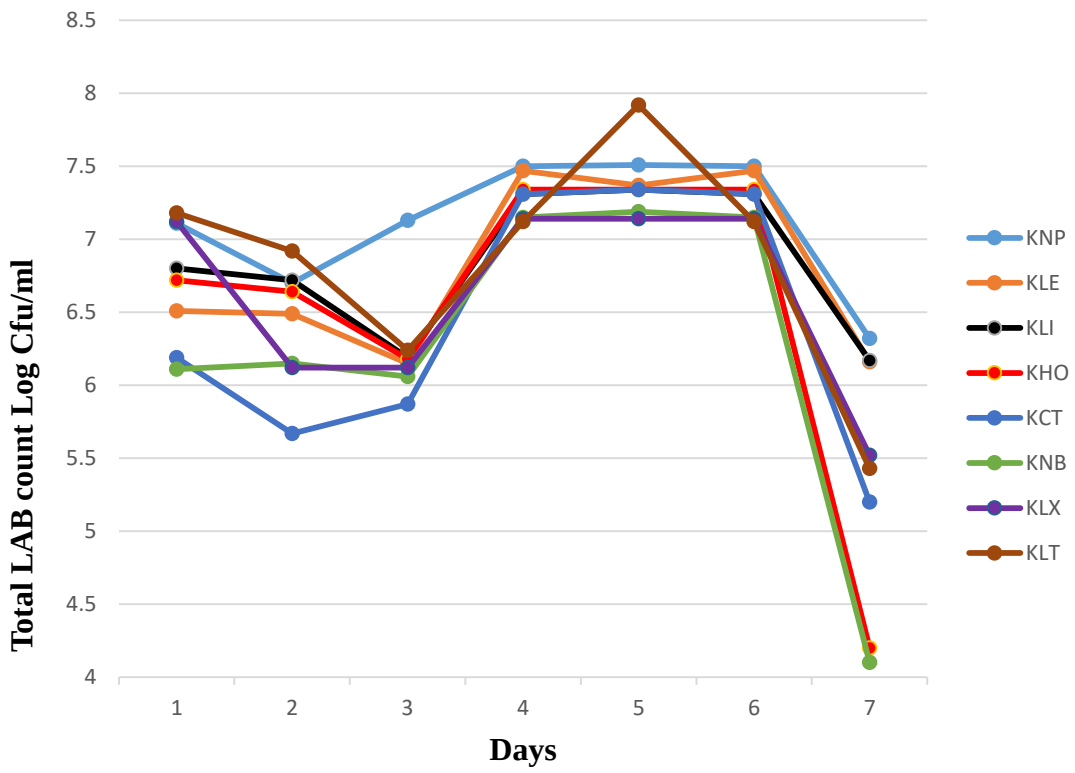

Figure 19: Total LAB bacteria count of *kunun-zaki* during storage

KNP: without preservative; KLE: 1% lemon essential oil; KLI: 1% lime essential oil; KHO: 1% hydrogen; KCT: 1% citric acid; KSB: 1% sodium benzoate; KLX: 1% lemon extract; KLT: 1% lime extraction

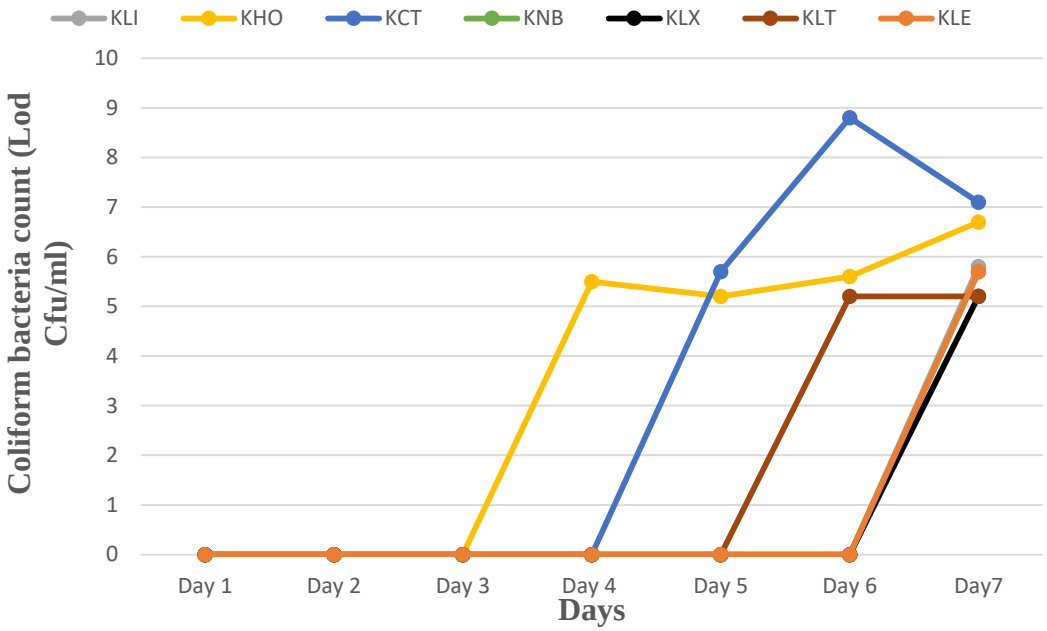

Figure 20: Total coliform bacteria count of *kunun-zaki* during storage

KNP: without preservative; KLE: 1% lemon essential oil; KLI: 1% lime essential oil; KHO: 1% hydrogen; KCT: 1% citric acid; KSB: 1% sodium benzoate; KLX: 1% lemon extract; KLT: 1% lime extraction

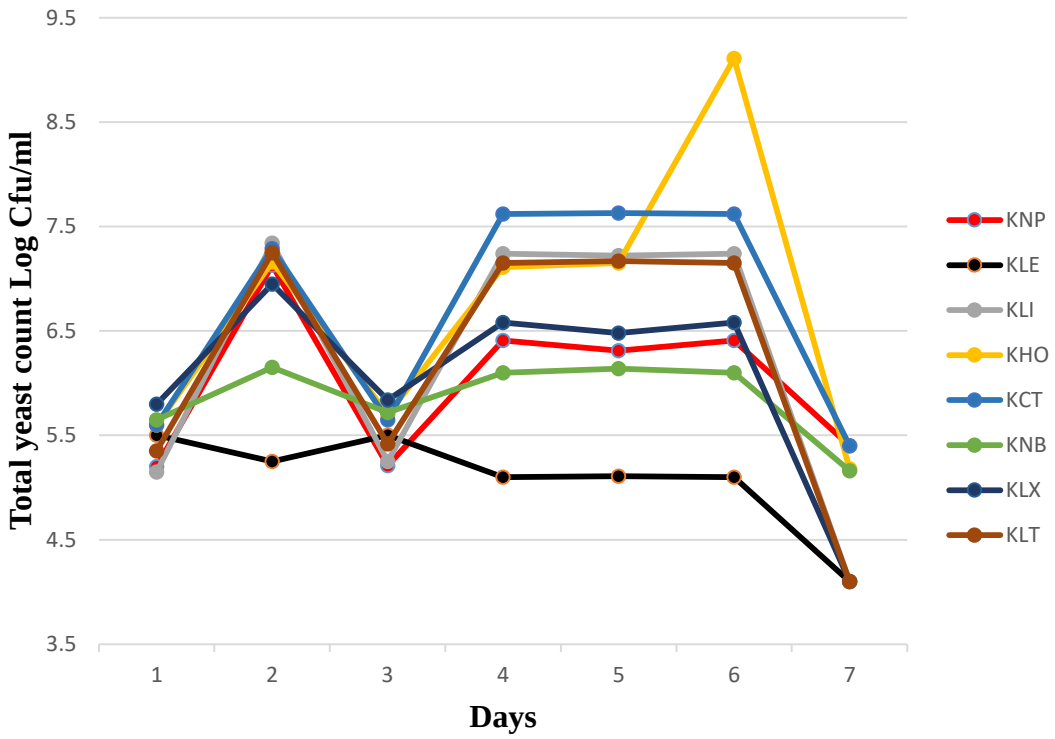

Figure 21: Total yeast count of *kunun-zaki* during storage

KNP: without preservative; KLE: 1% lemon essential oil; KLI: 1% lime essential oil; KHO: 1% hydrogen; KCT: 1% citric acid; KSB: 1% sodium benzoate; KLX: 1% lemon extract; KLT: 1% lime extraction

Before the addition of preservatives the pH and total titratable acidity of freshly *kunun-zaki* were 5.20 and 0.26 respectively. Sample KNB (*kunun-zaki*+0.07 mg/ml sodium benzoate) had the least TTA of 0.031 on day one and 0.047 on day seven of storage, which indicates less acid production during storage. While sample KCT (*kunun-zaki*+0.048 mg/ml citric acid) had the highest TTA of 0.139 on the first day but was over taken by sample KLI (*kunun-zaki*+1% lime essential oil) with 0.233 on the seventh day of preservation, which indicates more acid production (Figure 25) On the addition of preservatives the pH changed in almost all the samples except for sample KNB (*kunun-zaki* + 0.07 mg/ml sodium benzoate) which maintain a stable pH of 5.1 to 5.3 from day one to the seventh day, which was as a result of chemical preservative added that inhibited microbial growth thereby reduced the rate of acid production in the sample. And sample KCT (*kunun-zaki*+0.048 mg/ml citric acid) had the least pH of 2.8 which was caused by the presence of citric acid which impacted an acidic condition in the sample (Figure 26).

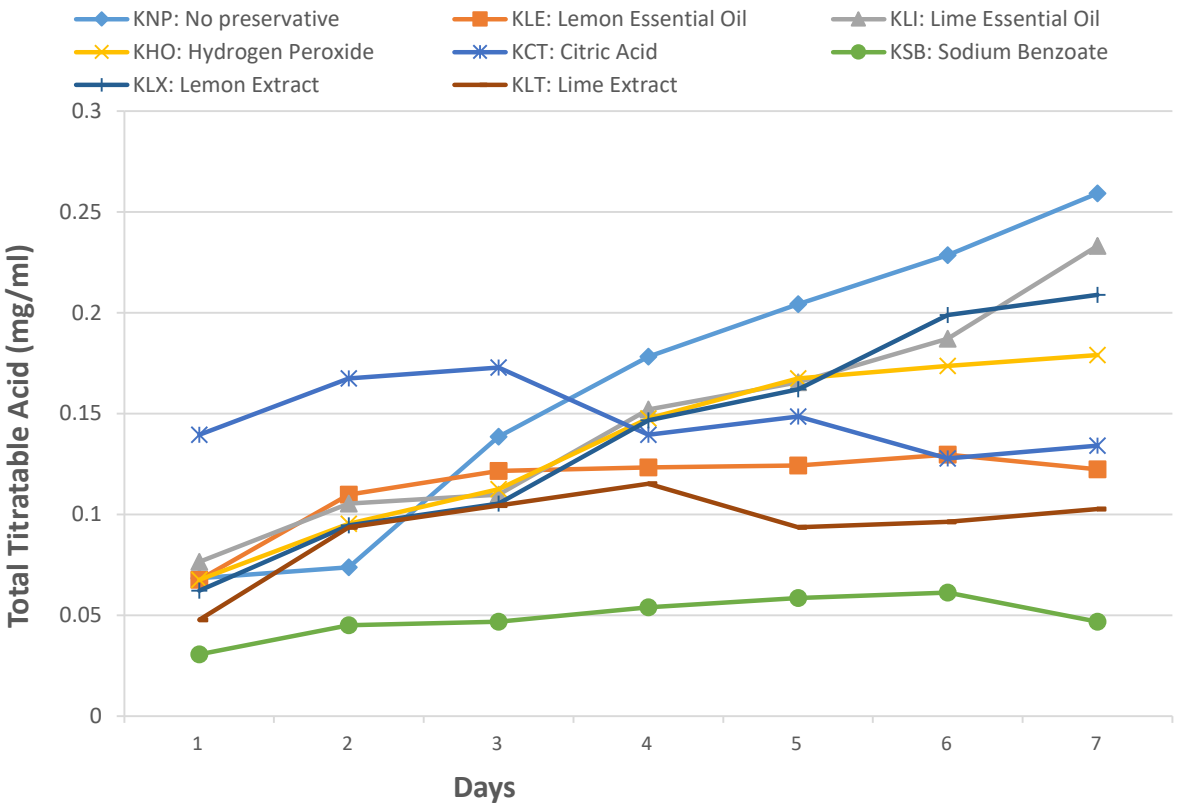

Figure 22: Total titratable acidity (TTA) of *kunun-zaki* during storage

KNP: without preservative; KLE: 1% lemon essential oil; KLI: 1% lime essential oil; KHO: 1% hydrogen; KCT: 1% citric acid; KSB: 1% sodium benzoate; KLX: 1% lemon extract; KLT: 1% lime extraction

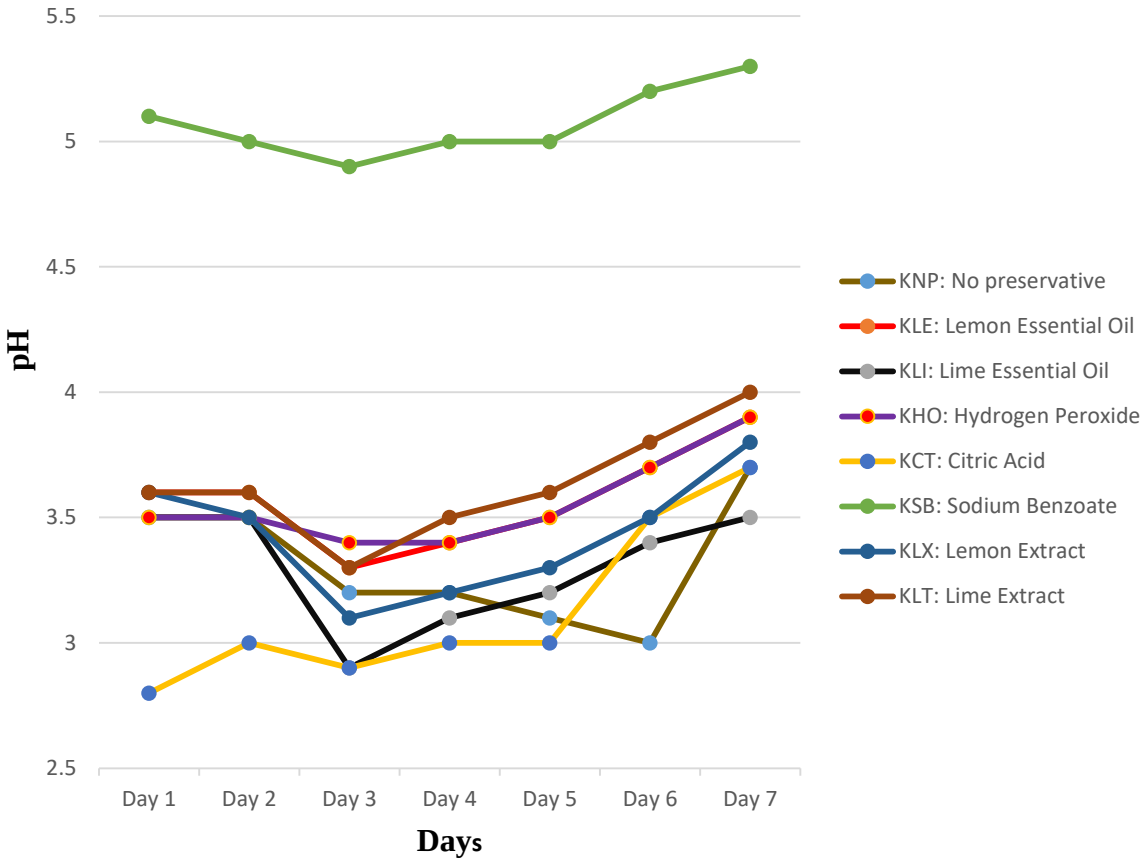

Figure 23: pH values of *kunun-zaki* during storage

KNP: without preservative; KLE: 1% lemon essential oil; KLI: 1% lime essential oil; KHO: 1% hydrogen; KCT: 1% citric acid; KSB: 1% sodium benzoate; KLX: 1% lemon extract; KLT: 1% lime extraction

4.2 Discussion

The proximate composition of millet/groundnut *kunun-zaki* showed that crude protein and crude fat generally increased along with increasing groundnut inclusion, while crude fiber and carbohydrate generally increased along increasing inclusion of millet. This result is comparable with the findings of Ajanaku, Ajanaku, Edobor-Osoh & Nwinyi (2012) which reported that low protein, ash and fat content of *ogi* was improved remarkably with groundnut inclusion. The increased protein composition of the blends in addition to the level of groundnut supplementation, may result from single cell protein from the microbial growth and proliferation, microbial structural proteins. The fat contents increase with level of groundnut inclusion may be as a result of high level of fat in the groundnut seeds (Tope, 2014).

The microbial counts of millet groundnut *kunun-zaki* indicates that the microbial succession showed geometric progression and the peak of activities occurred during fermentation, this agrees with the report of Adesulu-Dahunsi et al., (2020). There was no coliform count in all the plates after 48 hour incubation at 37 ^{o}C, this could be due to the fact that all aseptic techniques as stated by Akoma et al., 2010 were observed in the production process which eliminated possible sources of contamination in the beverage. Hazard analysis critical control point (HACCP) as described by Bello, Umoh, Galadima & Mohammed (2020) was carefully observed which reduced or eliminated pathogens and food spoilage microorganisms in the *kunun-zaki*. Owning also to the fact that LAB are majorly involved in the fermentation process of *kunun-zaki* reported by Adesulu-Dahunsi et al., (2019), which led to the production of lactic acid, this slowed down the growth of yeast and food spoilers in the *kunun-zaki*, this is comparable with the report of Hsouna et al., (2017).

Kunun-zaki a nonalcoholic fermented cereal drink which under goes lactic acid fermentation was produced with the addition of groundnut to get protein enriched cereal drink. The proximate analysis shows that there was increase in the protein content of *kunun-zaki* with groundnut addition, as increase in groundnut increased the protein content of the beverage, Verni, Rizzello, & Coda et al., (2019) reported that the presence of protein and dietary fiber in a food makes it functional food, hence the millet groundnut *kunun-zaki* can be a functional food/beverage. Moisture content of millet groundnut *kunun-zaki* ranged from 84.38±0.1 to 85.14±0.2. As the result in this work is comparable with the findings of Aderinola & Oluwamukomi, 2021.

From the result of the mineral compositions, it can be deduced that the level of each cation in the beverage correlates with the percentage of millet in the blend. This suggests that millet, the dominant component of these blends, is the higher source of the cations. The fluctuations observed in the mineral content of work is similar to that reported by Adegbehingbe, (2015).

Kunun-zaki produced from 50% millet and 50% groundnut (KE) is more nutritious as it has the highest crude protein from the proximate result (Table 1). But *kunun-zaki* produced from 80% millet and 20% groundnut was most significant ($P<0.05$) and preferred by the sensory panelist in taste, flavour, appearance and colour, the significance may be due to low percentage groundnut addition which did not alter the known traditional *kunun-zaki* taste but improved on the flavour, texture and overall quality of the product. Pan, Xiang, Diao, Ma, Shi et al., (2019) reported that ammonia nitrogen is produced by microbial decomposition of protein during fermentation. Thus, the product of protein fermentation (ammonia) may have impacted an off taste and

flavour on sample KE (50% millet + 50% groundnut) which could be the reason the sensory properties was not significant and not rated high by the panelist.

The pH of freshly prepared groundnut millet *kunun-zaki* in this study ranged from 4.8 to 6.9 (Tables 4, 8, 12 and 16). This may be due to the inclusion of groundnut in the *kunun-zaki*, as high pH were reported by Oyedoh, Oshoma, & Ikenebumeh. (2020) in fermentation of peanut and cowpea by *Lactobacillus spp*. Groundnut is fermented at pH range of 4.5-6.5, groundnut fermentation dragged the *kunun-zaki* pH to the values obtained in this result.

Sample NZK (18 hours steep) was most significant at confidence level of $P<0.05$ in taste, flavour, appearance and colour and over all acceptability with mean value of 7.13±1.5 compared with other samples steeped at 6 hours, 12 hours or 24 hours. Steeping or soaking is a household food processing method that improves on the bioaccessibility of micronutrients in grains as reported by Kumari & Platel (2020).

Usually, beverages produced from malted cereals have characteristic malty, cereal-like, sweetish, sour, and worty-like flavors as reported by Nsogning Dongmo et al. (2016), Salmeron, Rozada, Thomas, Ortega-Rivas, Pandiella (2015). This is due to the fact that germination improves the nutritional content of cereal and legumes, it activates endogenous enzymes like; $\alpha-$ amylases, phytases and other types of glucosidases that degrades anti nutritional factors in cereal and legumes, these enzymes break down complex macromolecules and present them in digestible forms. The findings of this research work has shown that using malted cereals improves on the proximate quality of *kunun-zaki*, as it agrees with consumers claim that *kunun-zaki* is medicinal as reported by Akoma *et al.* (2014).

Mineral element results of *kunun-zaki* from 100% starter culture (KCN) shows that the activities of LAB during fermentation help in making mineral elements available in the beverage as a result of microbial degradation of substrates as reported by Waters, Mauch, Coffey, Arendt, & Zannini (2015). But the starter culture did not really impact on the sensory quality of *kunun-zaki* as *kunun-zaki* from spontaneous fermentation (KAN) was most significant at confidence (P<0.05) in taste, flavour, colour and overall acceptability

Relatively low pH is indicative of acid production which prevented the growth of pathogenic microorganisms in the beverage, this is in agreement with the findings of Elmahmood and Doughari (2007). Sample KLE (*kunun-zaki*+1% lemon essential oil) had pH of between 3.6 and 3.9 from the first day to the seventh day on the bench while KLI had pH of 3.5 on the first day and 2.9 on the third day and 3.5 on day seven on the bench. Sample KLX (*kunun-zaki*+1% lemon extract) had pH of 3.6 and 3.8 on day one and day seven respectively while KLT (*kunun-zaki*+1% lime extract) had pH of 3.6 and 4.0 for day one and day seven respectively. The lemon and lime essential oil and extract impacted acidic condition in the *kunun-zaki* thereby inhibiting the growth of food spoilers and pathogens in the *kunun-zaki*. Kim & Ndegwa (2018) reported that pathogens and food spoiler's exhibit low growth rate in acidic environment than in alkaline environment. This work is also in agreement with the findings of Hsouna et al. (2017) which reported that citrus essential oils successfully inhibited the growth of *L. monocytogenes* in minced meat.

Lemon and lime preservatives impacted an acidic environment in the medium thereby inhibiting the growth of pathogens and food spoilers. Components of lemon are limonene, ß-pinene, $\alpha-$ terpinol, linalool and other essential components that proffers

antimicrobial activities against pathogens and are therefore explores as food preservatives (Hsouna et al., 2017).

There was no coliform count in all the preserved *kunun-zaki* from the first day of preservation but on the fourth day there was 5.5 (log cfu/ml) count on sample KHO (*kunun-zaki* + hydrogen peroxide). This may be due that to the fact hydrogen peroxide is more of antiseptic than preservative in foods Martin, Friedlander, Mok, Kent, Wiedmann & Boor, (2014), reported that hydrogen peroxide deteriorate quickly in fluid milk, hence its short preservative properties.

Lemon and lime essential oil and lemon extract preserved the *kunun-zaki* samples from coliform bacteria till the seventh day. This can be comparable with the findings of Hsouna et al. (2017), who reported preservative properties lemon essential oil on minced meat.

CHAPTER FIVE

5.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

Kunun-zaki, a traditional non-alcoholic fermented sweet-beverage in Nigeria, was produced using different production technologies in order to enhance its nutritional properties, sensory attributes and shelf life. The various technologies include fortification of millet with groundnut by varying the combination ratio, steeping of the millet at different durations, malting the millet, inclusion of commercial starter cultures in the fermentation process and addition of natural and chemical preservatives to the beverage.

A blend of 20% unmalted groundnut + 20% malted millet + 60% unmalted millet, steeped at eighteen hours, fermented spontaneously produced refreshing protein rich *kunun-zaki*. Moreover, lemon and lime essential oils and their extracts extended the shelf life of the product for seven days by inhibited the growth of spoilage and pathogenic microorganisms in the *kunun-zaki*.

5.2 Conclusions

Bearing in mind the growing consumer's interest in organic functional food and beverages without chemical additives, it can be confirmed that the outcomes of this study that:

- The addition of groundnut to *kunun-zaki* made from millet grains improved the ash, crude fat and crude protein of the fermented beverage and the fortified products had high acceptability by the consumers. In particular, the sample with 50% groundnut

addition had the highest overall consumer acceptability, while sample with 20% groundnut addition had the highest presumptive shelf life.

- Steeping the grains used for *kunun-zaki* production for 18 hours enhanced its appearance, taste, flavour, viscosity and overall acceptability.
- Combining 20% malted millet, 60% unmalted millet and 20% groundnut gave the best fortified *kunun-zaki* in terms of appearance, taste, flavour, viscosity and consumer's overall acceptability.
- Addition of the commercial starter culture had no significant effect on the appearance and viscosity of *kunun-zaki*. Also, the spontaneously fermented was better in overall acceptability.
- Lemon and lime essential oils and lemon extract extended the shelf life of the product by seven days on the bench comparable to the chemical preservative, sodium benzoate. Hence, lemon essential oils and lemon extract can be harnessed as natural (organic) preservative in *kunun-zaki* production.

Generally, this work showed that groundnut fortification improved the proximate contents of *kunun-zaki* and lemon and lime essential oils and lemon extract could be used as preservatives in the beverage.

5.3 Recommendations

Based on the nutritional profile of the beverage, groundnut should be added to millet in *kunun-zaki* production to improve the nutritional content of *kunun-zaki*. Lemon and lime essential oil can be harnessed as preservatives in *kunun-zaki* as they contain antimicrobial properties that can extent the shelf life of foods. Moreover, there is need to exploit other starter cultures in the production of *kunun-zaki* to ensure reproducibility.

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APPENDICES

Appendix 1: Sensory evaluation sheet

LANDMARK UNIVERSITY

MICROBIOLOGY DEPARTMENT

SENSORY EVALUATION SHEET

Name……..… (Optional)
Date…….

Product: ***Kunun-zaki***

Instruction: You are provided with five (5) samples formulated from malted millet, unmalted millet and ground nut. Please rank the products according to your degree of likeness or otherwise for their Appearance/colour, Taste, Flavour, Viscosity and Overall acceptability using the number indicator below:

1. Dislike extremely
2. Dislike very much
3. Dislike moderately
4. Dislike slightly
5. Neither like nor dislike
6. Like slightly
7. Like moderately
8. Like very much
9. Like extremely

Sample	Appearance/colour	Taste	Flavour	Viscosity	Overall acceptability
KZN					
KNZ					
NZK					
ZNK					
NKZ					

Comments……
……

Signature…….

APPENDIX 2: *Kunun-zaki* dispensed in universal bottles prior to analysis

APPENDIX 3:

TIME LINE OF RESEARCH WORK

TIME	ACTIVITY
June 2020	Collection of samples and materials from Bida, Niger State.
June 2020	Proposal presentation
July-September 2020	Extraction of essential oil from citrus fruits
September-October 2020	Production of *kunun-zaki*
October 2020	Determination of organoleptic properties of the product (sensory evaluation)
October-November 2020	Determination of proximate composition of *kunun-zaki*. samples produced
December2020	Microbial evaluation of *kunun-zaki* during production and storage.
February 2021	First and second progress report presentation
March 2021	Departmental presentation
April 2021	College presentation