EVALUATION OF WOUND-HEALING POTENTIALS OF PROBIOTICS ISOLATED FROM LABORATORY- PRODUCED 'KUNUN-ZAKI' ON STREPTOZOTOCIN-INDUCED DIABETIC RATS.

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CERTIFICATION

I certify that this dissertation titled "Evaluation of wound-healing potentials of probiotics isolated from laboratory- produced 'kunun-zaki' on streptozotocin-induced diabetic rats'' was carried out by VICTOR-ADULOJU, ADEYEMISI TOPE, Registration number: NAU/PG/Ph.D./ 2012487002F under the supervision of Prof. C. O. ANYAMENE in partial fulfilment of the requirements for the award of Doctor of Philosophy (Food Microbiology) in the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka Anambra State, Nigeria.

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APPROVAL

This research work titled "Evaluation of wound-healing potentials of probiotics isolated from laboratory-produced 'kunun-zaki' on streptozotocin-induced diabetic rats" has been approved for the award of the degree of Doctor of philosophy (Food Microbiology) by the Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka.

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DEDICATION

This dissertation is dedicated to the Almighty God, my helper and my strength.

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ABSTRACT

Diabetic ulcers is one of the most common complications in diabetic patients. They are considered as a high-risk factor for lower-limb amputation. The present study was conducted to evaluate the wound-healing potentials of probiotics isolated from laboratory-produced 'kunun-zaki' on streptozotocin-induced diabetic rats. The 'kunun-zaki' is a slurry produced from millet and sorghum with a thickener (paddy rice and sweet potatoes) at different substitution levels (50:50%, 80:20%) were evaluated for their physicochemical, proximate, vitamins, mineral, sensory and microbial parameters. The isolation of probiotics was carried out using **standard culture and biochemical techniques. These isolates were screened for their** probiotic properties using different values of pH, temperature and sodium chloride (1-10% concentration). The antibacterial activity of the *Lactobacillus* strains was evaluated using agar well diffusion method against three pathogens (*Pseudomonas aeruginosa, Staphylococcus haemolyticus* and *Klebsiella pneumoniae*) isolated from a chronic ulcer. The *Lactobacillus* with the highest inhibitory zone were molecularly identified using polymerase chain reaction method. Seventy five Wistar female rats were divided into 5 groups and each group was sub-divided into 3 of 5 rats each (Group A- normal control, B- diabetic control, C- diabetic treated with *Lactobacillus casei,* D- diabetic treated with *Lactobacillus plantarum* and group E- diabetic treated with gentamicin) assigned to sub-groups is the pathogenic organisms to be treated. A full-thickness wound (2.0 cm) was made at the upper paravertebral of each rat and infected with the pathogenic organisms. After 24 h of injury, body weight, blood glucose level, percentage wound-healing and the total red blood cell and white blood cell counts of rats were examined. The pH of the samples ranged from 4.30–5.67 and the total soluble solids content ranged from 9.47% - 13.93%. 'Kunun-zaki' produced using paddy rice prepared at 50:50% (millet/sorghum) had 86.07% moisture content, 4.45% ash, 0.34% fiber value, 1.90% protein and 6.80% carbohydrate. The sweet potatoes based 'kunun-zaki' had highest vitamin C of 32.25 mg/100ml, 18.21mg/100ml vitamin E, 154.67mg/100ml magnesium and 245mg/100ml potassium. Furthermore, the sensory quality characteristics (flavour and overall acceptability) of the 'kunun-zaki' processed with paddy rice and paddy rice + sweet potatoes at 50:50% and 80:20% (millet and sorghum) had the highest scores of 6.35 and significantly different ($P<0.05$) from other samples. A total of eight *Lactobacillus* strains viz: *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus delbruekii, Lactobacillus lactis, Lactobacillus helveticus* and *Lactobacillus bulgaricus* were isolated from eight 'kunun-zaki' samples. The *Lactobacillus plantarum* and *Lactobacillus casei* survived in a wide range of NaCl concentration (1-10%) and in pH 2. *Lactobacillus plantarum* isolated from 50:50 'kunun-zaki' with paddy rice had the highest inhibitory zone against all the pathogenic organisms and is significantly different ($P< 0.05$) from other strains. The molecular identification revealed the *Lactobacillus* species with highest zones of inhibition to be *Lactobacillus casei* and *Lactobacillus plantarum*. The body weight was slightly increased in group A (control) but decreased in group B on day 7. The fasting blood glucose level of untreated diabetic rats (233mg/dL) was significantly higher than other groups on day 7. *Lactobacillus plantarum* significantly decreased all infected wound areas as compared to other groups and increased wound healing. The control group had the highest haemoglobin of 13.69g/dL on day 15. All the treatment groups showed non-significant change ($P> 0.05$) in the percentage of neutrophil in rats infected with *Klebsiella pneumoniae* in day 7, however, there was a significant increase ($P< 0.05$) in neutrophils, in diabetic control rats in day 15.This study has shown that the use of 'kunun-zaki' can serve as a natural probiotic drink. The isolated *Lactobacillus plantarum* has been shown to be the most effective probiotic which promotes diabetic ulcer healing.

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CHAPTER ONE

INTRODUCTION

1.1. Background of the Study

Diabetic ulcers, one of the most common prevalent complications in diabetic patients, are becoming for serious concern worldwide. They are considered major source of morbidity and leading cause of hospitalization in patients with diabetes (Aalaa *et al*., 2012). Diabetic ulcer can lead to lower-limb amputation and even death if necessary care is not provided (Snyder and Hanft, 2009). Every year, more than a million lower limbs of diabetic patients are amputated as a consequence of wound advancement (Jaoa *et al*., 2012). Development and progression of diabetic ulcers are complicated and are different from other chronic wounds that involve the convergence of several pathological mechanisms such as neuropathy, foot deformity, gangrene, vascular diseases, as well as infections (Shahbazian *et al*., 2013; Guo and DiPietro, 2010; Zhao *et al*., 2010). Diabetic wound infection is one of the main factors disturbing inflammatory response and delaying wound healing process. Most diabetic wounds are generally infected with complex microorganisms that are composed of both Gram positive and Gram negative bacteria (Shaw *et al*., 2010). In current clinical practice, the treatment of diabetic foot infections includes blood sugar control, wound debridement, surgery, systemic antibiotics and advanced dressings (Alavi *et al*., 2014; Tallis *et al*., 2013; Zhao *et al*., 2010).

Diabetes mellitus occurs throughout the world but is more common in developed countries (Maria *et al*., 2013). The dramatic rise in diabetic ulcer is largely due to the rise in type 2 diabetes and factors driving it include overweight and obesity (Cichosz *et al*., 2013).

Probiotics are defined as live microorganisms which when administered in adequate amounts confer health benefits on the host (Gareau *et al*., 2010). Probiotic organisms are naturally present in food and water (Soccol *et al*., 2010). Probiotic species belong to the genera *Lactobacillu*s and *Bifidobacterium* (Gupta and Garg, 2009). These bacteria are generally regarded as safe because they can reside in the human body causing no harm. They are key microorganisms in milk fermentation and food preservation (Patrick, 2012). Several studies have shown that probiotic bacteria are not only associated with intestinal health (Durchschein *et al*., 2016) but they can also affect a wide range of other health factors: oral ecology, anticarcinogenic effects, modulation of gut immunological mechanisms, mucin production, down-regulation of inflammatory responses, inhibition of pathogens, mucosal adherence, stimulation of immunoglobulin A and the production of antimicrobial substances such as organic acids, hydrogen peroxide and bacteriocins (King *et al*., 2014; Rijkers *et al*., 2010; Azizpour *et al.,* 2009). The recent scientific investigation has supported the important role of probiotics as a part of a healthy diet for humans as well as for animals and may be an avenue to provide a safe, cost-effective, and 'natural' approach to reduce microbial infection (Grimoud *et al*., 2010). Probiotics adhere and colonize human body which may increase their retention time thus facilitating the prolonged probiotic activity (Lee and Salminen, 2009). These probiotic preparations may be presented in the form of powders, liquid, tablets, capsules, granules, pastes or sprays depending on the animal or human receiving the supplement and the condition to be treated (Hoque *et al*., 2010).

'Kunun', a traditional non-alcoholic beverage made mainly from cereals, is of low viscosity and has a sweet-sour taste depending on the level of fermentation, milky-cream in appearance and is popular with people of northern Nigeria (Odom *et al*., 2012; Adebayo *et al*., 2010). It is

produced mainly from *Pennisetum americarum* (Millet), although *Sorghum bicolor* (Sorghum), *Zea mays* (Maize), *Oryza sativa* (Rice) and other cereals can be used. It is normally flavoured with a combination of spices which include, *Zingiber officinale* (Ginger), *Eugenia aromatic* (Cloves), *Piper guineense* (Black pepper) and *Xylopia acthiopica* (cinnamon) (Adebayo *et al*., 2014; Oluwajoba *et al*., 2013; Nkama *et al*., 2010; Amusa and Odunbaku, 2009).

1.2. Statement of the Research Problem

Diabetes mellitus is an epidemic chronic disease that is frequently complicated by complex wound infections. Diabetic wounds are slow, non-healing wounds that can persist for years despite adequate and appropriate care. It causes great physical handicap and psychosocial disability. Such wounds are difficult and frustrating to manage. Diabetic wounds do not follow the precisely orchestrated course of events observed in normal healing and bacterial colonization. This disease has become the leading health challenge globally. Nowadays, the occurrence of multidrug-resistant organisms has emerged as a serious and common concern in diabetic wounds, leading to a longer hospital stay duration, increased economic burden, morbidity, and in some cases may lead to higher mortality (Trivedi *et al*., 2014). Furthermore, concern has been expressed as the degree of microbial resistance to indiscriminately prescribed and misused antibiotics increases. To combat these trends directly, the World Health Organization currently advocates the implementation of alternative disease control strategies, such as exploiting the prophylactic and therapeutic potential of probiotic bacteria (Amirreza *et al*., 2016). It is, therefore, necessary to develop a new non-antibiotics approach for the treatment of infected diabetic wounds.

1.3. Justification for the Study

To address the difficulties in treating infected diabetic ulcer, several approaches have been undertaken including intensifying research to develop new antibiotics, use of different antibiotic combinations and identification of alternative means. However, the scope for the development of new antibiotics that will be more effective than the existing antibiotics is very limited. Therefore, a lot of research is being carried out to develop non-antibiotics therapeutics against diabetic ulcer using probiotic isolated from diary products and traditional medicine but a few exhibit antibacterial effects under *in-vitro* condition but their antibacterial effects under *in-vivo* situations are largely unknown. A main concern with ayurvedic medicine mediated treatment is the dosage. Milad, (2016) reported the effects of probiotic on cutaneous wound healing in rats but could not elucidate the exact role of probiotics in wound-healing process. Probiotic *Lactobacillus* is also of interest because of its safety for use in humans. Till date, several studies have been done on production and nutritional composition of varieties of 'kunun-zaki' and many strains of probiotic bacteria have been isolated. However, not much work has been carried out on isolation of probiotic *Lactobacillus* strains from 'kunun-zaki' processed with sweet potatoes and paddy rice thickener. Thus so there is little or no information on the wound-healing potentials of probiotics on streptozotocin-induced diabetic rats. This study investigates the nutritional composition of millet and sorghum drinks processed with sweet potatoes and paddy rice thickener. Also, it assessed the wound-healing potentials of *Lactobacillus*.

1.4. Aim

The aim of this work was to evaluate wound-healing potentials of probiotics isolated from laboratory- produced 'kunun-zaki' on streptozotocin-induced diabetic rats.

1.5. Objectives.

The specific objectives of this work were to:

1. evaluate the physicochemical, proximate, vitamins and mineral components of the 'kunun-zaki',
2. investigate the sensory attributes of the 'kunun-zaki' samples, .
3. determine the microbial load of the 'kunun-zaki' samples,
4. isolate and identify *Lactobacillus* species from 'kunun-zaki' samples,
5. examine the probiotic potentials of *Lactobacillus* species isolated from 'kunun-zaki',
6. determine the antibacterial effects of the *Lactobacilli* on pathogenic microorganisms isolated from chronic skin ulcer,
7. classify the *Lactobacillus* species with the highest inhibitory zone and the pathogenic organism using molecular identification,
8. determine the body weight and blood glucose level of the streptozotocin-induced diabetic rats,
9. observe behavioural responses of the experimental rats,
10. assess the percentage wound healing,
11. estimate the total red blood cell and white blood cell counts of the experimental animals.

CHAPTER TWO

LITERATURE REVIEW

2.1. Probiotics

Probiotics refer to microorganisms with positive effects on health by assisting with food and nutrient digestion and having a direct antagonistic effect against specific groups of organisms to decrease their numbers or effect on their metabolism and to stimulate immunity (Gareau *et al*., 2010). They cooperatively maintain a delicate balance between the gastrointestinal tract and immune system (Moayyedi *et al*., 2010) and prebiotics–non digestible food ingredients that encourage the growth and activity of favourable intestinal bacteria are quickly gaining attention as functional foods. Probiotics may beneficially affect the host by augmenting its intestinal microbial population beyond the amount already existing, thus possibly inhibiting pathogens (Haller *et al*., 2010).

Traditionally, the most popular food delivery systems for these cultures have been freshly fermented dairy foods, such as yoghurts and fermented milks, as well as unfermented milks with cultures added (Saikali *et al*., 2008). Potential benefits may result from growth and action of the bacteria during the manufacture of cultured foods. Lactose malabsorption may compromise their intake of protein and calcium (Saikali *et al*., 2008) and these microflora are capable of providing numerous health benefits beyond basic nutritional value.

2.2. Composition of Probiotic Preparation

The most commonly used organisms in probiotic preparations are the lactic acid bacteria. These are found in large numbers in the gut of healthy animals. Organisms other than lactic acid bacteria which are currently being used in probiotic preparations include *Bacillus* species, yeasts (*Saccharomyces cerevisiae* and *Saccharomyces boulardii)* and filamentous fungi (*Aspergillus oryzae*) (Stanton *et al*., 2009). Probiotic products are now available in different formulations with, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Enterococcus faecium* and others with or without probiotic and fructooligosaccharides (FOS). Some of the most common probiotic products are *Lactobacillus acidophilus* and *Bifidus longum* with fructooligosaccharides, and *Bifidus infantis* (Stanton *et al*., 2009).

Probiotics must have good shelf- lives in food or preparations, containing a large number of viable cells at the time of consumption, and be nonpathogenic and nontoxic in their preparations. The most extensively studied and widely used probiotics are the lactic acid bacteria, particularly the *Lactobacillus* and *Bifidobacterium* species (Yateem *et al*., 2008).

2.3. *Lactobacillus*

The *Lactobacillus* genus consists of a genetically and physiologically diverse group of rod-shaped, gram-positive, non-spore forming, non-pigmented, catalase negative and microaerophilic to anaerobic (Yateem *et al*., 2008). Lactic acid bacteria have widespread use in fermented food production and generally recognized as safe (GRAS) organisms and can be safely used for medical and veterinary applications (Zago *et al*., 2011).

In the food industry, Lactic acid bacteria is widely used as starter cultures and has been cited to be part of human microbiota (Lee and Bak, 2011). Ingestion of live cells of certain species of lactobacilli in adequate amounts is believed to confer several beneficial physiological

effects on the host such as maintaining a healthy and equilibrated intestinal infection. Lactic acid bacteria have been used successfully to prevent antibiotic associated diarrhea and recurrent *Clostridium difficile* diseases and to treat various diarrhea illnesses (Peera, 2013).

2.4. Nutritional and Health Advantages of Lactic Acid Fermentation

Lactic acid fermentation of sorghum foods by lactic acid bacteria prior to cooking or consumption significantly alters their biochemical properties, rendering a food product with enhanced nutritional value and flavour (Towo *et al*., 2008). Lactic acid fermentation has been shown to reduce the level of anti-nutritive oligosaccharides, phytate and tannins (Adeyemo *et al*., 2016).

2.5. Factors Affecting Probiotics Viability in Foods.

Some factors, both intrinsic and extrinsic, may influence the survival of probiotics in food and so have to be considered in all stages of probiotic food manufacturing: physiological state of the added probiotic in the food; physicochemical conditions of food processing; physical conditions of product storage, such as temperature; chemical composition of the product, such as content of nutrients, oxygen or pH; interactions with other product components, that can be inhibitory or protective.

2.5.1. Physiological State.

The physiological state of bacteria when prepared and remaining in a product itself are important factors for survival of the probiotics. Dryness in a food product keeps the bacteria in a relatively quiescent state during storage, while a wet product establishes potentially active metabolism. Different probiotic strains have their own intrinsic tolerances to environmental conditions, including how the culture is prepared, and some cross- protection can be observed, providing protection against

other stresses by the exposure to only one stress. Stress responses can be explored to make probiotic strains more resilient and likely to survive in food matrices, with significant industrial importance (Takahashi *et al*., 2007).

2.5.2. Temperature

The temperature at which probiotic organisms grow is an important factor in food applications where fermentation is required, is also a critical factor influencing probiotic survival during manufacturing and storage (Lee and Salminen, 2009). The lower the temperature, the more stable probiotic viability in the food product (Rijkers *et al*., 2010).

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During processing, temperatures over 45-50°C will be detrimental to probiotic survival; this means that the higher the temperature, the shorter the time period of exposure required to severely decrease the numbers of viable bacteria, ranging from hours or minutes at 45–55°C to seconds at higher temperatures. Therefore, it is obvious that probiotics should be added downstream of heating, cooking and pasteurization processes in food manufacturing to avoid the high temperatures (Lee and Salminen, 2009). Elevated temperature also has a detrimental effect on stability during the product's process of shipping and storage. Again, the cooler a product can be maintained, the better probiotic survival will be, as in vegetative probiotic cells in liquid products, where refrigerated storage is usually essential. If the product is dried, the bacteria will be in a quiescent state, so acceptable probiotic viability can be maintained in dry products stored at ambient temperatures for 12 months or more. Producing and maintaining low water activities in the foods is the

key to maintaining probiotic viability during non-refrigerated storage because there is a remarkable interaction between temperature and water activity (Doleyres and Lacroix, 2008).

2.5.3. pH

Some bacteria like Lactobacilli and Bifidobacteria can tolerate lower pH level because they produce organic acid and products from carbohydrate metabolism. Indeed, numerous *in vitro* and *in vivo* studies have demonstrated that in gastric transit where the cells are exposed to low pH values and with a time of exposure relatively short, some probiotic organisms can survive. In fermented milks and yoghurts with pH values between 3.7 and 4.3. Lactobacilli are able to grow and survive, while *Bifidobacteria* tend to be less acid tolerant, with most species surviving poorly in fermented products at pH levels below 4.6 (Mortazavian *et al.*, 2010; Karna *et al.*, 2007).

2.5.4. Water Activity

For quiescent probiotic bacteria, water activity is a crucial determinant for the survival in food products during storage. The higher moisture levels and water activity, the lower survival of probiotics. There is a substantial interaction between water activities and temperature with respect to their impact on the survival of quiescent probiotics. As the storage temperature is increased, the detrimental impact of moisture is magnified. Here, the osmotic stresses appear to play a role, with the presence of smaller molecules resulting in poorer bacterial survival (Vesterlund, 2012).

2.5.5. Oxygen

Both Bifidobacteria and Lactobacilli are considered strict anaerobes and oxygen can be detrimental to their growth and survival. However, the degree of oxygen sensitivity varies considerably between different species and strains; for example, lactobacilli, which are mostly microaerophilic, are more tolerant of oxygen than bifidobacteria, to the point where oxygen levels are not an important consideration in maintaining the survival of lactobacilli (Ravinder *et al*., 2012). Most probiotic *Bifidobacteria* do not grow well in the presence of oxygen, although, many bifidobacteria have enzymatic mechanisms to limit the oxygen toxicity. For oxygen-sensitive strains, some strategies can be used to prevent oxygen toxicity in food products (Amund, 2016). Antioxidant ingredients have been shown to improve probiotic survival, as well as the use of oxygen barrier or modified-atmosphere packaging (Lee and Salminen, 2009).

2.5.6. Toxicity of Ingredients

Interactions between probiotics and other ingredients could happen and those interactions can be protective, neutral, or detrimental to probiotic ability (Lee and Salminen, 2009). Obviously, the inclusion of antimicrobial preservatives can inhibit probiotic survival and elevated levels of ingredients such as salt, organic acids, and nitrates can inhibit probiotics during storage, while starter cultures can sometimes inhibit the growth of probiotics during fermentation through the production of specific bacteriocins (Ravinder *et al*., 2012).

2.5.6.1. Growth Factors, Protective and Synergistic Ingredient.

Probiotic lactobacilli and bifidobacteria are only weakly proteolytic and grow relatively slowly or poorly in milk. The growth of bifidobacteria can be improved by the presence of suitable companion cultures, which can aid in protein hydrolysis and through the production of growth factors. Some growth substrates such as carbon sources, nitrogen sources, and growth factors or antioxidants, minerals, and vitamins can be added to improve growth. Finally, the food matrix itself can be protective like in the cheese, where the anaerobic environment, high fat content and buffering capacity of the matrix helps to protect the probiotic cells both in the product and during intestinal transit (Kanmani *et al*., 2013).

2.5.6.2. Freezing and Thawing.

The damages made to cell membranes by freezing probiotics is detrimental to survival, and also can make the cells more vulnerable to environmental stresses. To prevent or at least mitigate cell injury, protectants are usually added to cultures to be frozen or dried. Once frozen, probiotics can survive well over long shelf lives in products such as frozen yogurts and ice-cream. Using alternative methods of freezing, such as slow-cooling rates or pre-freezing stress, can significantly improve cell survival. Repeated freeze-thawing cycles are highly detrimental to cell survival and should be avoided (Akin *et al*., 2007).

2.5.6.3. Shear Forces.

Probiotic lactobacilli and bifidobacteria are gram-positive bacteria with thick cell walls that are able to tolerate the shear forces generated in most standard food production processes such as high-speed blending or homogenization that may result in cell disruption and losses in viability (Yong *et al*., 2016).

2.6. Health Benefit of Probiotics.

2.6.1. Nutrient Synthesis and Bioavailability

Lactic acid bacteria are known to release various enzymes and vitamins into the intestinal lumen. This exert synergistic effects on digestion, alleviating symptoms of intestinal malabsorption, and produced lactic acid, which lowers the pH of the intestinal content and helps to inhibit the development of invasive pathogens such as *Salmonella* species or strains of *Escherichia coli* (Azizpour *et al*., 2009). Bacterial enzymatic hydrolysis may enhance the bioavailability of protein and increase the production of free amino acids, short chain fatty acids (SCFA). Lactic acid, propionic acid and butyric acid are also produced by lactic acid bacteria. When absorbed, these short chain fatty acids contribute to the available energy pool of the host (Cencic and Chingwaru, 2010) and may protect against pathological changes in the colonic mucosa (Gijs *et al.*, 2013). Functional microorganisms transform the chemical constituents of raw materials of plant/animal sources during food fermentation thereby enhancing the bio-availability of nutrients, enriching sensory quality of the food, imparting bio-preservative effects and improvement of food safety, degrading toxic components and anti-nutritive factors, producing antioxidant and antimicrobial compounds, stimulating the probiotic functions,

and fortifying with some health-promoting bioactive compounds (Bourdichon *et al*., 2012).

2.6.2. Gastric and Intestinal Tract Effect of Probiotics

There are a number of studies in humans that suggest that lactic acid bacteria can decrease the incidence, duration and severity of some gastric and intestinal illnesses.

2.6.2.1. Preventive and Theurapeutic Effects Against Diarrhoea.

The well-known uses of probiotics are for diarrhoea diseases prevention and management of acute viral and bacterial diarrhoea as well as the control of antibiotic-associated diarrhoea. A number of specific strains, including *Lactobacillus reuteri, Saccaromyces boulardii, Bifidobacterium* species and others, have been shown to have significant benefit for diarrhoea (Guarino *et al*., 2015; Walker *et al*., 2013; Elizabeth, 2010; Brenner *et al*., 2009). The probiotic species that show the most promise in treating diarrhoea diseases in children include *Lactobacillus reuteri, Lactobacillus casei, Saccaromyces boulardii, Bifidobacterium bifidum* and *Streptococcus thermophilus* (Devrese and Marteau, 2007). Lactic acid bacteria are known to release various enzymes into the intestinal lumen that exert synergistic effects on digestion, alleviating symptoms of intestinal malabsorption.

2.6.2.2. Alleviation of Lactose Intolerance.

Lactic acid of the yoghurt alleviates the symptoms of lactose intolerance in lactase-deficient individuals. Probiotics have been shown to improve lactose

digestion by reducing the intolerance symptoms as well as by slowing orocecal transit (Oak and Jha, 2018).

2.6.2.3 Hepatic Disease.

Hepatic encephalopathy is a liver disease and its effects can be life threatening. The exact pathogenesis of hepatic encephalopathy still remains unknown. The probiotics, *Streptococcus thermophilus*, *Bifidobacterium species*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus bulgaricus*, and *Enteroccous faecum* containing therapeutic effect have multiple mechanisms of action that could disrupt the pathogenesis of hepatic encephalopathy and may make them superior to conventional treatment and lower portal pressure with a reduction in the risk of bleeding (Saji *et al*., 2011; Versalovic, 2007).

2.6.2.4. Inflammation/ Arthritis.

Probiotic supplementation has both direct and indirect effects. Probiotics exhibit direct effects locally in the gastrointestinal tract, including modulation of resident bacterial colonies and vitamin production (Roberfroid, *et al*., 2010). There are also indirect effects exerted at sites outside the gastrointestinal tract, including the joints, lungs, and skin. Indirect effects most likely result from an impact on immunity, via changes in inflammatory mediators such as cytokines. Modulation of inflammatory responses may be related to regulating or modulating the immune system both locally and in the gastrointestinal tract (Jeong *et al*., 2010).

It is speculated that inflammation associated with rheumatoid arthritis may be modulated by the use of probiotics. Modulation or down regulation of the immune system and subsequent reduction in gastrointestinal tract permeability can result from consuming probiotics (Orel and Trop, 2014).

2.6.2.5. Allegy/Eczema

Probiotic bacteria are important in down regulating inflammation associated with hypersensitivity reactions in patients with atopic eczema and food allergy (Cuello-Garcia, *et al*., 2015).

Perinatal administration of *Lactobacillus rhamnosus* decreased subsequent occurrence of eczema in at-risk infants by one-half. In newborn infants, the initial bacteria to colonize the sterile gastrointestinal tract may establish a permanent niche and have lasting impact on immune regulation and subsequent development of atopic disorders (Niers *et al*., 2009). It was suggested that probiotics may enhance endogenous barrier mechanisms of the gut and alleviate intestinal inflammation, providing a useful tool for treating food allergy (Boyle *et al*., 2009).

Probiotics may also be helpful in alleviating some of the symptoms of food allergies such as those associated with milk protein. Possibly by degrading these proteins to smaller peptides and amino acids, added to the diet of infants on a hydrolysed whey formula decreased the symptoms of atopic dermatitis (Cuello-Garcia, *et al*., 2015). Probiotics have also been found to upregulate anti-

inflammatory cytokines, such as interleukin-10, in atopic children (Pessi *et al*., 2009).

2.6.2.6. HIV and Immune Response

Children with HIV infections have episodes of diarrhoea and frequently experience malabsorption associated with possible bacterial overgrowth (Guandalini, 2011). Administration of *Lactobacillus plantarum* can be given safely to immunocompromised hosts; may have a positive effect on immune response, and has the potential to improve growth and development (Chiara *et al*., 2017). The immune response may further be enhanced when one or more probiotics are consumed together and work synergistically, as seems to be the case when *Lactobacillus* is administered in conjunction with *Bifidobacterium* (Klatt *et al*., 2013; Yan and Polk, 2011).

The effect of probiotics on the immune response has been comprehensively reviewed (Yan and Polk, 2011; McNaught *et al*., 2008). The majority of evidence from *in vitro* systems, animal models and humans suggests that probiotics can enhance both specific and nonspecific immune responses (Kang and Im, 2015).

2.6.3. Hypertension

Preliminary evidence indicates that probiotic bacteria or their fermented products may also play a role in blood pressure control, with animal and clinical studies documenting antihypertensive effects of probiotic ingestion (Pablo *et al*., 2015). Elderly hypertensive patients who consumed fermented milk with a starter containing *Lactobacillus delbrueckii* and *Saccaromyces cerevisiae* experienced

reductions in systolic and diastolic blood pressure (Moro *et al*., 2013). Decreased systolic and diastolic blood pressure and heart rate of hypertensive patients were altered when administered with powdered probiotic cell extracts (Manuel *et al.*, 2015). Some studies have indicated that consumption of milk fermented with various strains of LAB may result in modest reductions in blood pressure, an effect possibly related to the ACE inhibitor-like peptides produced during fermentation (Dong *et al*., 2013).

2.6.4. Cancer

In general, cancer is caused by mutation or activation of abnormal genes that control cell growth and division (De-Moreno and Persigon, 2010). Most of these abnormal cells do not result in cancer as normal cells usually out-compete abnormal ones. Also, the immune system recognizes and destroys most abnormal cells. Many processes or exposures can increase the occurrence of abnormal cells. Precautions that minimize these exposures decrease the risk of cancer. Among the many potentially risky exposures are chemical exposures. Cancer-causing chemicals can be ingested or generated by metabolic activity of microbes that live in the gastrointestinal system. It has been hypothesized that probiotic cultures might decrease the exposure to chemical carcinogens by: (i) detoxifying ingested carcinogens. (ii) altering the environment of the intestine and thereby decreasing populations or metabolic activities of bacteria that may generate carcinogenic compounds. (iii) producing metabolic products which improve a cell's ability to die when it should die (a process known as apoptosis or programmed cell death). (iv) producing compounds that inhibit the growth of tumour cells. (v) stimulating

the immune system to better defend against cancer cell proliferation (Isolauri, 2009; Murch, 2008).

Colorectal cancer (CRC) is a major cause of death from cancer in the western world. Approximately 70% of colorectal cancer is associated with environmental factors, probably mainly the diet. There is interest in the potential protective role of fermented milks containing probiotic cultures against colorectal cancer from humans, animal and in vitro studies (Fernandes *et al*., 2013).

Lactobacillus rhamnosus and *Lactobacillus bulgaricus* have demonstrated anti-mutagenic effects thought to be due to their ability to bind with heterocyclic amines, which are carcinogenic substances formed in cooked meat (Vong *et al.*, 2014). Dietary supplementation with a strain of *Lactobacillus acidophilus* significantly suppressed the total number of colon cancer cells in rats in a dose-dependent manner (Elahe *et al*., 2017). Lactic acid bacteria and their fermented food products are thought to confer a variety of important nutritional and therapeutic benefits on consumers, including antimutagenic and anticarcinogenic activity (Lee *et al*., 2011).

2.6.5. Control of Blood Cholesterol and Hyperlipidaemia.

Cholesterol is essential for many functions in the human body. It acts as a precursor to certain hormones and vitamins and it is a component of cell membranes and nerve cells. However, elevated levels of total blood cholesterol or other blood lipids are considered risk factors for developing coronary heart disease. Hyperlipidaemic patients who were administered *Lactobacillus*

sporogenes experienced a mean 32% reduction in total cholesterol and 35% reduction in LDL cholesterol over a 3-month period. Because *in-vitro* studies have shown that bacteria can remove cholesterol from culture media (Parvez *et al*., 2009) much attention has been given to the cholesterol-lowering potential of probiotics in humans (Tamime, 2008).

2.6.6. Condition of the Genitourinary Tract.

In a recent study of bacterial cultures isolated from women with recurrent episodes of bacterial vaginosis, four different strains of lactobacilli demonstrated inhibitory activity against the bacterial species, possibly by producing an acidic environment (Martin *et al.*, 2009). In addition, a number of observational studies have correlated vaginal health with the presence of lactobacilli (Brotman *et al.*, 2010; Falagas *et al*., 2007). The colon might thus be a source of beneficial as well as harmful bacteria for the urinary and genital tracts. Both oral probiotics and vaginal suppositories of probiotics have been shown to reduce the incidence of recurrent urinary tract infection (Oakley *et al*., 2008; Saunders *et al*., 2007).

2.6.7. *Helicobacter pylori* Infections.

There is a preliminary evidence that probiotic bacteria may inhibit the gastric colonization and activity of *Helicobacter pylori*, which is associated with gastritis, peptic ulcers and gastric cancer (Zhang *et al*., 2015). *Lactobacillus salivarius* was found to inhibit *Helicobacter pylori* colonization in in-vitro studies as well as in mice (Nele *et al*., 2016). The use of probiotics in the field of *Helicobacter pylori* infection has been proposed for improving eradication rate and tolerability and for

compliance of multiple antibiotic regimens used for the infection (Zhu and Liu, 2017; Patel *et al*., 2014). An inhibition of *Helicobacter pylori* infection was also shown in humans consuming *Lactobacillus johnsonii* (Zhang *et al*., 2015; Yang *et al*., 2014).

2.6.8. Inflamatory Bowel Disease.

Studies have shown an improvement in symptoms of inflamatoory bowel disease and ulcerative colitis with consumption of certain strains of lactobacilli (Preidis *et al*., 2012; Caplan and Schrezenmeir, 2009). Lactic acid bacteria may improve intestinal mobility and relieve constipation possibly through a reduction in gut pH (Panigrahi *et al*., 2007). It has also been reported that probiotic combination therapies may benefit patients with inflammatory bowel disease (Sekhon and Jairath, 2010). *Saccharomyces boulardii* in patients with Crohn's disease was found to extend remission time and reduce relapse rates.

2.6.9. Irritable Bowel Syndrome.

Probiotics exhibit a direct effect in the gut, in the treatment of inflammatory and functional bowel disorders. In one of the most common functional bowel disorders, irritable bowel syndrome, *Lactobacillus plantarum* were shown in clinical trials to reduce abdominal pain, bloating, flatulence, and constipation (Roberfroid *et al*., 2010). Randomized controlled trials (RCTs) in healthy adults indicated that probiotic intervention or probiotics-fermented products resulted in changes in intestinal microbiota composition or diversity (Plaza-Diaz *et al.*, 2015).

2.6.10. Vitamin Production.

One of the multiple benefits that probiotics have is the capacity to synthesize vitamins. *In vitro* studies and in humans have demonstrates the capacity of some probiotic strains to synthesize Vitamin K, folic acid and vitamin B12 (Jones *et al*., 2013; Strozzi and Mogna, 2008).

2.6.11. Oral Health

2.6.11.1. Dental Caries

Dental caries is a multifactorial disease of bacterial origin that is characterized by acid demineralization of the tooth enamel. Probiotics have a beneficial effect in limiting or preventing dental caries by adhering to dental surface and integrating into the bacterial communities making up the dental biofilm (Robert *et al*., 2017; Haukioja, 2010).

2.6.11.2. Halitosis

Halitosis or bad breath, is a condition affecting comparatively large section of the population. Bad breath in the oral cavity is mainly ascribed to the production of volatile sulfur compounds predominantly by gram negative anaerobes residing in periodontal pockets and on the tongue dorsum. (Bollen and Beikler, 2012). The replacement of bacteria implicated in halitosis by colonization with probiotic bacterial strains from indigenous oral microbiota of healthy humans may have potential

applications as adjuncts for the prevention and treatment of halitosis (Iwamoto *et al.*, 2010; Scully and Greenman, 2008).

2.7. Diabetes Mellitus.

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period (Rippe and Richard, 2010). Symptoms of high blood sugar include frequent urination, increased thirst, and in creased hunger. If left untreated, diabetes can cause many complications. Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes (Tripathy *et al.*, 2012). Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced (Gardner and Shoback, 2017).

2.7.1. Types of Diabetes

2.7.1.1. Type 1

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the pancreatic islets, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which a T cell-mediated autoimmune attack leads to the loss of beta cells and thus insulin (Kenny, 2014). Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults. Type 1 diabetes can be accompanied by irregular and unpredictable high blood sugar

levels, frequently with ketosis, and sometimes with serious low blood sugar levels. Other complications include an impaired counter regulatory response to low blood sugar, infection, gastroparesis (which leads to erratic absorption of dietary carbohydrates), and endocrinopathies (Verrotti *et al*., 2012).

Type 1 diabetes is partly inherited, with multiple genes, including certain HLA genotypes, known to influence the risk of diabetes. The increase of incidence of type 1 diabetes reflects the modern lifestyle (Butalia *et al.*, 2016).

2.7.1.2. Type 2

Type 2 is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion (Vivian and Fonseca, 2009). The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. Type 2 is the most common type of diabetes mellitus (Pasquel and Umpierrez, 2014*).* In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, high blood sugar can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce the liver's glucose production (Ganguly *et al.*, 2015).

Type 2 is primarily due to lifestyle factors and genetics (Riserus *et al*., 2009). A number of lifestyle factors are known to be important to the development of type 2 including obesity, lack of physical activity, poor diet, stress, and urbanization (Shoback, 2017). Consumption of sugar-sweetened drinks in excess is associated with an increased risk (Malik et al., 2010). Type of fats in the diet is also important, with saturated fat and trans fats increasing the risk and polyunsaturated and

monounsaturated fat decreasing the risk (Risérus *et al*., 2009). Eating lots of white rice also may increase the risk of diabetes (Hu *et al*., 2012). A lack of physical activity is believed to cause 7% of cases (Lee *et al.*, 2012).

2.7.1.3. Gestational Diabetes

Gestational diabetes mellitus (GDM) resembles type 2 in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2–10% of all pregnancies and may improve or disappear after delivery. However, after pregnancy approximately 5-10% of women with gestational diabetes are found to have diabetes mellitus, most commonly type 2 (De-la Monte, 2014). Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy (Eman and Alfadhli, 2015). Management may include dietary changes, blood glucose monitoring, and in some cases, insulin may be required. Though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital heart and central nervous system abnormalities, and skeletal muscle malformations. Increased levels of insulin in a fetus's blood may inhibit fetal surfactant production and cause respiratory distress syndrome (Lee *et al.*, 2012).

Prediabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 (Kitabchi *et al*., 2009). Many people destined to develop type 2 diabetes mellitus spend many years in a state of prediabetes. Some cases of diabetes are caused by the body's tissue receptors not responding to insulin (even when insulin levels are normal, which is what separates

it from type 2 diabetes); this form is very uncommon. Genetic mutations (autosomal or mitochondrial) can lead to defects in beta cell function. Abnormal insulin action may also have been genetically determined in some cases. Any disease that causes extensive damage to the pancreas may lead to chronic pancreatitis and cystic fibrosis (Selvin *et al.*, 2010). Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes. Many drugs impair insulin secretion and some toxins damage pancreatic beta cells (Tuomi *et al.*, 2014).

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes.

2.7.2. Signs and Symptoms

The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger). Symptoms may develop rapidly (weeks or months) in type 1 DM, while they usually develop much more slowly and may be subtle or absent in type 2 DM. Several other signs and symptoms can mark the onset of diabetes although they are not specific to the disease. In addition to the known ones above, they include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes (De la Monte, 2014).

2.7.3. Diabetic Emergencies

Low blood sugar is common in persons with type 1 and type 2 diabetes mellitus. Most cases are mild and are not considered medical emergencies. Effects can range from feelings of unease, sweating, trembling, and increased appetite in mild cases to more serious issues such as confusion, changes in behavior such as aggressiveness, seizures, unconsciousness, and (rarely) permanent brain damage or death in severe cases (Verrotti *et al*., 2012). Moderate hypoglycemia may easily be mistaken for drunkenness; rapid breathing and sweating, cold, pale skin are characteristic of hypoglycemia but not definitive (Kitabchi *et al*., 2009). Mild to moderate cases are self-treated by eating or drinking something high in sugar. Severe cases can lead to unconsciousness and must be treated with intravenous glucose or injections with glucagon.

People may also experience episodes of diabetic ketoacidosis, a metabolic disturbance characterized by nausea, vomiting and abdominal pain, the smell of acetone on the breath, deep breathing known as Kussmaul breathing, and in severe cases a decreased level of consciousness (Verrotti *et al*., 2012). Diabetes mellitus is characterized by recurrent or persistent high blood sugar (De-la Monte, 2014). This is diagnosed by demonstrating any one of the following:

i. Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl).
ii. Plasma glucose ≥ 11.1 mmol/l (200 mg/dl) two hours after a 75 g oral glucose load as in a glucose tolerance test
iii. Symptoms of high blood sugar and casual plasma glucose ≥ 11.1 mmol/l (200 mg/dl).

iv. Glycated hemoglobin (HbA_{1C}) $\geq$ 48 mmol/mol ($\geq$ 6.5 DCCT %) (Vijan, 2010).

2.7.4. Complications

All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10-20) but may be the first symptom in those who have otherwise not received a diagnosis before that time. The major long term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease (Sarwar, 2010). Other "macrovascular" diseases are stroke, and peripheral artery disease.

The primary complications of diabetes due to damage in small blood vessels include damage to the eyes, kidneys, and nerves (Kitabchi *et al*., 2009). Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and blindness. Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplantation. Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes (Kitabchi *et al.*, 2009). The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems may occur and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle atrophy and weakness.

2.7.5. Pathophysiology

Insulin production is more or less constant within the beta cells. Its release is triggered by food, chiefly food containing absorbable glucose. Insulin is the principal hormone that regulates the uptake of glucose from the blood into most cells of the body, especially liver, adipose tissue and muscle, except smooth muscle, in which insulin acts via the IGF-1. Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus (Robert, 2012).

The body obtains glucose from three main places: the intestinal absorption of food; the breakdown of glycogen, the storage form of glucose found in the liver; and gluconeogenesis, the generation of glucose from non-carbohydrate substrates in the body. Insulin plays a critical role in balancing glucose levels in the body. Insulin can inhibit the breakdown of glycogen or the process of gluconeogenesis, it can stimulate the transport of glucose into fat and muscle cells, and it can stimulate the storage of glucose in the form of glycogen (Shoback and David, 2011).

Insulin is released into the blood by beta cells (β-cells), found in the islets of Langerhans in the pancreas, in response to rising levels of blood glucose, typically after eating. Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Lower glucose levels result in decreased insulin release from the beta cells and in the breakdown of

glycogen to glucose. This process is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin (Shoback and David, 2011).

When the glucose concentration in the blood remains high over time, the kidneys will reach a threshold of reabsorption, and glucose will be excreted in the urine (glycosuria) (Robert, 2012). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst.

2.7.6. Prevention

There is no known preventive measure for type 1 diabetes (Ripsin *et al*., 2009). Type 2 diabetes which accounts for 85-90% of all cases can often be prevented or delayed by maintaining a normal body weight, engaging in physical activity, and consuming a healthful diet. Higher levels of physical activity (more than 90 minutes per day) reduce the risk of diabetes by 28% (Kyu *et al*., 2012). Dietary changes known to be effective in helping to prevent diabetes include maintaining a diet rich in whole grains and fiber, and choosing good fats, such as the polyunsaturated fats found in nuts, vegetable oils, and fish. Limiting sugary beverages and eating less red meat and other sources of saturated fat can also help prevent diabetes. Tobacco smoking is also associated with an increased risk of diabetes and its complications, so smoking cessation can be an important preventive measure as well (Willi *et al*., 2007). The relationship between type 2 diabetes and the main modifiable risk factors (excess weight, unhealthy diet, physical inactivity and tobacco use) is similar in all regions of the world. There is growing evidence that the

underlying determinants of diabetes are a reflection of the major forces driving social, economic and cultural change: globalization, urbanization, population aging, and the general health policy environment (Willi *et al*., 2007).

2.7.7. Management

Diabetes mellitus is a chronic disease, for which there is no known cure except in very specific situations (Selvin *et al.*, 2010). Management concentrates on keeping blood sugar levels as close to normal, without causing low blood sugar. This can usually be accomplished with a healthy diet, exercise, weight loss, and use of appropriate medications (insulin in the case of type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes). Learning about the disease and actively participating in the treatment is important, since complications are far less common and less severe in people who have well-managed blood sugar levels (Kitabchi *et al.*, 2009). Attention is also paid to other health problems that may accelerate the negative effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise (Lawlor, 2010). Specialized footwear is widely used to reduce the risk of ulceration, or re-ulceration, in at-risk diabetic feet. Evidence for the efficacy of this remains equivocal (Cavanagh, 2008).

2.7.7.1. Lifestyle

People with diabetes can benefit from education about the disease and treatment, good nutrition to achieve a normal body weight, and exercise, with the goal of keeping both short-term and long-term blood glucose levels within acceptable

bounds. In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications are recommended to control blood pressure (Cichosc *et al*., 2013; Inzucchi *et al*., 2012).

2.7.7.2. Medications

Medications used to treat diabetes do so by lowering blood sugar levels. There are a number of different classes of anti-diabetic medications. Some are available by mouth, such as metformin, while others are only available by injection such as GLP-1 agonists (Garry *et al*., 2011). Type 1 diabetes can only be treated with insulin, typically with a combination of regular and NPH insulin, or synthetic insulin analogs (Wu *et al*., 2017).

Metformin is generally recommended as a first line treatment for type 2 diabetes. It works by decreasing the liver's production of glucose (Roger *et al*., 2018). Several other groups of drugs, mostly given by mouth, may also decrease blood sugar in type 2.

When insulin is used in type 2 diabetes, a long-acting formulation is usually added initially, while continuing oral medications (Brunstrom and Carlberg, 2016). Since cardiovascular disease is a serious complication associated with diabetes, some have recommended blood pressure levels below 130/80 mmHg. However, evidence supports less than or equal to somewhere between 140/90 mmHg to 160/100 mmHg; the only additional benefit found for blood pressure targets beneath this range was an isolated decrease in

stroke risk, and this was accompanied by an increased risk of other serious adverse events (Brunstrom and Carlberg, 2016).

Comment [H2]: Is it dverse Effects or Events?

Aspirin is also recommended for people with cardiovascular problems, however routine use of aspirin has not been found to improve outcomes in uncomplicated diabetes (Pignone *et al*., 2010).

2.7.7.3. Surgery

A pancreas transplant is occasionally considered for people with type 1 diabetes who have severe complications of their disease, including end stage kidney disease requiring kidney transplantation (Picot *et al.*, 2009). Weight loss surgery in those with obesity and type two diabetes is often an effective measure (Frachetti and Goldfine, 2009). Many are able to maintain normal blood sugar levels with little or no medications following surgery and long-term mortality is decreased.

There is, however, a short-term mortality risk of less than 1% from the surgery. The body mass index cutoffs when surgery is appropriate are not yet clear. It is recommended that this option be considered in those who are unable to get both their weight and blood sugar under control (Schulman *et al.*, 2009).

2.8. Streptozotocin

Streptozotocin is a chemotherapeutic alkylating agent and a cytotoxic glucose analogue (Har and Ja, 2007). It is the most prominent diabetogenic agent which is used to experimentally induce insulin-dependent diabetes mellitus in laboratory animals. Streptozotocin induced diabetes mellitus is caused by extensive, severe necrosis and degeneration of the pancreatic β-cell islets. This state could lead to an insulinopenia syndrome known as "Streptozotocin-induced diabetes mellitus". Streptozotocin and related alkylating chemicals cytotoxic activity depends on their cellular appearance (Shafrir, 2010). Because of nitrosoureas high chemical affinity to lipids, streptozotocin cellular uptake through the plasma membrane is so swift and it would be accumulated in pancreatic β-cells selective. Insulin-producing cells which do not express GLUT2 glucose transporter on their membrane would be streptozotocin resistant (Damasceno *et al*., 2011). Streptozotocin also damages other tissues expressing GLUT2 glucose transporter, specifically liver and kidney (Lenzen, 2008). Streptozotocin cytotoxicity is because of its ability to have DNA alkylating and methylating activity and also protein glycosylation induced by this chemical agent (Scheen, 2010). After DNA damage induced by streptozotocin cellular uptake, DNA repair mechanisms will be activated in response to DNA damages (Zafar and Naqvi, 2010).

2.9. 'Kunun'

Fermented cereal foods and drinks have been used from ancient times in Africa as weaning foods for infants and refreshing drinks for adults (Adebiyi *et al*., 2018). Apart from being enjoyed for their refreshing and taste-quenching properties, these fermented drinks have been reportedly used for medicinal purposes because of the presence of some health-promoting bacteria which have

been reported to contribute to health and wellbeing of the consumers (Lamsal and Faubion, 2009; Adejuyitan *et al*., 2008). In developing countries like Nigeria, alcoholic and non-alcoholic fermented beverages play a very important role in the dietary pattern of people serving as after-meal or refreshing drinks (Akoma *et al*., 2013). Most of these beverages are often made from submerged fermented cereals, mixed with sugar, flavouring agents and sometimes preservatives. Some of these drinks include burukutu, pito, 'kunun' among others (Uvere and Amazikwu, 2011). The fermentation of these drinks has been reported to involve probiotics which confer health benefits to their consumers. They are regarded as after meal drinks or refreshing drinks and are served at social gatherings. 'Kunun' is widely accepted as a food drink in some urban center especially in the Hausa land and it is consumed anytime by both adult and children as a breakfast drink or food complement. The additive that is used, sweet potatoes, contains essential amino acids and is a rich source of vitamins (Adebayo *et al*., 2010).

The quality and quantity of the product largely depend on the quality of the ingredients and its proper handling in the course of production by the producer (Ayo-Omogie and Okorie, 2016). The products could be obtained qualitatively after two days and it could be stored for another three days when refrigerated.

'Kunun', however, seems to be highly nutritious with relatively low cost of production. It is a considerably cheap beverage drink because the ingredients used for the preparation are cheap and available anywhere in the market and stores (Amusa and Odunbaku, 2009). The hydrolytic enzyme (amylase) in the malted cereal aids in digesting the thick slurry, thereby converting the complex carbohydrate to simple sugars (Agarry *et al*., 2010).

Millet used as a major ingredient in the production is believed to lower cholesterol; phytate on the other hand, 'kunun-zaki' are rich in phytochemical and phytic acid which are cancer reductant (Achi and Ukwuru, 2015).

2.9.1. Types of 'Kunun'

2.9.1.1. 'Kunun- gyada'

'Kunun-gyada' is one of the most important home- prepared weaning foods. The main ingredient for preparing kunun-gyada are partially roasted groundnuts in combination with rice, millet, maize or sorghum flours, sugar and tamarind fruit pulp extract. During the preparation of kunun-gyada, the proportion of groundnut to cereals by weight varies.

2.9.1.2. 'Kunun- dawa' (from *Sorghum bicolor*)

The major cereal used in the preparation of kunun-dawa is *Sorghum bicolor*. It is a non-alcoholic fermented beverage mostly consumed by the northern people in Nigeria.

2.9.1.3. 'Kunun-tsamiya'

'Kunun-tsamiya' is produced from millet (*Pennisetum typoidum*), sorghum (*Sorghum bicolor*), or maize (*Zea mays*) with tamarind (Tsamiya) as the main ingredient for flavouring (Sengev *et al*., 2012). Tsamiya is one of the widely used spice condiments found in the average northern Nigeria kitchen. It has a delicately sweet and sour taste and is mostly used in adding flavor to food. It's very similar to black velvet tamarind (Icheleku igbo) and taste alike.

2.9.1.4. 'Kunun-zaki'

'Kunun-zaki', a non -alcoholic beverage produced from whole grains of millet, sorghum or corn, spiced (ginger, black pepper, clove) and sweetened with sugar. It is a highly nutritious drink rich in protein and fibre. It is a popular drink especially among the northerners. The variety of the drink made from sorghum is a milky light brown colour whilst that which is made from millet and maize is whitish in colour (Amusa and Ashaye, 2009).

2.9.1.5. 'Kunun-aya'

'Kunun-aya' is produced from tiger nut also known as Aki Awusa in Igbo, Aya in Hausa, Isip in Efik/Ibibio, Ofio in Yoruba. Tiger nuts are actually small tubers which are abundant in northern Nigeria. It is milky, nutritious and refreshing. It has lots of health benefits and the milk can be used as a substitute for the regular cow's milk (Rowland *et al*., 2017).

2.9.2. Raw materials used in 'Kunun- zaki' Production

The raw materials used in production of 'kunun-zaki' include the following:

2.9.2.1. *Pennisetum glaucum* L. R.Br. (Millet)

Millets are a group of small seeded species of cereal crops or grains, widely grown around the world especially in east Asia for the last 10,000 years for food and fodder (ICAR, 2018; Hariprassana, 2017). The grain colour can be nearly white, pale-yellow, brown, grey, stale blue or purple (Saleh *et al*., 2013). Millet is a good source of minerals such as calcium, iron, zinc, copper and manganese. The average carbohydrates contents of millets varies from 56.88 to 72.97 g/100g and

thus serve as a high energy food (Singh and Raghuvanshi, 2012). It has a high percent of indigestible fibre because the seeds are enclosed in hulls, which are not removed by ordinary processing methods (Tripathi and Platel, 2011). In Nigeria, millet is mostly used in pap making (Omemu *et al*., 2007). The cracked grains may be used and cooked like rice. Beverages like burukutu, fura and 'kununzaki' are also made from millet. In addition to their nutritive value, several potential health benefits such as preventing cancer and cardiovascular diseases, reducing tumor incidence, lowering blood pressure, risk of heart disease, cholesterol and rate of fat absorption, delaying gastric emptying, and supplying gastrointestinal bulk have been reported for millet (Ashwani *et al*., 2018).

Millet grains are usually processed by commonly used traditional processing techniques which include decorticating, malting, fermentation, roasting, flaking, and grinding to improve their edible, nutritional, and sensory properties (Saleh *et al*., 2013). However, negative changes in these properties during processing are not avoidable because industrial methods for processing of millets are not as well developed as the methods used for processing of wheat and rice (FAO, 2017; FAO, 2012). Therefore, with value-added strategies and appropriate processing technologies, the millet grains can find a place in the preparation of several value-added and health food-products, which may then result in high demand from large urban populations and nontraditional millet users (Devi *et al*., 2014).

2.9.2.2. *Sorghum bicolor* L. Moench (Sorghum)

Sorghum bicolor, native to Africa with many cultivated forms. It is an important crop worldwide, used for food (as grain and in sorghum syrup or sorghum

molasses), animal fodder, the production of alcoholic beverages, and biofuels (Arun *et al*., 2010). Most varieties are drought and heat-tolerant, and are important in arid regions, where the grain is one of the staples for poor and rural people. *Sorghum bicolor* is an important food crop in Africa, Central America, and South Asia, and is the fifth-most important cereal crop grown in the world (Arun *et al*., 2010; Mutegi *et al.*, 2010). Sorghum out performs other cereals under various environmental stress and is thus generally more economical to produce (Valeria *et al*., 2015). The nutrient composition of sorghum indicates that it is a good source of energy, proteins, carbohydrates, vitamins and minerals including trace elements particularly iron and zinc. Sorghum grain contains minerals such as phosphorus, potassium and magnesium in varying quantities (Ab*e et al*., 2012).

The bran layer of the sorghum grains contain important antioxidants that are not found in many other types of food. These antioxidants have been directly connected to a reduced chance of developing various types of cancer, including esophageal cancer, particularly in comparison to people who regularly eat wheat and corn (Imran *et al*., 2014). Antioxidants are the beneficial compounds that neutralize and eliminate free radicals in the body, which often cause healthy cells in the body to mutate into cancer cells (Tripathi *et al*., 2012).

Sorghum is one of the best foods for dietary fiber. A single serving of sorghum contains 48% of daily recommended intake of dietary fiber. It's more

than 12 g, and prevent things like cramping, bloating, constipation, stomach aches, excess gas, and diarrhea (Adetuyi, 2007).

Magnesium, copper and iron are found in high quantities in sorghum, which means that calcium levels will be properly maintained (Caroline and Camila) *et al*., 2017). These two minerals are also integral to the development of bone tissue and speeds up the healing of damaged or aging bones. This can prevent conditions like osteoporosis and arthritis (Solange *et al*., 2014). Sorghum contains 28% of the recommended niacin intake per day. The use of sorghum as a main constituent of the diet is usually associated with under- nourishment. This is mainly due to it lacking some essential amino acids and the presence of anti-nutritional factors (Awadalkareem, 2008). However, fermentation, facilitated mainly by lactic acid bacteria (LAB), greatly improves the nutritive value and sensory properties of sorghum (Kalui, *et al*., 2010).

The nutrient composition of millet and sorghum as shown in the Table 1.

Table 2.1: Nutritional values of Millet and Sorghum per 100 g

	Milllet	Sorghum
Energy	1,582 kJ (378 kcal)	1377KJ
Carbohydrates	72.8 g	72.1
Dietary fiber	8.5 g	6.7g
Fat	4.2 g	3.5g
Protein	11.0 g	10.6g
Vitamins		
Thiamine (B1)	0.42 mg	0.33mg
Riboflavin (B2)	0.29 mg	0.1mg
Niacin (B3)	4.72 mg	3.7mg
Pantothenicacid (B5)	0.85 mg	0.4mg
Vitamin B6	0.38 mg	0.44mg
Folate (B9)	85 µg	20µg
Minerals		
Calcium	8 mg	13mg
Iron	3.0 mg	3.4mg
Magnesium	114 mg	165mg
Manganese	1.6 mg	1.6mg
Phosphorus	285 mg	289mg
Potassium	195 mg	363mg
Sodium	5 mg	2mg
Zinc	1.7 mg	1.7mg

Source: Amadou, (2013).

2.9.2.3. *Zingiber officinale* (Ginger)

Ginger is an underground stem of the ginger plant *Zingiber officinale*. The flesh of the ginger rhizome can be yellow, white or red in colour, depending on the variety. The addition of ginger to kunun-zaki extends shelf life as ginger acts as a preservative (Odom *et al*., 2012). Ginger contains gingerols which increases antibacterial, sedative and antipyretic properties (Jalal and Nasroallah, 2014).

Ginger causes sweating which results to warming on a cold day. It also breaks fevers by warming the body and increasing perspiration (Baliga *et al.*, 2011). Ginger stimulates blood circulation, removes toxins from the body, cleaning the bowels and kidneys and nourishing the skin (White, 2007). Ginger induces cell death in ovarian cancer cells by direct colon cancer cell growth suppression and inhibition of the tumor via angiogenesis (Shukla and Singh, 2007).

The chemical composition of ginger is as shown on Table 2.2.

Table 2.2: Chemical Composition of Ginger Root per 100g of Raw Edible Portion.

Component	Quantity
Energy	20Kcal= 80KJ
Protein	1.82g
Carbohydrate	17.7g
Sugar	1.7g
Dietary fiber	2.0g
Fat	0.75g
Vitamin B 1	0.025mg
Vitamin B 2	0.034mg
Vitamin B 3	0.870mg
Pantothenic acid	0.203mg
Vitamin B 6	0.16g
Folate	11ug
Vitamin C	5mg
Calcium	16mg
Phosphorous	34mg
Magnesium	43mg
Iron	0.6mg
Potassium	415mg
Zinc	4.89mg

Source: Shirin and Jamuna, (2010).

2.9.3. Thickeners used in the Production of 'Kunun-zaki'

2.9.3.1. *Ipomea batatas* (Sweet potatoes)

The *Ipomoea batatas* Linn Lam (Sweet potatoes) is a dicotyledonous plant that belongs to the family Convolvulaceae. Its large, starchy, sweet-tasting, tuberous roots are a root vegetable. Sweet potato is an extremely versatile and delicious vegetable that possesses high nutritional value. Sweet potato tubers are mainly an energy source due to their high carbohydrate content, which accounts for 80-90% of the dry weight. These carbohydrates consist of starch, sugars and small amounts of pectins, hemicelluloses and cellulose (Lebot, 2009). Sweet potato is now considered a valuable source of unique natural products, including some that can be used in the development of medicines against various diseases and in making industrial products (Mohanraj and Sivasankar, 2013). Sweet potatoes are cultivated for food in more than 100 countries, sometimes as a staple food but usually as an alternative food. Because of their fast growing period and low input and work requirements. Sweet potatoes are often planted in Africa as a security crop or famine prevention crop (Lebot, 2009). The starchy tubers are used as a vegetable and can be boiled, baked, fried (to make chips), dried and ground into flour to make biscuits, beer, bread and other pastries (Sandeep *et al*., 2015; Odebode, 2008). They can be cooked and frozen. Leafy tops are eaten as a vegetable. Some sweet potato tubers grown in the USA are used as a natural source of dye, or valued as a healthy food due to their high beta-carotene content. Their very high-grade starch is appreciated for food and by the pharmaceutical industries (Oluwaseyi, 2013).

2.9.3.2. *Oryza sativa* (Paddy rice)

Paddy rice is the individual rice kernels that are in their natural, unprocessed state.

Sometimes referred to as rough rice, it is harvested directly from rice fields or paddies and transported to a processing site. As part of the process, the protective hull is removed, leaving only the actual rice kernel for consumption (Orthoefer, 2008).

Paddy rice is a dietary staple food and one of the most important cereal crops, especially for people in Asia, but the consumption outside Asia has increased, recently (Orthoefer, 2008). In fact, rice has greater variability of the glycemic index depending on type and cooking method. The unique taste of rice provides an easy way to combine rice with the other food, to achieve better taste and nutritional balance. Some studies revealed some health effects of rice and its products (Chaudhari, 2018; Orthoefer, 2008). The pigment of certain rice can inhibit the formation of atherosclerotic plaque, because it has anti-oxidative or anti-inflammatory effects (Ruozhi *et al*., 2018). Rice is also one of the foods which is considered to be a potential food vehicle for the fortification of micronutrients because of its regular consumption (Thankachan, 2012).

The germ and bran contain high levels of minerals, protein and vitamins (Verma and Srivastav, 2017). Therefore, removal of the germ and bran from the brown rice produces will decrease the nutrients compared to brown rice itself (uncooked). Parboiled milled rice showed 18% ash enrichment in comparison with milled rice, and has higher contents of potassium and phosphorus (Faustina and Cleopatra, 2017).

As a consequence, rice is fortified with some minerals such as iron (Fe) and zinc (Zn), to prevent diseases associated with mineral deficiencies (Siwaporn *et al*.,

2013; Sperottoa *et al*., 2012).

2.9.3.2.1. Rice in Diabetes Prevention and Treatment

Foods with a high glycemic index (GI) have been associated with increased risk of type-2 diabetes, because they are rapidly digested and can cause dramatic increase in blood sugar levels (Wolever, 2013). Glycemic index is a widely accepted measure of the effect of carbohydrate foods including rice on human health (Wolever, 2013). The glycemic load value incorporates the amount of rice in a serving in order to better gauge the impact of a diet on postprandial glucose response (Atkinson *et al*., 2008). Based on glycemic index, the diets including rice are grouped into three categories, namely low glycemic index (55 or less), medium (56 - 69), and high (70 or more). Furthermore, based on glycemic load, the diets are classified into low glycemic load (10 or less), medium (11 - 19), and high (20 or more) (Dodd *et al.*, 2011). Glycemic index predicts the ranking of the glycemic potential of different meals in individual subjects. Low-glycemic index diets result in modest improvements in overall blood glucose control in patients with insulin-dependent diabetes (type I) and non-insulin-dependent diabetes (Chen *et al*., 2015). The high glycemic load of rice has a significantly higher glycemic index than medium one (Fatema *et al.,* 2010). Because of this atherogenic role of insulin, it is desirable to control the blood glucose of patients and keep their insulin level as low as possible (Fatema *et al*., 2010).

Table 2.3: Chemical Composition of Sweet Potatoes and Paddy Rice per 100g.

Nutrient component	Sweet potatoes	Paddy rice
Energy (KJ)	360	1549
Protein (g)	1.6	1.9
Fat (g)	0.05	2.92
Carbohydrate (g)	20	77
Fiber (g)	3.0	3.5
Sugar	4.18	0.85
Calcium	30	23
Iron (mg)	0.61	1.47
Magnesium	25	143
Phosphorus	47	333
Potassium	337	223
Sodium	55	7
Zinc	0.3	2.02
Manganese	0.26	3.74
Vitamin C	2.4	0
Thiamin B1 (mg)	0.08	0.40
Riboflavin B2 (mg)	0.06	0.09
Niacin B3 (mg)	0.56	5.09
Panthothenic B5 (mg)	0.80	1.49
Vitamin B6	0.21	0.51
Folate	11	20
Vitamin A (IU)	7	0
Vitamin E	0.26	0.19
Saturated fatty acids (g)	0.02	0.58
Monosaturated fatty acids	0	1.05
Polyunsaturated fatty acids	0.01	1.04

Source: FAO, (2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Collection of Raw materials for 'Kunun-zaki' Production

Pennisetum glaucum (Millet), *Sorghum bicolor* (Sorghum), *Zingiber officinale* (Ginger) *Capsicum annum* (Pepper), *Eugina caryophyllata* (Clove), *Ipomea batatas* (Sweet potatoes) and *Oryza sativa* (Paddy rice) were all purchased from Eke Awka market in Awka, Awka South Local Government, Anambra State. These grains were sorted, cleaned and stored in plastic containers before used.

3.2. Production of 'Kunun-zaki'

3.2.1. Preparation of Ground Malted Rice Paste.

The 250g of paddy rice used was washed with tap water and soaked in 500 ml of tap water (1:2w/v) for 12 h and then drained. The drained grains were couched by covering them with moist cloth for 5 days at ambient temperature (30^0C) to germinate and then dried in the sun for 3 days. The dried malted rice was washed and ground (Agarry *et al*., 2010).

3.2.2. Preparation of Sweet potatoes

Ipomea batatas (Sweet potatoes) were peeled, washed, sliced into 2 cm with knife and sundried for 7 days then grinded into powder.

Comment [H3]: Incomplete. Sundried for how long?

3.2.3. Preparation of 'Kunun-zaki'

'Kunun-zaki' was prepared from a ratio 50:50 and 80:20% mixture of millet and sorghum with a modification on ingredients according to Adelekan *et al*., (2013). Five hundred

grams of the cereal grains mixture were washed with potable water, drained and steeped in 1000 ml of tap water (1:2 w/v) in a bucket for 8 h after which the grain was washed and mixed with 60g of spices (ginger 40g, clove 10g, red pepper 10g); these were washed and then grinded to a paste. The slurry was divided into two unequal portions (1:3 w/w). The larger portion was gelatinized by the addition of boiling water (1:1w/v) in a plastic container, and 100g of the grounded thickner (Paddy rice/sweet potatoes) was added, stirred vigorously for 3 minutes and then cooled to about 50^{o}C. The slurry was allowed to sediment and ferment for 12 h at 30 ± 2^{0}C. The fermented samples were sieved using a clean muslin cloth (mesh 350 µm). The supernatant liquid was decanted and the filtrate, 'Kunun-zaki', was packaged inside sterile container for subsequent analysis. The paddy rice and sweet potatoes based 'kunun-zaki' were produced differently by applying the above procedures. 'Kunun-zaki' produced without no thickener served as control.

The flow chart for the preparation of 'kunun-zaki' is shown in Figure 1.

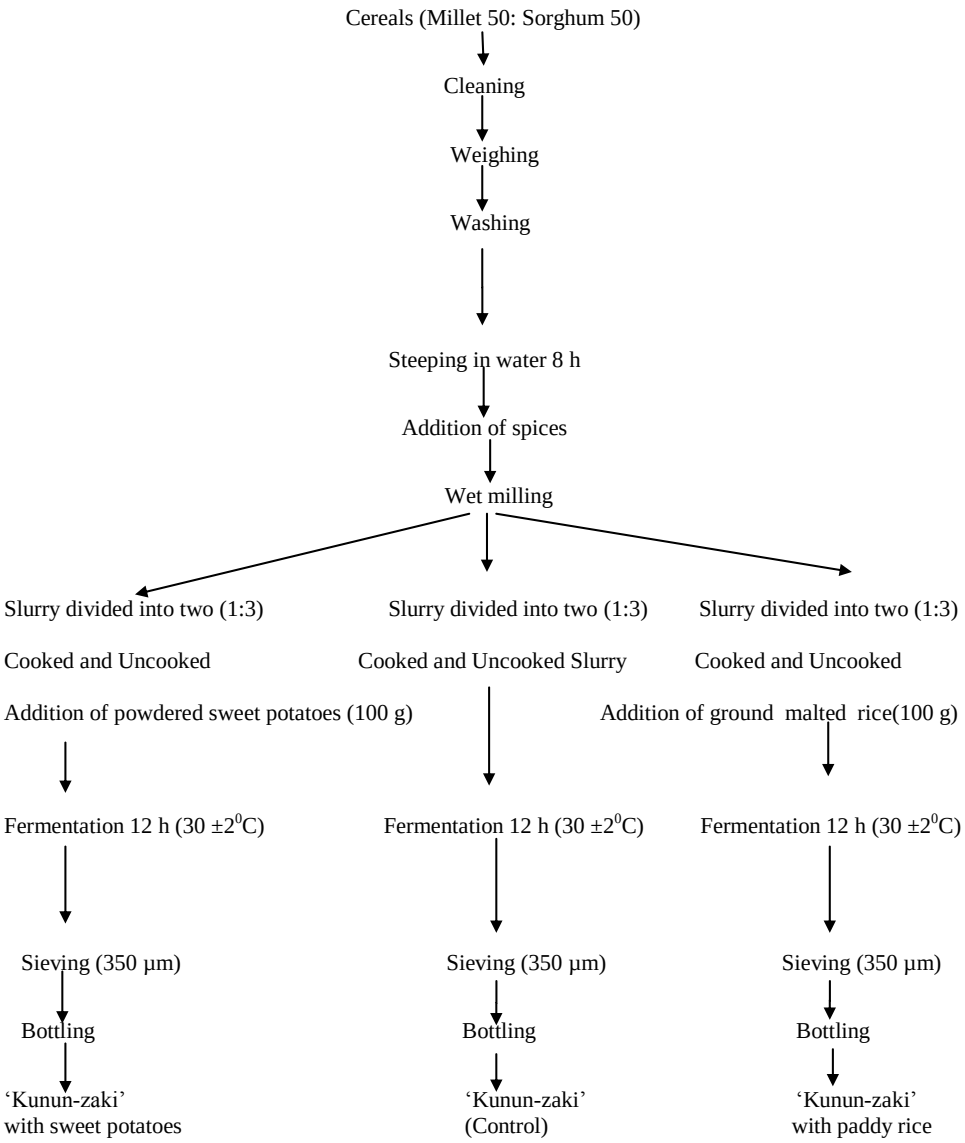


Figure 3.1: Flow chart for the production of different 'kunun-zaki' samples at 50:50 millet/ sorghum.

Source: Adelekan *et al*., (2013).

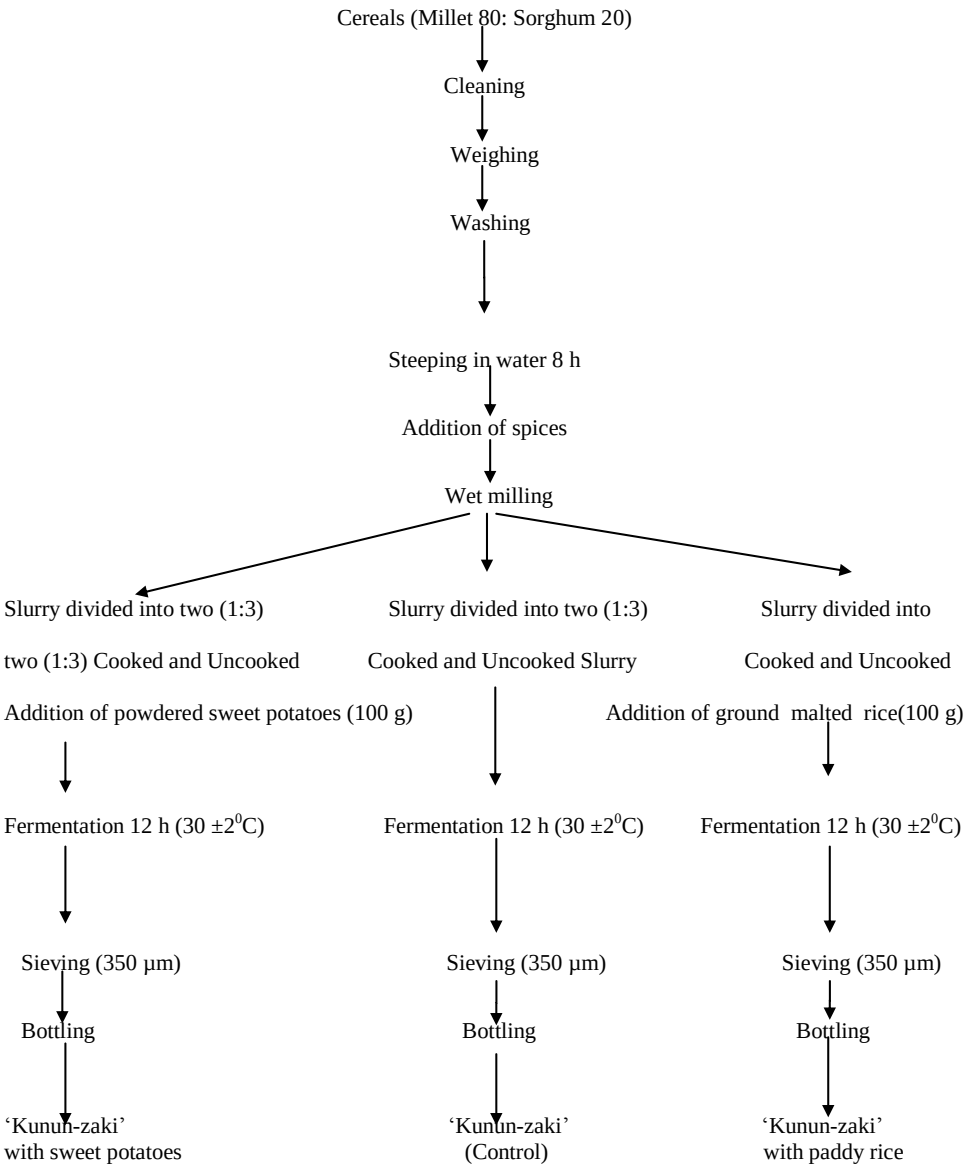


Figure 3.2: Flow chart for the production of different 'kunun-zaki' samples at 80:20 millet/ sorghum.

Source: Adelekan *et al.*, (2013).

3.3. Physicochemical Tests

3.3.1. pH Determination

The pH values of the 'kunun- zaki' was monitored by using an electronic pH meter (Hanna model 209 Electronic Limited, England) that was initially standardized with appropriate buffer of pH 4 and 7. The sample was homogenized and placed into 50 ml clean glass beaker and the electrode of a standardized pH meter was inserted. The values were read and recorded according to the procedure described by Association of Official Analytical Chemists (AOAC, 2010).

3.3.2. Determination of Brix

The brix value of the sample was determined in triplicates using Abbe (PZO-RL1), Warszawa, Poland refractometer. The refractometer was caliberated with deionized water (refraction index = 1.3330 and 0^0 Brix at 20^0C) and the reading of the sample were performed by placing a few drops of the sample on the prism of the refractometer, with the prism pointing towards the direction of light. The readings of the various samples were automatically taken and recorded (Robert, 2010).

3.3.3. Determination of Total Soluble Solid

Total solid was determined by evaporating 25 ml of 'kunun-zaki' to dryness in a boiling water bath and then dried to constant weight to constant weight in an oven at 130^0C for 3 h (AOAC, 2010).

Calculation:

$$\% \text{ Total solid} = \frac{\text{Dry weight}}{\text{Weight of sample}} \times 100$$

3.3.4. Determination of Total Titratable Acidity

This measures the acidity in samples. It was determined according to the procedure described by Association of Official Analytical Chemists, 2010. A 10 ml sample of the 'kunun-zaki' was measured into a dried, weighed conical flask, and 50 ml of distilled water was added. A 0.5 ml phenolphthalein indicator was added to the sample and titrated against 0.1 M NaOH. The end point was reached when a faint pink colour was observed.

Calculation:

$$\text{Total titratable acidity} = \frac{\text{TV} \times \text{lactic acid factor} \times 0.01 \times 100}{\text{Volume of sample}}$$

Where TV is the titre value.

3.3.5. Determination of Specific Gravity

This was carried out using pyconometer bottle according to AOAC (2010). A 50ml pycometer bottle was thoroughly washed, dried and weighed. The bottle was first filled with water and weighed. After drying the bottle again, it was filled with the sample and weighed.

Calculation:

$$\text{Specific gravity} = \frac{\text{density of object}}{\text{density of water}}$$

$$\text{Where density} = \frac{\text{mass}}{\text{volume.}}$$

3.4. Proximate Analyses

3.4.1. Moisture Determination:

The Petri dishes were first weighed. A 10 ml of sample was weighed in triplicate into each Petri dish using on an analytical balance. The Petri dishes containing the sample were then placed in a hot air oven at a temperature of 100^0C for 2 h. After drying, the Petri dishes were retracted from the oven and placed in a desiccator to cool after which the Petri dishes containing the dried samples were then weighed and average value recorded (Robert, 2010).

Calculations:

The percentage moisture of a food sample was calculated thus;

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where:

W_1 = Weight of empty Petri dish

W_2 = Weight of sample +Petri dish

W_3 = Weight of sample +Petri dish after drying.

$W_2 - W_1$ = Weight of the sample.

3.4.2. Ash Determination

A crucibles was washed, rinsed with water and dried in an oven at 100^0C. The dried crucible was labelled and weighed and their weights was recorded. The crucible

containing the sample was placed on a water bath until all its moisture evaporated. The crucible that contained the sample was then placed on a hot plate in a fume cupboard until no more smoke was given off and the sample turned black. The crucible was then placed on a muffle furnace operating at 550^0C for 3 h until the sample turned light grey in colour. The crucible was removed from the furnace and placed in the desiccator to cool. Once cooled, the crucibles containing the ash sample was weighed. Other samples were similarly treated (AOAC, 2010).

Calculation:

$$\%Ash = \frac{\text{Weight of Ash}}{\text{Weight of Sample}} \times 100$$

$$= \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where:

W_1= Weight of empty crucible

W_2=Weight of crucible and sample

W_3= Weight of crucible and ash

W_2-W_1= weight of the sample

W_3-W_1= Weight of ash.

3.4.3. Determination of Crude Fibre

A 0.5g celite was weighed into a dried crucible and the crucible placed in muffle furnace at 550^0C. The crucible containing celite was then placed in the desiccator

to cool. The sample was concentrated by weighing 20 ml into crucible and then placed on a water bath until all its moisture evaporates. 2g of the evaporated sample was then added into the crucible containing the celite. The crucible was then placed on a cold extraction unit where acetone was added. After 5 minutes, the acetone in each sintered crucible was drained. A 1.25% of sulphuric acid (H_2SO_4) was dispensed into each crucible and boiled under reflux for 30 minutes. After 30 minutes, the 1.25% of sulphuric acid was drained off and the sample was washed with deionized water. The residue was transferred into a beaker and boiled for 30 minutes with a 200 ml solution containing 1.25% of carbonated NaOH. The final residue was filtered and dried in a furnace at 450^0C for 3 h, after which the crucibles were cooled in a desiccator and weighed (AOAC, 2010).

Calculation:

$$\% \text{ Crude fibre} = \frac{W_2 - W_3}{W_1} \times 100$$

Where:

W_1 = Weight of sample taken

W_2= Weight of crucible after oven drying

W_3= Weight of crucible after ashing in furnace

3.4.4. Determination of Protein

This was carried out using Kjedahl method according to Association of Official Analytical Chemists (AOAC, 2010). The sample placed on a water bath until all its moisture evaporated. 2g of the sample was weighed into a Kjeldahl flask. 5g of anhydrous sodium sulphate was added followed by addition of 1g copper sulphate and 1 tablet of kjeldahl catalyst. Into the mixture, 25 ml concentrated sulphuric acid and 5 glass bead were added. In the fume cupboard, the mixture was heated gently at temperature of 42^0C for 30 minutes until the solution changed colour to green. It was cooled and the particles at the mouth and neck of the flask were washed down using distilled water. The mixture was re-heated until the green colour disappeared. After cooling, the digest was transferred with several washing into a 250 ml markham distillation flask containing 25 ml of 4M Sodium hydroxide solution and then distillied. The distillate was collected in the receiver containing 10 ml of boric acid indicator solution. A 0.1M Hydrochloric acid solution was used to titrate to pale end point. The crude protein was calculated using a conversion factor 5.8 for paddy rice and 6.25 for sweet potatoes.

$$\% \text{ nitrogen} = \frac{0.00014 \times \text{titre value} \times 100}{\text{Weight of sample}}$$

% crude protein = % nitrogen x 5.8 (Paddy rice based samples)

% crude protein =% nitrogen x 6.25 (Sweet potatoes based samples)

3.4.5. Determination of Total Carbohydrate

This was carried out using the method of Association of Official Analytical Chemists (AOAC, 2010) by subtracting the total sum of the percentage of fat, moisture, ash, crude fibre and protein content from hundred.

3.4.6. Determination of Total Lipids

The total lipid was determined by evaporating 25ml of kunun-zaki on a boiling water bath which was followed by drying to constant weight in an oven at 130^0C for 3 h till a constant weight was obtained. The weight of water lost and dried solids obtained was determined by subtraction and this was used to calculate the total amount of fat on wet weight basis. Two grams (2 g) of the dried sample was weighed into each of the two paper thimbles. The thimbles were sealed and placed in soxhlet extractors. About 150 ml of petroleum ether was poured into each of the two previously dried and weighed round bottom flasks attached to the extractor. Extraction took place for 16 h. After this, the petroleum ether was recovered from the soxhlet with only a small amount left in the flasks. The flask was removed and placed in an oven for ether to completely evaporate. The flasks were cooled in the dessicator, weighed and the fat content was calculated on wet weight basis. Association of Official Analytical Chemists (AOAC, 2010).

Calculation:

$$\% \text{ Fat} = \frac{m_f \times W_D}{m_S \times W_T} \times 100$$

Where:

m_f = mass of fat extracted

m_S = mass of dried sample taken for extraction

W_D = mass of total dried sample

W_T = mass of wet sample originally taken and dried

3.5. Determination of Vitamins Concentrations

3.5.1. Determination of Vitamin C Concentartion

Vitamin C was determined based upon the quantitative decolouration of 2, 6-dichlolorophenol indophenol by ascorbic acids. The excess dye was extracted with xylene and the colour intensity was measured at 500nm and compared with standard ascorbic acid (0.80g/mL) (Nielsen, 2010).

3.5.2. Determination of Vitamin E Concentration

This was performed using gas chromatography according to Ronald *et al*. (2010). Flame ionization detection was performed after separation on a fused silica DB5-MS capillary column. The heating gradients were 180^0C for 2 minutes and 280^0C for 10 minutes. The injector temperature was 260^0C and the detector temperature was 300^0C. Nitrogen was used as carrier gas. A suitable portion (1µl) of the sample preparation was injected into suitable gas chromatography. The chromatogram was recorded and the areas under the first (alpha tocopherol) and second major (hexadecyl hexadecanoate) peaks. The values were recorded as a_U and a_D respectively.

Calculation:

$(50C_D/F)(a_U/a_D)$

Where,

C_D= Concentration in mg/ml

F= the relative response factor

3.6. Determination of Mineral Contents

The dry ashing procedure was used for mineral content determination. Five grams of the samples was accurately weighed into porcelain crucible and pre-ashed until the sample was completely charred on a hot plate. The pre-ashed sample was thereafter ashed in the muffle furnace at 500^0C for 2 h till the ash was white. After ashing, the crucible was transferred into the desiccator to cool and be reweighed. The sample was quantitatively transferred into volumetric flasks by carefully washing the crucible with 1 ml nitric acid, then with portions of dilute nitric acid. The washing was transferred to a volumetric flask, and the procedure repeated twice the washing procedure twice. The solution was diluted to volume with deionized water, and was used for individual mineral determination using the appropriate standards and blank. The content of the minerals; calcium, magnesium, potassium, zinc, iron and sodium were determined with the Atomic Absorption Spectrophotometer (AAS, Hitachi Z6100, Tokyo, Japan) at different wavelength for each mineral element. All determinations were carried out in triplicates (Miller and Rutzke, 2010).

3.7. Sensory Evaluation

The sensory attributes (taste, colour, mouthfeel, flavour and overall acceptability) of 'kunun-zaki' produced were evaluated by 20 member panel, comprising trained in the Department of Food Science and Technology, Nnamdi Azikiwe University, Awka, Anambra State, using a seven- point hedonic scale where 1= dislike extremely, 2 = dislike very much, 3= dislike slightly, 4=neither like nor dislike, 5 =like slightly, 6=like very much and 7= like extremely (Amihud, 2009).

3.8. Isolation of Microorganisms

3.8.1. Culture Media Preparation

A total of 2.8g of Nutrient agar, 6.5g of Sabouraud dextrose agar, 4.9g of MacConkey agar, 6.7g of de Man Rogosa Sharpe agar, 2.4g of yeast extract agar, 1.6g tryptophan broth, 4.0g tryticase soy agar, 2.4g of Simmons citrate agar were measured using tripple beam balance and each dispensed into clean and dry 250 ml conical flasks. 30 ml of distilled water was poured into each flask and heated with continuous agitation to dissolve the consituents, more distilled water was added to make the volume 100 ml. After the agar had been prepared, the complete dissolution of the agar was then cotton plugged and sealed with an aluminium foil. Distilled water was used as diluents and 9ml each was dispensed in McCartney bottles and sterilized along with the conical flask in the autoclave at 121^{o}C for 15 minutes at 15psi. The already prepared agar was allowed to cool to 45^{0}C before dispensing into sterilized Petri dish and allowed to solidify (Ashraf *et al*., 2009).

3.8.2. Isolation of Microorganisms from 'Kunun-zaki'

Ten-fold dilutions of each 'kunun-zaki' sample was made using distilled water and 0.1 mL of 10 5 dilution was cultured by pour plate method was measured from dilution factor five and pour plated in triplicate plates on Nutrient agar for viable count, MacConkey agar for coliform count, de Man Rogosa and Sharpe agar (MRS) supplemented with 0.02% Sodium azide for lactic acid bacteria count, Sabouraud dextrose agar, with chloramphenicol (250 µg) was used for fungi while yeast agar for yeast count. The yeast agar medium was adjusted to pH 3.5 with tartaric acid. All plates were incubated for 48 h at 35°C except for Sabouraud dextrose agar and de Man Rogosa and Sharpe agar that were incubated at 26°C for 5 days. Colony counts were made using digital illuminated colony counter (Gallen kamp model). Pure cultures of each isolates were obtained by streaking the specific colonies on suitable media. These were then maintained in agar slants in MaCartney bottles. The result was recorded in cfu/ml (Pundir *et al*., 2013).

3.8.3. Collection of Pathogenic Organisms

Three pathogens *(Staphylococcus haemolyticus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) were collected from Department of Microbiology, Chwukwuemeka Odumegwu Ojukwu Teaching Hospital, Awka, Anambra State. The isolates were sub-cultured on Nutrient Agar plates and incubated for 24 h at 28ºC. The colonies were picked and stored on slants until when needed.

3.8.4. Identification of Bacterial Species

3.8.4.1. Morphological Characteristics

The appearance of the colony of each isolate on the agar media was studied according to Phumudzo *et al.* (2013). Characteristics observed include shape, edge, colour, elevation and texture.

3.8.4.2. Gram Staining Technique

The Gram staining involved the preparation of smear of 24 h culture of an organism on clean grease-free glass slide. The smear was heat fixed by passing the reverse side of slide slightly over flame three times. The heat fixed smear of cells was stained with 0.20% crystal violet staining reagent for 60 seconds. The slide was then flooded with Lugols iodine (a mordant) and left for 30 seconds before it was washed off with distilled water. The slide was decolorized again with 95% (vol/vol) ethanol for 10 seconds and flushed with distilled water to prevent excessive discolouration and then counter-stained with 0.25% safranin solution for 1 minute, washed off with water and allowed to dry. A drop of immersion oil was added to the stained slide and examined under the oil immersion objective lens of the microscope as described by Leboffe, (2014).

3.8.4.3. Motility

This was determined by hanging drop preparation using broth culture of the bacterium. A loopful of 24 h broth culture of the bacteria was gently placed on a clean dried concave glass slide and covered with a coverslip that has its edge sealed with vaseline. The concave glass slide was gently turned upside down with the oil drop placed over the depression and this preparation was then examined

under high power objective lens through the coverslip. Motile organisms show unidirectional movements while non-motile have Brownian motion-like movement (Claus, 2007).

3.8.4.4. Sugar Fermentation

The Nutrient broth and 0.01% Phenol red was dissolved in 100 ml distilled water and transfered into conical flasks. 1% of desired carbohydrate was added into the flask and dispensed in test tubes. Durham tubes were inverted into the test tubes and then sterilized in the autoclave at 121^0C for 15 minutes at 15 psi. The 24 h culture of the test organism were inoculated aseptically into the labelled test tubes and incubated at 37^0C for 24 h. This set up was observed for acid and gas production. The sugars used were lactose, sucrose, maltose, glucose, fructose and mannitol (Tortora *et al*., 2010).

3.8.4.5. Catalase Production

The test organisms were inoculated on Nutrient agar plates and incubated at 37^0C for 24 h. After incubation, 3 drops of hydrogen peroxide solution were added using Pasteur pipette. Effervescence caused by the liberation of oxygen as gas bubbles indicated catalase production by the bacteria (Cheesbrough, 2009).

3.8.4.6. Starch Hydrolysis

The soluble starch was dissolved in a small amount of water and was heated slowly with constant stirring. Then weighed Agar agar was added into it and transferred into a conical flask and sterilized by autoclaving at 121°C for 15 minutes. The sterilized agar medium was poured into the sterilized Petri plates and allowed to solidify. Each plate was inoculated at the center with the bacterial

inoculum. Plates were incubated at 37°C for 48 h. To test for hydrolysis of starch, each plate was then flooded with iodine solution and left for 30 seconds. The excess iodine solution was poured off and the clear zone around the line of bacterial growth was examined (Tille, 2014; Sigmon, 2008).

3.8.4.7. Spore Stain

A smear of 48 h pure culture of the organism was made on a slide, flooded with malachite green solution and heated over a beaker of boiling water for 3 minutes. The slide was cooled in air for 5 minutes and rinsed with a gentle stream of tap water for 5 seconds. The slide was counter stained with safranin for 30 seconds, washed off with tap water for 5 seconds and allowed to air dry. The stained slide was examined under oil immersion objective lens. The with spores stained green and the vegetative cell portion being red (Ammann *et al*., 2011).

3.8.4.8. Indole Production

Tryptophan broth in test tube was inoculated with the broth culture of bacteria and incubated for 48 h at 37^0C. After the incubation period, Kovac s reagent (0.5 ml) was added gently to the test tube and allowed to stand for 20 minutes. Formation of a red ring at the reagent layer, indicated indole production (Murray *et al*., 2007).

3.8.4.9. Oxidase Test

A single line streak inoculation of a 24 h test organism on the agar surface was made on Trypticase Soy agar plates. The plates were incubated in an inverted position for 24 h at 37^0C. N, N-Dimethyl- p- phenylenediamine (DMPD) was

added to the surface of the growth of the each organism. For positive test, colour change in 20 seconds; negative test, no colour change (Murray *et al*., 2007).

3.8.4.10. Coagulase Test

From a 24 h culture of the organism, an inoculum was picked using an inoculating loop and placed on the clean slide. A drop of human plasma was added to an emulsion of the test organism and rocked gently. The production of coagulase enzyme was shown by the coagulation of the plasma indicated by granule formulation (Leboffe, 2014).

3.8.4.11. Urease Production

The slant containing Christensen's urea agar was prepared by dissolving the ingredients in 100 ml of distilled water and filtered using sterilized 0.45 mm pore size. The agar was suspended in 90 ml of distilled water and boiled to dissolve completely. The dissolved agar was autoclave at 121^0C for 15 minutes at 15 psi. The agar was cooled to 50^0C. A 100 ml of filtered sterilized urea base was added aseptically to the cooled agar solution and mixed thoroughly. 5 ml was measured into each sterile tube and the tubes were slanted during cooling until solidified. The complete media slant was inoculated with each organism and incubated at 35^0C for 7 days and observed daily for any colour change. Where there was active urease, urea was hydrolyzed to ammonia which increased the pH as indicated by colour changes in the medium from yellow to pink (Forbes *et al.*, 2007).

3.8.4.12. Citrate Utilization Test

Simmon's citrate agar slants was inoculated with the test organism by streaking. The slant was incubated at 37^0C for 48 h. After incubation, citrate positive test

was identified by the presence of growth on the surface of the slant, which was accompanied by blue colouration. Citrate negative cultures show no growth and the medium remain green (Forbes *et al*., 2007).

3.9. Screening of Isolated *Lactobacillus* species for Probiotic Properties

3.9.1. Sodium Chloride Tolerance

Lactic actic acid bacteria cultures were inoculated into 10 ml sterile de Man Rogosa and Sharpe broth in test tubes containing varying concentration of NaCl (1-10) and incubated at 37ºC for 48 h. Growth was monitored by visual observation of the test tubes and NaCl tolerance was confirmed after 1 ml of broth culture was plated out on using sterile de Man Rogosa and Sharpe agar (MRS), allowed to set and incubate at 37ºC for 48 h. Positive control experiments were made of tubes containing lactic acid bacterial cultures without NaCl, while negative control experiments were made of tubes with added NaCl but without lactic acid bacterial cultures (Tambekar and Bhutada, 2010).

3.9.2. Sensitivity to Temperature Test

Some selected lactic acid bacteria cultures were inoculated into 10 ml sterile de Man Rogosa and Sharpe brothin test tubes and incubated anaerobically at varying temperatures from 15-45ºC for 48-72 h. Thereafter, 1 ml inoculum was cultured on de Man Rogosa and Sharpe agar plates by pour plate method and incubated at 37ºC for 48 h. The growth of lactic acid bacteria on de Man Rogosa and Sharpe agar plates was used to designate isolates as temperature tolerant (Tambekar and Bhutada, 2010).

3.9.3. Tolerance of Isolated Lactic Acid Bacteria to Acidic pH

The tolerance of the probiotic bacteria to acidic pH was tested *in vitro* as described by Pelinescu *et al*. (2009). 1 ml of each latic acid bacterial culture at 1×10^8 cfu/ml was inoculated into sterile de Man Rogosa and Sharpe broth tubes of pH2 and pH3 (broth was adjusted by a pH meter using HCl of NaOH) and incubated anaerobically at 37ºC for 24 h. After incubation,1 ml inoculum from each tube was inoculated into de Man Rogosa and Sharpe agar medium using pour plate technique and incubated anaerobically at 37ºC for 48 h. Presence or absence of growth of lactic acid bacteria on agar de Man Rogosa and Sharpe agar was used to designate isolate as pH tolerant.

3.10. Antibiotic Sensitivity Test of the Isolated Pathogenic Organisms

The Kirby-Bauer test, also known as disc diffusion method was used to determine the effect of standard antibiotics on the bacterial isolates as described by Bano *et al*., (2012). This method involved the use of filter paper disc that had been impregnated with antibiotics of known concentration. The agar was seeded with 18hour-old pure culture of the following bacteria, *Pseudomonas aeruginosa*, *Staphylococcus haemolyticus*, and *Klebsiella pnuemoniae.* The discs were applied unto the seeded plates and incubated for 24 h at 37^0C. The plates were observed for clear zones of inhibition. Seeding the agar plate with 24 h old pure isolates without antibiotics serve as control experiment. The zones of inhibition were measured. Each experiment was prepared in triplicates and the results were expressed as average values. The standard antibacterial agents, erythromycin (10µg), gentamycin (10µg), ampiclox (30µg), vancomycin (30µg), amoxacillin (30µg), ciprofloxacin (10µg), streptomycin (30µg), septrin (30µg), augmentin (30µg), cephalexin (30µg) were used as control for bacteria.

3.11. Assay for Antimicrobial Activity

Antimicrobial activities of probiotics were determined by the agar well diffusion method as described by Tajehmiri *et al.*, (2014). A 0.2 ml of a 24 h broth culture was aseptically introduced into the sterile Petri dishes. The sterilized medium at 45^0C was poured into petri dishes. The agar depth was 4 mm. (26 ml medium was used for plate with 90 mm diameter). Wells were made on the agar plates using a sterile cork borer of 5 mm diameter. A 100µl of the supernatants of isolated probiotics were placed into each well. A negative control was 100µl of the broth without organisms. The cultured plate was incubated at 37^0C for 48 h and the resulting zones of inhibition were measured using a ruler calibrated in millimeter. Each experiment was replicated three times and the results were expressed as average values. Isolates which gave an inhibition zone bigger than 10 mm was determined to have antimicrobial activity and the isolate was further identified using molecular procedure.

3.12. Molecular Identification

Five bacterial isolates were sent to Macron Inc, Amsterdam Netherlands for molecular identification using sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA.

3.12.1. Genomic DNA Isolation

Pellet was suspended in 100 µl of TE buffer (10% w/v sucrose, 25 mM Tris-HCL pH 8.0, nM EDTA, 10mg/ml, freshly made lysozyme and 40 µgml RNase A) and incubated for 30 minutes at 37^0C. The protoplast cells were immediately lysed by adding 600 µl of lysis buffer (100mM Tris-HCl pH 8.0, 100mM EDTA, 10 mM NaCl and 1% w/v SDS) and incubated for 15 minutes at room temperature. The lysates were

treated with 10 µl of proteinase K (10 mg/ml) and incubated for 15 minutes at 37^0C. After incubation at 80^0C for 5 minutes and cooling down to room temperature for 5 -10 minutes, 200µl of sodium acetate (3 M, pH 5.2) was added, chilled on ice for 15 minutes and centrifuged at 6,000 rpm for 10 minutes. The supernatant was decanted and 600 µl 0f isopropanol was added to it to precipitate the DNA. Finally, genomic DNA was dissolved in distilled water and maintained at 20^0C for further studies (Vanysacker *et al.,* 2010).

3.12.2. Polymerase Chain Reaction and Gel Electrophoresis

The primers were synthesized and provided in a lyophilized form, which were re-dissolved with TE buffer (pH 8) to a final concentration of 100 pmol/µl and stored at -20^0 C. The sequence of the primer set Lacto -16S forward – was 5'…..GGA ATC TTC CAC AAT GGA CG…..3' and the primer set Lacto -16S reverse – was 5'….CGC TTT ACG CCC AAT AAA TCC GG…. 3' (Abdulamir *et al*., 2010); amplifications were carried out in 30 µl volumes containing (10 pmol/µl) of each primer, 2x taq PCR PreMix, and 200 ng genomic DNA. Amplification was achieved in 40 cycles according to the manufacturer's instruction; using a GTC thermal cycler (Cleaver Scientific, UK). Prior to the first cycle, DNA was denatured at 95^0 C for 3 minutes subsequently, and each cycle consisted of denaturation at 95^0C for 30 seconds, followed by annealing at 61^0C for 40 seconds. Elongation was carried out at 72^0C and the extension time at 1 minute subsequently. A final elongation was performed at 72^0C for 5 minutes, and the holding temperature was for 10 seconds. PCR product was separated by electrophoresis on 1.5% (w/v) agarose gel containing ethidium bromide (0.5 µg/ml). 12 µl of each PCR product and 4µl of 6x loading dye was loaded in to the agarose gel and run in 1 x TBE buffer. A 100 bp plus

DNA ladder was used as a molecular weight standard and a positive control was run together with the PCR products. The PCR products were separated by electrophoresis at a constant voltage of 70V for 40 minutes. Then, DNA bands were visualized by ultraviolet (UV) illuminator system, and the photographic pictures were taken for each gel (Tortora, *et al.*, 2013).

3.12.3. Sequence and Phylogenetic Analyses

All raw sequence files were inspected and corrected, where necessary, using Chromas Lite 2.0 (Technelysium) and BioEdit v. 5.0.9. To match the sequences obtained for individual 16S rRNA V3 fragments excised from DGGE gels with those obtained for the complete 16S rRNA gene of the pure cultures, these sequences were compared in BioEdit. All sequences were also compared to those in the nucleotide database of the National Centre for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/) using blastn to obtain preliminary identifications for the isolated bacteria.

Alignments for the complete 16S rRNA gene and pheS data determined in this study were generated using Multiple sequence Alignment based on Fast Fourier Transform (Katoh and Frith, 2012). These alignments also included the sequence information for the relevant type strains of species in the genera *Lactobacillus* which were obtained from GenBank. To determine the best-fit evolutionary models for the datasets, PAUP v. 4.0b1 together with Modeltest v. 3.7 were used.

3.13. Preparation of Supernatants

Probiotics were inoculated in de Man Rogosa and Sharpe broth medium and incubated under anaerobic condition at 37^0C for 48 h. The bacteria cells were removed by

centrifuging the culture at 5000g for 20 minutes at 4^0C. The pH values of supernatants were adjusted to pH 6.5-7.0 by the addition of 1N NaOH, the supernatants were membrane filtered (Millipore, 0.22µm) and store at 4^0C.

3.14. *In-vivo* Study

3.14.1. Housing and Maintenance of Rats

The 75 Female *Rattus norvegicus* (Wistar albino rats) with an initial weight of 200 ± 50g were obtained from the Chris Farm Mgbakwu Village, Anambra State. The animals were housed in metabolic cages under controlled environmental conditions (25^0C, 12 h light/dark cycle with controlled humidity of 50% to 70%). They all had free access to pulverized standard rat pellet food and water for one week before feeding with experiment diet (Hassan *et al*., 2012).

3.14.2. Grouping of Experimental Rats

The animals were divided into 5 groups of 15 animals each. Each group was further divided into three sub groups of 5 rats each. Assigned to each was the pathogenic organisms to be treated.

Group A: Normal control group with wound alone.

Group B: Diabetic control group with infected wound

Group C: Diabetic infected rats treated with *Lactobacillus casei.*

Group D: Diabetic infected rats treated with *Lactobacillus plantarum*

Group E: Diabetic infected rats treated with gentamicin i.p (120 mg/kg).

3.14.3. Induction of Diabetes Mellitus.

Animals of group B, C, D and E were fasted overnight, their body weight and their blood glucose levels were determined. After a 12 h fast, rats received a single intraperitoneal injection of streptozotocin (65 mg/kg; Merck Chemical, Darmstadt, Germany) freshly prepared in 0.1 M sodium citrate buffer (pH 4.5). At three days after streptozotocin injection, blood glucose measurement was performed on tail-vein blood to ensure the induction of diabetes by using a glucometer (Accu-Chek Active, Roche Diagnostics, Penzberg, Germany). Rats whose blood glucose levels exceeded 200 mg/dL (13.9 mmol/dL) were considered diabetic. Weights of the rats were monitor throughout the study (Somashekar and Sudhakar, 2013).

3.14.4. Induction of Wounds

The 75 female *Rattus norvegicus* (Wistar albino rats) were anaesthetized; their backs were shaved using surgical blade. A 2 cm longitudinal full thickness excision were made in the upper paravertebral region of each rat. Wounds were traced on 1 mm 2 graph paper on the day of wounding and subsequently on the alternate days, until healing was complete. Changes in wound area were calculated, giving an indication of the rate of wound contraction. All rats received sterile sodium chloride to prevent dehydration. After fully recovering from anesthesia, rats were placed in individual cages (Romana *et al*., 2009).

3.14.5. Assessment of Contamination.

Bacteria collection was performed by using the one-point method with the sterile swab, the center surface of each wound was scrubbed carefully by rotating the swab 3 times clockwise with enough manual pressure to produce a small amount of exudate. The inoculated swab was inserted into a tube and transported to the laboratory for immediate processing. The swab collection tube was vortexed (with the swab inside) for 5 seconds, and a 100-µL aliquot of the suspension was used for serial dilutions. Quantification of the viable bacteria present in the swab was performed by using the 10-fold serial dilution method and 100 µL of each dilution was plated onto tryptone soy agar (Biokar Diagnostics, Pantin Cedex, France). The plates were incubated under aerobic conditions at 37°C for 24 h, after which colony- forming units were counted (Fraccalvieri *et al*., 2011).

3.14.6. Preparation of Probiotics

The probiotics were prepared according to Walencka *et al.*, (2008). The strain was cultivated in 1 litre of de Man Rogosa and Sharpe (MRS) broth for three days at 37^0C at static conditions. Medium containing the probiotics was given to animals at a concentration of 100 ml/kg food.

3.14.7. Administration of Probiotics to Rats

The probiotics were given daily to the rats orally through the feeding bottles until day 15. Changes in wound area were calculated (Mirjana *et al*., 2017).

3.14.8. Behavioural Observation

The animals were observed twice daily for clinical signs exhibited by them in different groups. The behavioural alterations were observed subjectively by observing alertness, attraction to feed and water responsiveness to external stimulus and activity (Lee *et al.*, 2012).

3.14.9. Assessment of Wound-Healing

The wound area and percentage wound- healing at different times after treatments were assessed in rats according to the method described by Vinay and Dinesh, (2014) . The length and width of wound (2 mm) were measured in days 1, 3, 7 and 15. The percentage wound- healing was calculated as:

$$\%\text{wound closure} = \frac{(\text{Wound area in first day}) - (\text{Wound area in specific day})}{(\text{Wound area in first day})} \times 100$$

3.14.10. Determination of Haematological Parameters.

3.14.10.1. Collection of Blood Samples

The rats were anesthetized using chloroform on day 1, 3, 7, and day 15 after the initial infection. Blood was collected from each rat into a sterile 2.5 mL tubes containing EDTA (anticoagulant) and immediately mixed well to avoid clotting and left to stand at room temperature (20^0C) (Muhammad *et al.*, 2012; Cheesbrough, 2009).

3.14.10.2. Determination of Red Blood Cell

Blood films are made by placing a drop of blood on one end of a slide, and using a spreader slide to disperse the blood over the slide's length. The monolayer was found in the "feathered edge" created by the spreader slide as it draws the blood forward. The slide was left to air dry, after which the blood was fixed to the slide by immersing it briefly in 95% (vol/vol) methanol. The fixed slide was stained with giemsa stain to distinguish the cells from each other. These stains were allowed for the detection of white blood cell, red blood cell, and platelet abnormalities. After staining, the monolayer was viewed under a microscope using magnification up to 1000x. Individual cells are examined and their morphology was characterized and recorded (Alam, 2015; Peter *et al*., 2009).

3.14.10.2.1. Determination of Haemoglobin

The Hb square tube was filled to 20% with 0.1 N HCl and Hb pipette was filled with the blood micro liter. The blood was added into the acid in the square tube and the pipette was rinsed three times with water by drawing in and discharging into the blood-acid mixture. The blood- acid was mixed with hematin solution in the tube with a glass rod and the tube allowed to stand for 20 minutes. The solution was diluted by adding distilled water drop by drop and stirring the mixture all the time with glass rod. The comparator was held against good day light and distilled water was added till the colour of the solution matches perfectly with that of standards. The reading was recorded in g/dL and the result was read at the bottom of the meniscus (Cheesbrough, 2009).

3.14.10.2.2. Hematocrit (Packed Cell Volume) Determination.

The capillary tube was filled two-thirds to three quarters full with well-mixed, oxalated venous blood. One end of the tube was sealed with clay and the filled tube was placed in the microhematocrit centrifuge with the plugged end away from the center of the centrifuge. The tube was centrifuge at a preset speed of 12,000 rpm for 5 minutes. After centrifuge, the tube was placed in the microhematocrit reader and the hematocrit was read by following the manufacturer's instruction on the microhematocrit reading device (Cheesbrough, 2009).

3.14.10.2.3. Determination of Mean Cell Volume (MCV)

The mean cell volume (MCV) was calculated by dividing packed cell volume by red blood count. The latter was determined by visual counting of cells using an impoved Neubauer haemocytometer (Cheesbrough, 2009).

$$\text{MCV (fL)} = \frac{\text{Hematocrit (\%)} \times 10}{\text{RBC } (\times 10^{12}/\text{L})}$$

3.14.10.2.4. Determination of Mean Cell Haemoglobin (MCH)

This was calculated as reported by Cheesbrough, (2009). It involves dividing haemoglobin concentration by the value of red blood cell count.

$$\text{MCH (pg)} = \frac{\text{Hb (g/dL)} \times 10}{\text{RBC } (\times 10^{12}/\text{L})}$$

3.14.10.2.5. Determination of Mean Cell Haemoglobin Concentration (MCHC)

The mean cell haemoglobin concentration was calculated by dividing the haemoglobin concentration value by that of the packed cell volume (Cheesbrough, 2009).

$$\text{MCHC (g/dL)} = \frac{\text{Hb (g/dL) x 100}}{\text{Hematocrit (\%)}}$$

3.14.10.3. Determination of White Blood Cell

The total white blood cell count was estimated by visual count method (Cheesbrough, 2009). The blood sample (0.02 ml) was added to 0.38 ml of Turks solution in a plain cuvette and thoroughly mixed. 0.01 ml of the resultant mixture was loaded into the counting chamber, covered with the cover slide and allowed to settle (2 minutes) before placing it under the microscope and viewed using x10 objective lens. The white cells present in the four corners and the central 1 mm^2 areas were counted using hemocytometer counter. From the result, the total white blood cell count was deduced by calculation.

3.14.10.4. Differential White Blood Cell Count/ Cell Morphology

The Leishman staining technique as reported by Cheesbrough, (2009) was adopted in the differential count and cell morphology analysis. A drop of the EDTA anti-coagulated blood was placed on dry grease-free glass slide. A thin film of blood was made on the slide, which was allowed to air dry. The Leishman stain was then used to cover the film (drop wise). This was followed 2 minutes later by the addition of the sorenscens buffer before mixing and allowed to stand

for 8 minutes on a flat surface. The stain was gently washed off using tap water and the slide was allowed to dry in air The dried slide was then viewed under oil immersion with a x100 objective, the various cells were counted and the requisite morphological notes taken.

Neutrophils - When viewed under the microscope appeared spherical in shape with a dark stained nucleus that is segmented (2 to 5 lobes).

Monocytes - monocytes showed a bean or kidney shaped.

Lymphocytes - lymphocytes is a large round nucleus that takes up much of the cell volume. It is of two types small and large.

Eosinophils - eosinophils showed a bi-lobed (two lobes) nucleus that is shaped like a horse-shoe.

Basophil- when viewed under the microscope appeared spherical in shape with granules stained brick red.

3.15. Statistical Analysis of Results

Data were subjected to one way analyses of variance and the differences between means were evaluated by Duncan's multiple range test using SPSS statistic programme version 23. Significant difference was expressed at $P < 0.05$.

CHAPTER FOUR

RESULTS

4.1. Physicochemical Composition

The results of variation in pH, brix, total soluble solids, total titratable acidity, specific gravity and viscosity of the 'kunun-zaki' samples are presented in Table 4.1. The pH values obtained for all the samples ranged from 4.30 to 5.67. The pH values of the controls from at 50:50% and 80:20% were not significantly different while the pH of other samples 4.40, 4.60, 4.30, 4.45, 4.60 and 4.35 respectively were significantly different ($P<0.05$) from those of the control.

The brix values found in the samples ranged between 5.22±0.08% and 6.65±0.03%. 'Kunun-zaki' with sweet potatoes (KS) prepared with 50:50% had the highest brix value of 6.65±0.03% and was significantly different from those of other samples (5.65±0.03%, 5.60±0.10%, 5.86±0.03%, 5.32±0.09%, 5.22±0.08% and 5.93±0.02%).

The total soluble solids (TSS) content of 'kunun-zaki' samples ranged from 9.47±0.04 - 13.93±0.03%. The values obtained is significantly different ($P<0.05$) from each sample. The control from 80:20% had the lowest value of 9.93±0.07% while the 'kunun-zaki' with sweet potato from 50:50% had the highest total soluble solids of 13.93±0.03%.

The total titratable acidity (TTA) found in 'kunun-zaki' samples varied considerably, ranging from 0.53±0.02 to 0.75±0.01%. The samples were not significantly different ($P>0.05$) but when compared to the control, they were different. The samples with combination of paddy rice and sweet potatoes had the highest value of 0.75±0.01%.

The specific gravity of all the samples from 50-50% were not significantly different ($P>0.05$) except KS. The control from 80:20% had the highest value of 1.02±0.01%.

Table 4.1: Physicochemical Composition of 'Kunun-zaki' Samples.

SAMPLE	pH	Brix (%)	TSS (%)	TTA (%)	SG (%)
KO	5.57±0.09^{a}	5.65±0.03^{c}	9.93±0.07^{e}	0.53±0.02^{b}	0.98±0.00^{ab}
KP	4.40±0.00^{c}	5.60±0.10^{c}	13.72±0.09^{bc}	0.67±0.03^{a}	0.98±0.00^{ab}
KS	4.60±0.00^{b}	6.65± 0.03^{a}	13.93±0.03^{a}	0.67 ±0.04^{a}	0.98 ±0.00^{b}
KA	4.30±0.03^{c}	5.86 ±0.03^{b}	12.63±0.07^{d}	0.75±0.01^{a}	0.97±0.00^{ab}
KOI	5.67±0.02^{a}	5.32±0.09^{d}	9.47±0.04^{f}	0.53±0.02^{b}	1.02±0.01^{a}
KPI	4.45±0.05^{c}	5.22±0.08^{d}	13.65±0.03^{c}	0.67±0.03^{a}	0.92±0.01^{c}
KSI	4.60±0.04^{b}	6.53±0.06^{a}	13.84± 0.02^{ab}	0.67 ±0.04^{a}	0.89± 0.004^{c}
KAI	4.35±0.05^{c}	5.93±0.02^{b}	12.71±0.03^{d}	0.75± 0.01^{a}	0.84± 0.06^{d}

Values are mean scores of three replicated samples. Values in columns with different superscript letters are significantly different ($P < 0.05$).

Keywords:

TSS-Total Soluble Solids.

TTA- Total Titrable Acid.

SG- Specific Gravity.

KO-'kunun-zaki' (Control) at 50:50% Millet and Sorghum.

KP- 'kunun-zaki' + Paddy rice at 50:50%.

KS-'kunun-zaki' + Sweet potatoes at 50:50%.

KA- 'kunun-zaki' +Paddy rice+ Sweet potatoes at 50:50%.

KOI- 'kunun-zaki' (Control) at 80:20% millet and sorghum.

KPI- 'kunun-zaki' + paddy rice at 80:20%.

KSI- 'kunun-zaki' + sweet potatoes at 80:20%.

KAI- 'kunun-zaki' + Paddy rice + Sweet potatoes at 80:20%.

4.2. Proximate Analysis

The proximate composition of 'kunun-zaki' samples revealed the moisture content of the products to be 88±0.06, 86.07±0.03, 83.69±0.16, 86.23±0.03, 88.30±0.31, 87.00±0.00, 84.17±0.07 and 86.29±0.32% for KA,KP,KS,KA,KOI,KPI,KSI and KAI respectively. The moisture contents of the control (88.30±0.31%) was observed to be higher and significantly different ($P< 0.05$) from other samples. (Table 4.2). 'Kunun-zaki' processed with sweet potatoes from 50:50% (KS) had the lowest moisture content of 83.69±0.16% (Appendix VII).

The ash contents of the 'kunun-zaki' processed with paddy rice from 50:50 and 80:20% millet/sorghum ratios were found to be significantly different ($P<0.05$) and were given as 4.45±0.08% and 4.34±0.02% respectively. The control sample from 80:20% had the lowest value of 0.57±0.09% (Table 4.2). The crude fiber contents ranged from a mean value of 0.24 to 0.34%. The crude fibre of the samples from 80:20% differed significantly ($P<0.05$). The highest fiber was obtained from 50:50% 'kunun-zaki' with paddy rice.

'Kunun-zaki' produced with paddy rice for 50:50% and 80:20% had higher protein value of 1.90±0.08% compared to the control (1.14±0.01%).

The carbohydrate contents of the 'kunun-zaki' samples obtained in KO, KA, KSI and KAI were not significantly different (Table 4.2). The 'kunun-zaki' with paddy rice for 80:20% had the lowest carbohydrate of 5.34±0.08% while the 'kunun-zaki' processed with sweet potatoes for 50:50% had the highest carbohydrate value of 9.65±0.27%.

The fat contents of the control for 50:50% (KO) was 0.85±0.01% and was significantly different ($P<0.05$) from the other samples. The control for 80:20% had the least fat content of 0.84±0.01% (Table 4.2). The 'kunun-zaki' processed with sweet potatoes for 50:50% had the highest fat content of 1.40±0.12%.

Table 4.2: Proximate Composition of 'Kunun-zaki' Samples

Sample	Moisture (%)	Ash (%)	Fiber (%)	Protein (%)	Carbohydrate (%)	Fat (%)
KO	88.00±0.06^{a}	0.58±0.02^{d}	0.26±0.00^{d}	1.23±0.01^{cd}	9.08±0.08^{ab}	0.85±0.01^{c}
KP	86.07±0.03^{c}	4.45±0.08^{a}	0.34±0.09^{a}	1.90±0.04^{a}	6.80±0.15^{c}	1.06±0.02^{b}
KS	83.69±0.16^{e}	3.43±0.09^{b}	0.30±0.00^{bc}	1.54±0.09^{b}	9.65±0.27^{a}	1.4±0.12^{a}
KA	86.23±0.03^{c}	2.0±0 0.06^{c}	0.27±0.01^{d}	1.23±0.02^{cd}	9.10±0.07^{ab}	1.17±0.01^{b}
KOI	88.3±0.31^{a}	0.57±0.09^{d}	0.24±0.00^{e}	1.14 ±0.01^{d}	8.88 ±0.36^{b}	0.84± 0.01^{c}
KPI	87.00±0.00^{b}	4.34±0.02^{a}	0.32±0.01^{ab}	1.90±0.08^{a}	5.34±0.08^{d}	1.10±0.04^{b}
KSI	84.17±0.07^{d}	3.35±0.08^{b}	0.28±0.01^{cd}	1.55±0.02^{b}	9.09±0.18^{ab}	1.21±0.02^{b}
KAI	86.29±0.32^{c}	1.80±0.28^{c}	0.26±0.01^{d}	1.34±0.05^{c}	9.09±0.18^{ab}	1.21±0.02^{b}

Values are mean scores of three replicated samples. Values in columns with different superscript letters are significantly different ($P < 0.05$).

KO-'kunun-zaki' (Control) at 50:50% Millet and Sorghum.

KP- 'kunun-zaki' + Paddy rice at 50:50%.

KS-'kunun-zaki' + Sweet potatoes at 50:50%.

KA- 'kunun-zaki' +Paddy rice+ Sweet potatoes at 50:50%.

KOI- 'kunun-zaki' (Control) at 80:20% millet and sorghum.

KPI- 'kunun-zaki' + paddy rice at 80:20%.

KSI- 'kunun-zaki' + sweet potatoes at 80:20%.

KAI- 'kunun-zaki' + Paddy rice + Sweet potatoes at 80:20%.

4.3. Vitamin

The vitamin composition of the eight types of 'kunun-zaki' evaluated in this study are shown in Figure 4.1. The 'kunun-zaki' processed with sweet potatoes for 50:50% millet and sorghum was observed to have the highest vitamin C content (32.25 mg/100ml) and vitamin E contents (18.24 mg/100ml). There were significant differences ($P < 0.05$) between the ascorbic acids contents of all the samples.

4.4. Minerals

Analysis of the mineral contents of the 'kunun-zaki' samples indicated that sample with paddy rice and sweet potatoes for 80:20% millet and sorghum had the lowest value (13.11 mg/100ml) for calcium while the highest (19.77 mg/100ml) was obtained for a sample containing sweet potatoes (Table 4.3). There was no significant difference ($P > 0.05$) in the calcium contents of the control and 'kunun-zaki' processed with paddy rice and sweet potatoes for 80:20% (Appendix VIII). Likewise, no significant differences in the magnesium and potassium composition of 'kunun-zaki' processed with sweet potatoes for 50:50%. The potassium value of the control for 50:50% of 'kunun-zaki' was significantly different ($P< 0.05$) from those of all the samples obtained for 50:50%. In terms of zinc and iron content, the mean values ranged from 1.81 to 3.24 and 2.73 to 3.91 mg/100ml respectively. The 'Kunun-zaki' processed with paddy rice had the highest zinc concentration (3.31 mg/100ml) and iron (3.91 mg/100ml). The zinc and iron contents of sweet potatoes for 50:50% were not significantly different (Table 4.3).

The lowest value for sodium was observed for 'kunun-zaki' processed with sweet potatoes.

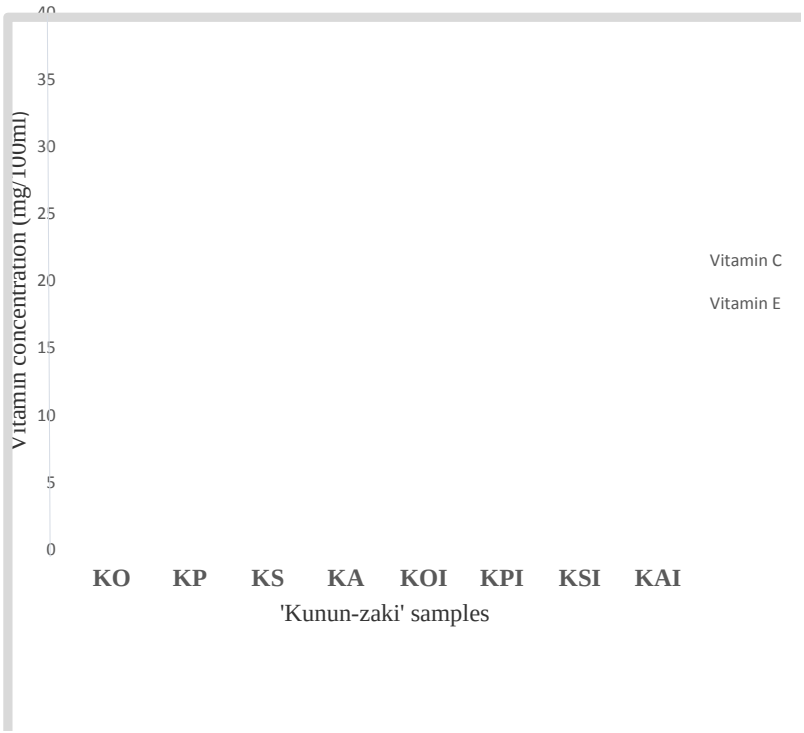


Figure 4.1: Vitamin Contents of the 'Kunun- zaki' Samples.
Keywords:

KO-'kunun-zaki' (Control) at 50:50% Millet and Sorghum.

KP- 'kunun-zaki' + Paddy rice at 50:50%.

KS-'kunun-zaki' + Sweet potatoes at 50:50%.

KA- 'kunun-zaki' +Paddy rice+ Sweet potatoes at 50:50%.

KOI- 'kunun-zaki' (Control) at 80:20% millet and sorghum.

KPI- 'kunun-zaki' + paddy rice at 80:20%.

KSI- 'kunun-zaki' + sweet potatoes at 80:20%.

KAI- 'kunun-zaki' + Paddy rice + Sweet potatoes at 80:20%.

Table 4.3: Mineral Contents of the 'Kunun- zaki' Samples

Samples	Minerals (mg/100ml)					
	Calcium	Magnessium	Potassium	Zinc	Iron	Sodium
KO	14.40±0.05^{f}	125.33±1.53^{e}	184.00±4.00^{d}	2.25±0.25^{b}	2.77±0.59^{de}	21.07±0.47^{g}
KP	18.13±0.13^{d}	145.67±1.15^{b}	220.00±18.03^{b}	3.31±0.08^{a}	3.91±0.53^{a}	22.27±0.09^{e}
KS	18.78±0.29^{b}	154.67±4.51^{a}	245.00±5.00^{a}	1.81±0.32^{c}	2.94±0.53^{c}	23.67±0.02^{b}
KA	15.21±0.36^{e}	131.67±2.88^{d}	202.00±7.21^{c}	1.92±0.25^{c}	2.73±0.11^{e}	22.64±0.07^{c}
KOI	13.2±0.05^{g}	110.33±4.51^{f}	189.00±3.6^{cd}	1.83±0.12^{c}	2.88±0.07^{bcd}	21.18±0.03^{f}
KPI	18.28±0.03^{c}	141.00±3.61^{b}	195.67±3.21^{cd}	3.24±0.03^{a}	3.56±0.036^{b}	22.49±0.04^{d}
KSI	19.77±0.06^{a}	140.33±2.52^{bc}	226.67±2.89^{b}	1.89±0.02^{c}	2.84±0.02^{cde}	24.11±0.01^{a}
KAI	13.11±0.10^{g}	134.33±5.13^{cd}	183.33±2.89^{d}	1.85±0.62^{c}	2.85±0.07^{cde}	22.72±0.09^{c}

Values are mean scores of three replicated samples.

Values in columns with different superscript letters are significantly different ($P < 0.05$).

Keyword:

KO-'kunun-zaki' (Control) at 50:50% Millet and Sorghum.

KP- 'kunun-zaki' + Paddy rice at 50:50%.

KS-'kunun-zaki' + Sweet potatoes at 50:50%.

KA- 'kunun-zaki' +Paddy rice+ Sweet potatoes at 50:50%.

KOI- 'kunun-zaki' (Control) at 80:20% millet and sorghum.

KPI- 'kunun-zaki' + paddy rice at 80:20%.

KSI- 'kunun-zaki' + sweet potatoes at 80:20%.

KAI- 'kunun-zaki' + Paddy rice + Sweet potatoes at 80:20%.

4.5. Sensory Evaluation Test

Table 4.4 showed the sensory properties of 'kunun-zaki' samples. 'Kunun-zaki' processed with paddy rice and sweet potatoes from 50:50% millet and sorghum ratio was significantly different ($P < 0.05$) in taste from other samples. There was no significant difference in the colour of 'kunun-zaki' processed with paddy rice and 'kunun-zaki' processed with paddy rice and sweet potatoes for 50:50%. Also, there was no difference in the colour of the control and the sweet potatoes based 'kunun-zaki' for 80:20%. There was no significant difference in the mouthfeel of both control for 50-50% and 80:20% respectively but the controls were significantly different from other samples. The 'kunun-zaki' processed with paddy rice and 'kunun-zaki' processed with paddy rice and sweet potatoes for 50:50% millet had the highest mean scores in flavour and acceptability while the control for 50:50% had the least score (Appendix IX).

4.6. Total Viable Counts

The total viable counts of the 'kunun-zaki' samples are reported in Table 4.5. The nutrient agar count of 'kunun-zaki' processed with sweet potatoes for 50:50% millet to sorghum ratio had the highest count of 2.26±0.05 CFU/ml and is significantly different ($P < 0.05$) to other samples in 50:50%. The control of 80:20% millet and sorghum was significantly higher from other 'kunun-zaki' samples processed for 80:20% except sweet potatoes based 'kunun-zaki'. No coliforms counts were detected in the beverage samples. The yeast agar count ranged from 1.18 to 1.52 (Table 4.5). There was no yeast count in the 'kunun-zaki' processed with paddy rice and sweet potatoes both for 50:50 and 80:20%. The least count of yeast was observed in the control for 80:20% and sweet potatoes based 'kunun-zaki'. The highest fungal count of 1.36±0.05 CFU/ml was observed in samples processed with sweet potatoes for 50:50% and 80: 20.

Table 4.4: Sensory Attributes of the 'Kunun-zaki' Samples

SAMPLE	TASTE	COLOUR	MOUTHFEEL	FLAVOUR	ACCEPTABILITY
KO	5.84±0.02^{c}	6.12±0.01^{b}	5.09±0.05^{f}	5.09±0.01^{d}	5.35±0.06^{d}
KP	6.38±0.01^{b}	5.67±0.04^{d}	6.77±0.12^{a}	6.35± 0.02^{a}	6.35±0.02^{a}
KS	5.82±0.03^{cd}	5.21±0.04^{f}	5.49±0.01^{e}	5.7±70.02^{b}	5.37±0.01^{cd}
KA	6.54±0.02^{a}	5.63±0.03^{d}	6.57±0.07^{b}	6.47±0.08^{a}	6.42±0.01^{a}
KOI	5.84±0.02^{c}	6.34±0.02^{a}	5.07±0.01^{f}	5.19±0.01^{d}	5.14±0.09^{e}
KPI	6.34±0.02^{b}	5.32±0.05^{e}	5.71±0.03^{d}	5.71±0.08^{b}	6.03±0.02^{b}
KSI	5.71±0.09^{de}	6.30±0.03^{a}	5.40±0.03^{e}	5.53±0.04^{c}	5.48±0.02^{c}
KAI	5.64±0.04^{e}	5.86±0.03^{c}	6.30 ±0.04^{c}	6.36±0.05^{a}	6.07±0.04^{b}

Values are mean scores with standard error of three replicated samples.
Values in columns with different superscript letters are significantly different ($P < 0.05$).
Each value is the mean with standard error of 20- member panelist using 7 point hedonic scale.

Keyword:

KO-'kunun-zaki' (Control) at 50:50% Millet and Sorghum.

KP- 'kunun-zaki' + Paddy rice at 50:50%.

KS-'kunun-zaki' + Sweet potatoes at 50:50%.

KA- 'kunun-zaki' +Paddy rice+ Sweet potatoes at 50:50%.

KOI- 'kunun-zaki' (Control) at 80:20% millet and sorghum.

KPI- 'kunun-zaki' + paddy rice at 80:20%.

KSI- 'kunun-zaki' + sweet potatoes at 80:20%.

KAI- 'kunun-zaki' + Paddy rice + Sweet potatoes at 80:20%.

Table 4.5: Microbial Load of the 'Kunun-zaki' Samples

			x10^5 CFU/ml		
Samples	Nutrient Agar	MacConkey Agar	de Manrogosa sharpe Agar	Yeast Agar	Sabroud Dextrose Agar
KO	1.46±0.47^b	ND	6.19±0.08^b	1.39±0.04^b	1.13±0.15^b
KP	1.38±0.03^b	ND	8.41±0.05^a	1.52±0.03^a	1.01±0.04^d
KS	2.26±0.05^a	ND	7.43±0.03^a	1.43±0.09^b	1.36±0.05^a
KA	1.22 ±0.03^c	ND	6.79±0.04^b	ND	ND
KOI	2.05±0.13^a	ND	5.89±0.05^c	1.19±0.01^c	1.06±0.06^c
KPI	1.3±8 0.04^b	ND	6.53±0.01^b	1.45±0.03^a	1.05±0.03^c
KSI	2.22±0.35^a	ND	6.53±0.06^b	1.18±0.03^c	1.35±0.03^a
KAI	1.27± 0.56^c	ND	6.11 ±0.01^c	ND	1.03 ±0.15^d

Values are mean scores of three replicated samples. Values in columns with different superscript letters are significantly different ($P < 0.05$).

Keyword:

ND = Non Detectable.

KO-'kunun-zaki' (Control) at 50:50% Millet and Sorghum.

KP- 'kunun-zaki' + Paddy rice at 50:50%.

KS-'kunun-zaki' + Sweet potatoes at 50:50%.

KA- 'kunun-zaki' +Paddy rice+ Sweet potatoes at 50:50%.

KOI- 'kunun-zaki' (Control) at 80:20% millet and sorghum.

KPI- 'kunun-zaki' + paddy rice at 80:20%.

KSI- 'kunun-zaki' + sweet potatoes at 80:20%.

KAI- 'kunun-zaki' + Paddy rice + Sweet potatoes at 80:20%.

4.7. Morphological and Biochemical Characteristics of Isolated Lactic Acid Bacteria.

The morphological and biochemical characteristics of the isolated lactic acid bacteria are shown in Table 4.6 and Table 4.7. All the lactic acid bacteria isolates were Gram positive rods with entire edges, raised elevation, cream in colour and smooth in texture (Table 4.6). *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus brevis, plantarum, Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus delbruekii, Lactobacillus lactis, Lactobacillus helveticus* and *Lactobacillus bulgaricus* were isolated from 'kunun-zaki' samples (Table 4.7).

4.8. Types of Lactic Acid Bacteria in the Samples

Table 4.8 presents the presence of lactic acid bacteria found in the 'kunun-zaki' samples. *Lactobacillus plantarum* had the highest occurrence followed by *Lactobacillus casei.*

Table 4.6: Morphological Characteristics of the Isolated Lactic Acid Bacteria.

ISOLATE	SHAPE	COLOUR	EDGE	ELEVATION	TEXTURE
L1	Rod	Cream	Entire	Raised	Smooth
L2	Rod	Cream	Entire	Raised	Smooth
L3	Rod	Cream	Entire	Flat	Smooth
L4	Rod	Cream	Entire	Raised	Smooth
L5	Rod	Cream	Entire	Raised	Smooth
L6	Rod	Cream	Entire	Raised	Smooth
L7	Rod	Cream	Entire	Raised	Smooth
L8	Rod	Cream	Entire	Raised	Smooth

Table 4.7. Biochemical Characteristics of Lactic Acid Bacteria Isolated from "Kunun –zaki"

Isolate Identity	Gram reaction	Motility	Sugar fermentation									Biochemical test					Probable organism
			Glucose	Lactose	Sucrose	Mannitol	Fructose	Maltose	Sorbitol	Galactose	Arabinose	Catalase	Starch	Spores	Indole	Oxidase	
L1	+	-	+	+	+	-	+	+	-	+	+	-	-	+	-	-	*Lactobacillus acidophilus*
L2	+	-	+G	+	-	+	+	+	+	+	+	-	-	-	-	-	*Lactobacillus casei*
L3	+	-	+G	-	+	-	+	-	-	-	+	-	-	-	-	-	*Lactobacillus brevis*
L4	+	-	+G	A+	+	+	+	+AG	+	+	+	-	-	-	-	-	*Lactobacillus plantarum*
L5	+	-	+	+	+	-	+	-	-	+	+	-	-	-	-	-	*Lactobacillus fermentum*
L6	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	*Lactobacillus delbruekii*
L7	+	-	+	-	+	+	+	-	-	-	-	+	+	-	-	-	*Lactobacillus lactis*
L8	+	+	+	-	+	-	-	+	+	-	-	-	-	-	-	-	*Lactobacillus helveticus*

+ = positive reaction,- = negative, G= gas formation.

Table 4.8: Types of Lactic Acid Bacteria found in "Kunun-zaki" Samples

Samples	*Lactobacillus acidophilus*	*Lactobacillus casei*	*Lactobacillus brevis*	*Lactobacillus plantarum*	*Lactobacillus fermentum*	*Lactobacillus delbruece*	*Lactobacillus lactis*	*Lactobacillus helveticus*
KO	-	+	+	+	-	-	+	-
KP	+	+	+	+	+	+	+	-
KS	+	+	+	+	-	+	+	+
KA	+	+	-	+	+	-	+	+
KOI	-	-	+	+	+	+	-	-
KPI	+	-	+	+	+	-	+	+
KSI	-	+	+	+	-	+	-	+
KAI	+	+	-	+	-	+	-	+

- =Not present, + = Present.

KO-'Kunun' (Control) at 50:50% Millet and Sorghum.

KP- 'Kunun-zaki' + Paddy rice at 50:50%.

KS- 'Kunun-zaki' + Sweet potatoes at 50:50%.

KA- 'Kunun-zaki' +Paddy rice+ Sweet potatoes at 50:50%.

KOI-'Kunun- zaki' (Control) at 80:20% millet and sorghum.

KPI- 'Kunun-zaki' + paddy rice at 80:20%.

KSI- 'Kunun-zaki' + sweet potatoes at 80:20%.

KAI- 'Kunun-zaki' + Paddy rice + Sweet potatoes at 80:20%

4.9. Sodium Chloride Tolerance of Lactic Acid Bacteria

Sodium chloride tolerance are represented in Table 4.9. All the strains were not able to tolerate 10% osmotic concentrations of NaCl except control A and *Lactobacillus plantarum*. In the sodium chloride test, all of the tested isolates exhibited good growth and visible growth in 1% and 2% but only *Lacobacillus bulgaricus*, showed resistance. The control B showed no growth in the test while in control A, good growth was observed (Table 4.9).

4.10. Sensitivity to pH and Temperature Test

The sensitivity to pH and temperature are shown in Table 4.10. *Lactobacillus acidophilus* showed no tolerance at pH 2 and pH 3. All of the strains consistently showed tolerance at pH 2-6 after 2 and 4 hours of incubation except *Lactobacillus fermentarum* and *Lactobacillus lactic* which showed no growth at pH2. All the strains grew at 15 and 45^0C at 2 h and 4 h of incubation except *Lactobacillus delbruekii* and *Lactobacillus helveticus* which showed resistance at 15^0C (Table 4.10).

Table 4.9: Evaluation of Sodium Chloride Tolerance of the Isolates

LAB	2 hours				4 hours				24 hours	
	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
A	|++	+	+	+	+	+	+	+	+	+
B	-	-	-	-	-	-	-	-	-	-
L1	|++	+	+	+	+	+	+	+	+	-
L2	++	+	+	+	+	+	+	+	+	-
L3	++	+	+	+	+	+	+	+	+	-
L4	++	+	+	+	+	+	+	+	+	+
L5	++	+	+	+	+	+	+	+	+	-
L6	++	+	+	+	+	+	+	+	+	-
L7	++	+	+	+	+	+	+	+	+	-
L8	-	-	+	+	+	+	+	+	+	-

Keyword:

++ = Good growth,

+ = visible growth,

- = no growth.

A- Positive control (Tubes with lactic acid bacteria cultures without NaCl)

B- Negative Control (Tubes with NaCl but without cultures)

LAB- Lactic Acid Bacteria.

Table 4.10: Tolerance of Lactic Acid Bacteria to pH and Temperature.

Probable	pH2		pH 3		pH4		pH5		pH6		15^0C	45^0C
Organisms	2hours	4hours	2hours	4hours	2hours	4hours	2hours	4hours	2hours	4hours		
Lactobacillus acidophilus	-	-	-	-	+	+	+	+	+	+	+	+
Lactobacillus casei	+	+	+	+	+	+	+	+	+	+	+	+
Lactobacillus plantarum	+	+	+	+	+	+	+	+	+	+	+	+
Lactobacillus fermentum	-	-	+	+	+	+	+	+	+	+	+	+
Lactobacillus delbruekii	+	+	+	+	+	+	+	+	+	+	-	+
Lactobacillus lactis	-	-	+	+	+	+	+	+	+	+	+	+
Lactobacillus helveticus	+	+	+	+	+	+	+	+	+	+	-	+
Lactobacillus bulgaricus	+	+	+	+	+	+	+	+	+	+	+	+

Keywords:

+ = Growth present.

- = Growth absent**.**

4.11. Antibiotics Sensitivity Test Against Pathogenic Organisms

The sensitivity of pathogenic organisms to different antibiotics are shown in Table 4.11. *Staphylococcus haemolyticus* showed high susceptibility (10.55±0.01) toward gentamicin and significantly different (P<0.05) from other antibiotics used. *Pseudomonas aeruginosa* was resistance to ampiclox but sensitive to other antibiotics. There was no significant difference (P>0.05) in the sensitivity of *Staphylococcus haemolyticus* to vancomycin and amoxacillin. Erythromycin and ampiclox had the least zone of inhibition of 5.36±0.05mm and 5.46±0.02mm on *Klebsiella pneumoniae.*

4.12. Antimicrobial Activity of Lactic Acid Bacteria Isolates against Three Selected Pathogens.

The results of probiotics antimicrobial effect on selected pathogenic organisms are shown in Table 4.12. The comparison of the inhibitory zones caused by the eight strains of probiotics (*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus delbruekii, Lactobacillus lactis* and *Lactobacillus helveticus*) shows that the antimicrobial effects of *Lactobacillus plantarum* and *Lactobacillus casei* were significantly higher (P< 0.05) from other strains. *Lactobacillus plantarum* had the highest zone of inhibition against all the pathogenic organisms

Lactobacillus delbruekii exhibited the lowest zones of inhibition on *Pseudomonas aeruginosa* with 5.15±0.02mm (Table 4.12). Antibacterial activity of *Lactobacillus lactis* and *Lactobacillus helveticus* against *Staphylococcus haemolyticus* and *Klebsiella pneumoniae* showed the lowest zones of inhibition of 7.57± 0.02mm and 6.82 ±0.03mm respectively.

Table 4.11: Zones of Inhibition (in mm) by Antibiotics Against the Pathogenic Organisms.

ISOLATE	ANTIBIOTICS/ ZONES OF INHIBITION (mm)									
	E	CN	APX	VAN	AM	CPX	S	SXT	AU	CEP
Pseudomonas aeruginosa	7.14±0.04^{g}	9.65±0.04^{b}	NI	5.26±0.03^{j}	8.50±0.03^{e}	7.57±0.02^{f}	8.67±0.04^{d}	8.76 ±0.01^{c}	8.92 ±0.01^{b}	6.83± 0.03^{h}
Staphylococcus haemolyticus	7.24±0.03^{d}	10.55±0.01^{a}	6.20±0.01^{f}	5.37±0.06^{g}	5.34±0.08^{g}	6.31±0.03^{f}	7.20±0.03^{d}	8.54±0.02^{b}	6.59±0.04^{e}	8.35±0.07^{c}
Klebsiella pneumoniae	5.36±0.05^{f}	8.39±0.02^{b}	5.46±0.02^{f}	6.34±0.03^{e}	7.39±0.03^{d}	8.44±0.05^{b}	7.66±0.07^{c}	8.33±0.06^{b}	9.06±0.07^{a}	7.43±0.07^{d}

Each value is a mean of three replicates with standard deviation.
Values in rows with different superscript letters are significantly different ($P < 0.05$).

Keywords:

NI- No inhibition.

E – Erythromycin (10µg).

CN - Gentamycin (10µg).

APX - Ampiclox (30µg).

VAN - Vancomycin (30µg).

AM- Amoxacillin (30µg).

CPX- Ciprofloxacin (10µg).

S- Streptomycin (30µg).

SXT- Septrin (30µg),AU- Augmentin (30µg), CEP- Cephalexin (30µg).

Table 4.12: Zones of Inhibition (in mm) by Lactic Acid Bacteria against the Pathogenic Organisms

Organisms	*Lactobacillus acidophilus*	*Lactobacillus casei*	*Lactobacillus brevis*	*Lactobacillus plantarum*	*Lactobacillus fermentum*	*Lactobacillus delbruekii*	*Lactobacillus lactis*	*Lactobacillus helveticus*
Pseudomonas aeruginosa	9.71 ±0.55^{c}	13.57±0.02^{b}	8.69± 0.04^{e}	13.86± 0.03^{a}	9.46 ±0.04^{d}	5.15 ±0.02^{h}	8.43± 0.05^{f}	7.63± 0.07^{g}
Staphylococcus haemolyticus	9.42± 0.06^{c}	14.03± 0.03^{b}	8.46± 0.06^{e}	14.25± 0.03^{a}	8.14 ±0.01^{f}	9.23 ±0.01^{d}	7.57± 0.02^{g}	7.19± 0.02^{h}
Klebsiella pneumoniae	8.46± 0.02^{d}	11.83± 0.02^{b}	8.80± 0.04^{c}	13.19± 0.03^{a}	7.25± 0.04^{e}	7.04± 0.06^{f}	6.82 ±0.03^{g}	6.98± 0.10^{f}

Each value is a mean of three replicates with standard deviation.

Values in the row with different superscript letters are significantly different ($P < 0.05$).

4.13. Genotypic Identification of the Isolated Pathogenic Organisms and *Lactobacillus* strains

The isolated pathogenic bacteria and the *Lactobacillus* strains that had the highest antimicrobial were submitted to further biochemical characterization using molecular identification. The pathogenic organisms isolated were identified as *Pseudomonas aeruginosa, Staphylococcus haemolyticus and Klebsiella pneumoniae* (Appendix II, III and IV). The DNA from the isolated strains were used as a template. The Polyacrylamide gel electrophoretic image of the *Lactobacillus* strains are shown in Plate 1. Genotypic methods of identification of pathogenic bacteria and the *Lactobacillus* strains with an alignment of 16S rDNA nucleotide sequences in the gene bank database are shown in appendix vi. The method described in this work allows the amplification of specific PCR products. This enables direct sequencing of unknown regions without the need for DNA cloning but makes use of analysis of microbial genetic elements.

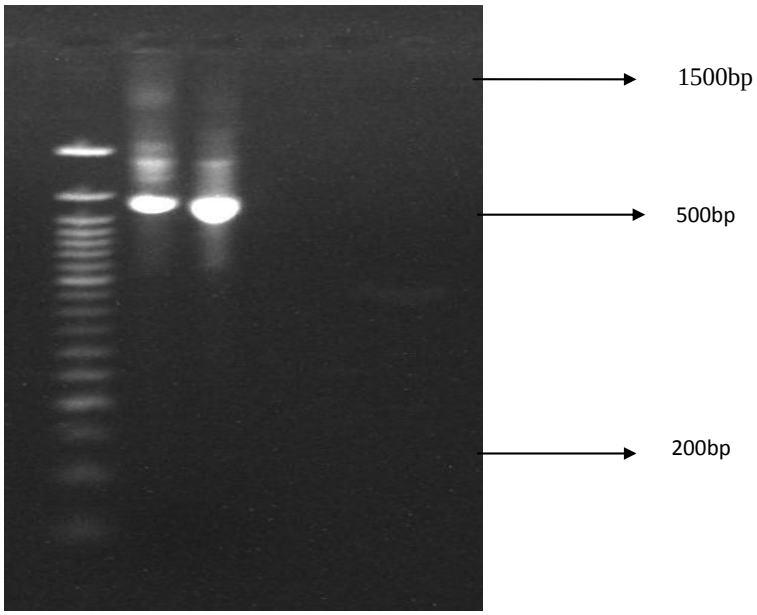


Plate 1: Restriction patterns obtained after digestion with 5 units Alu I for amplified 16S rDNA of *Lactobacillus* species after running in 2% agarose gel. 100 bp ladder was used as a standard size marker.

4.14. Body Weight of the Rats.

The body weight of the animals in day 1 ranged from 204 to 218g (Figure 4.2). There was no significant difference (P>0.05) in the weights of the rats infected with *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in all the groups in day 3 (Figure 4.3). The body weight of rat infected with *Staphylococcus haemolyticus* in group E on day 3 increased to 211g. On day 7, the body weight of rat was moderately increased in all the infected rats in group A (control) and treated rat groups but in group B, *Pseudomonas aeruginosa*- infected rat decreased to 202g and *Klebsiella pneumoniae*- infected rat decreased to 214g (Figure 4.4). On day 15, all the infected rats in group D had the highest body weight ranging from 218g to 227g while group B rats had the lowest body weights (Figure 4.5) There was no significant difference (P>0.05) in the body weights of rats infected with *Pseudomonas aeruginosa* and *Staphylococcus haemolyticus* in the control group and group D in day 15 (Figure 4.5).

4.15. Blood Glucose Level

The measurement of blood glucose level in experimental animals are presented in Figure 4.6. On day 1, all the groups had normal blood glucose level ranging from 80 mg/dL to 94 mg/dL. The fasting blood glucose level in the rats infected with *Staphylococcus haemolyticus* in control C had the highest glucose level of 94 mg/dL compared to other groups. There was no significant difference (P>0.05) in the fasting blood glucose level of all the groups in day 3 except group A (control). Fasting blood glucose level of untreated diabetic rats in group B was significantly higher than other groups in day 7 and it ranged from 219 mg/dL to 224 mg/dL (Figure 4.6). There was a significant decrease in blood glucose level in rats after treatments with

Lactobacillus plantarum and *Lactobacillus casei* in group C and gentamicin in group E but still in the category of diabetic rats.

The treatments showed significant antihyperglycemic activity by bringing down the blood glucose level on day 15 in diabetic rats. No hypoglycemic effect was observed in normal tested rats (Figure 4.6). The blood glucose level of *Pseudomonas aeruginosa* and *Staphylococcus haemolyticus* infected rats in group C and group E ranged from 210 mg/dL to 214 mg/dL.

4.16. Establishment of Infection in Rats

The number of colony forming unit recovered from the wounds after application of *Pseudomonas aeruginosa, Staphylococcus haemolyticus and Klebsiella pneumoniae* are shown in Figure 4.7. There was significant difference ($P<0.05$) in the numbers of colony forming unit of each organism in group A. *Pseudomonas aeruginosa* had the least count of 6.55±0.12 CFU/ml in group B. The bacterial colony count of all infected rats in group C were significantly different ($P<0.05$) and the count ranged from 6.93±0.05 CFU/ml to 7.26±0.02 CFU/ml. The highest colony count of 6.50±0.05 CFU/ml was observed in *Staphylococcus haemolyticus*- infected rats in group E.

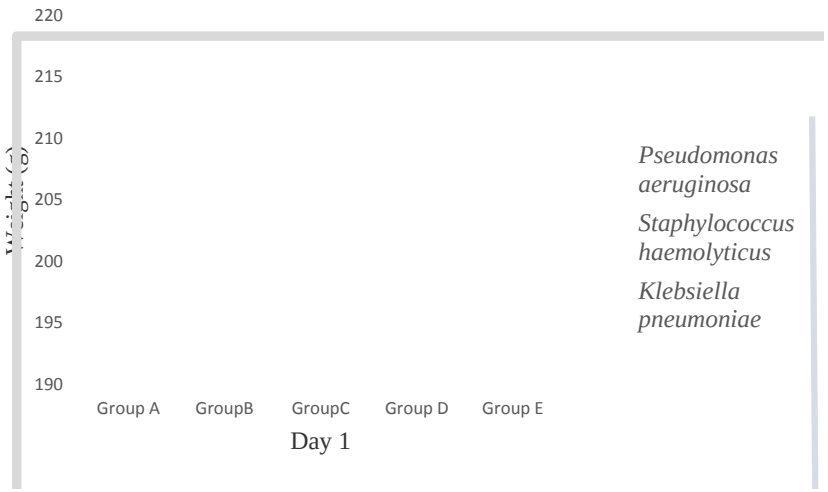


Figure 4.2: Body Weight of the Rats in Day 1

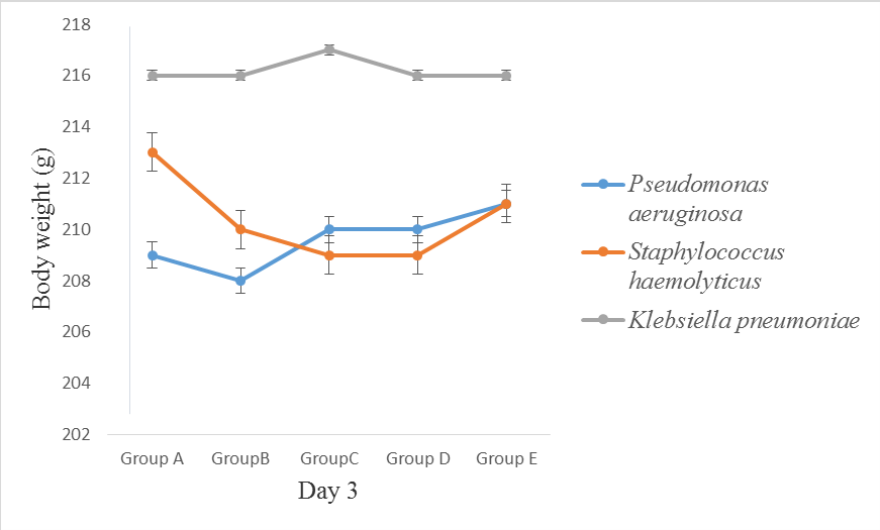


Figure 4.3: Body Weight of Rats in Day 3.

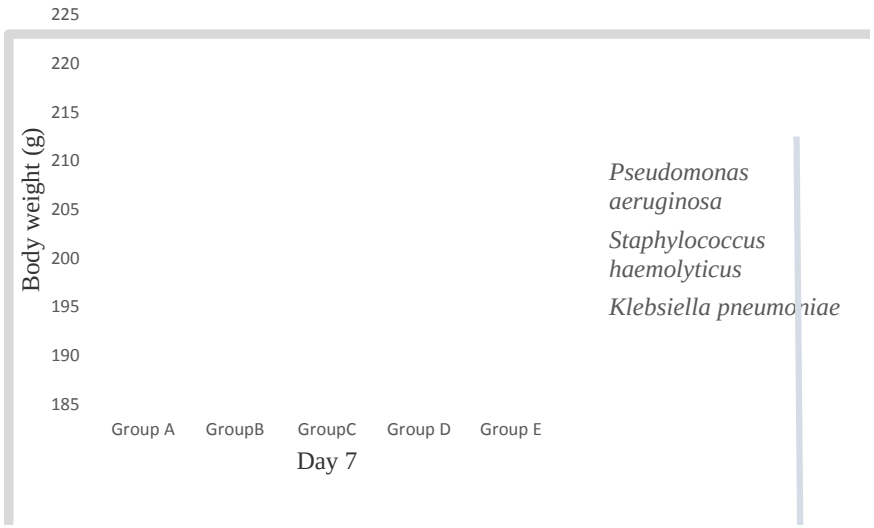


Figure 4.4: Body Weight of Rats in Day 7.

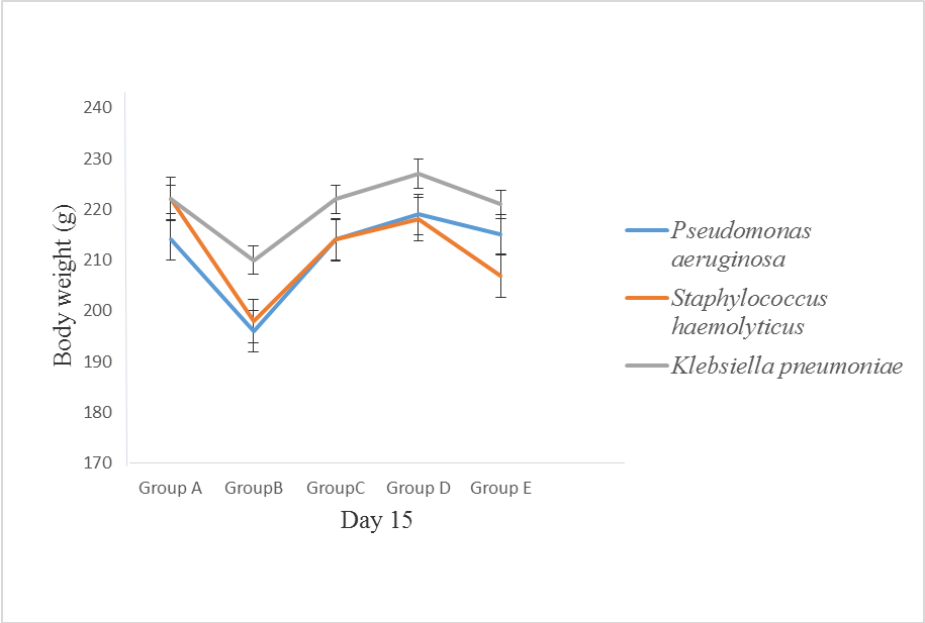


Figure 4.5: Body Weight of Rats in Day 15.

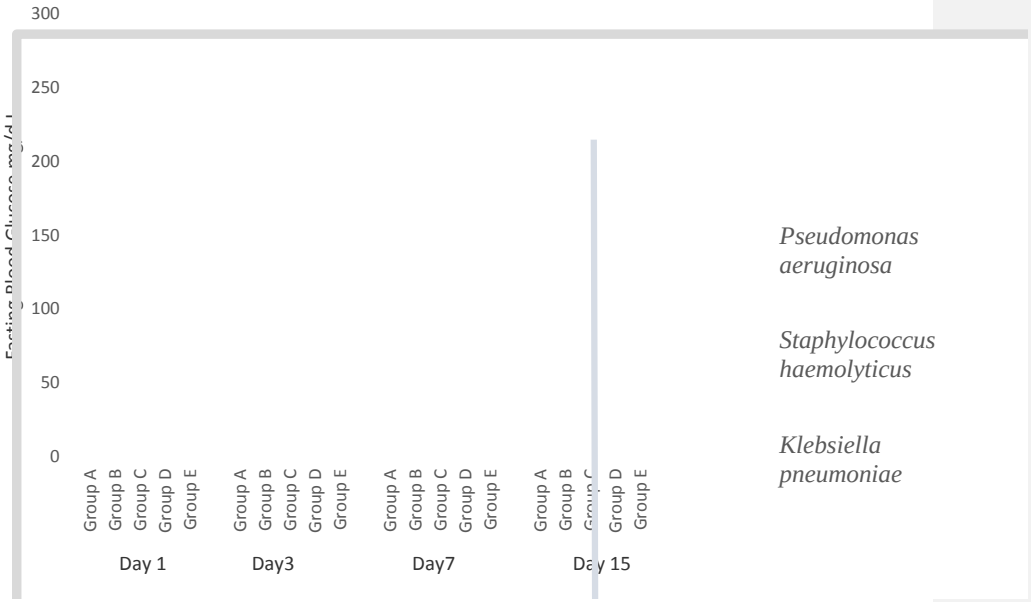


Figure 4.6: Blood Glucose Measurement

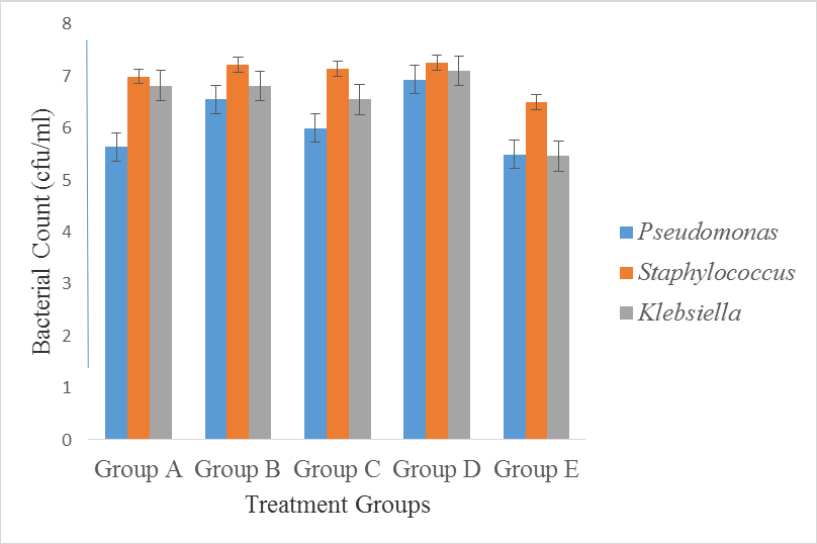


Figure 4.7: Bacterial Colony Counts from Swab Culture of Wounds

4.17. Behavioural Responses of the Rats

All the rats in group A were generally healthy throughout the experiment and showed no abnormality. The animals treated with streptozotocin in groups B, C, D and E appeared ill-looking. There was a decrease in food intake by diabetic control group because of injurious effects of streptozotocin. Some of the rats displayed signs of deeper infections with pus (Plate 2).

4.18. Percentage Wound Healing

The percentage wound healing on day 1 is shown in Figure 4.8. There was a significant difference ($P<0.05$) in wound area of rat infected with *Pseudomonas aeruginosa* in the normal control group and treated group on day 3 (Figure 4.9). During the first 3 days after infection, the wound area increased to 110.6% of the original size in the *Pseudomonas aeruginosa* infected rat in group B and 102.3% of the original size in the *Staphylococcus haemolyticus* infected rats. The wound healing on day 3 of treatment was not significant in either the control or experimental groups. Thereafter, there was a progressed increase in ulcer healing on day 7 in normal control group and animals treated with *Lactobacillus plantarum* (Figure 4.10). The rats infected with *Klebsiella pneumoniae* had 66.7% wound healing and is not significantly different ($P<0.05$) from *Pseudomonas aeruginosa*-infected rat in control group. On day 15, all the wounds were completely healed in the normal control group (Plate 3) and the rats infected with *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in group D and group E (Plate 4). Throughout the experiment, the percentage wound healing in the diabetic control group was significantly lower than those in the treated group. Wounds of infected rats (*Staphylococcus haemolyticus* and *Klebsiella pneumoniae*) in group C were not significantly different ($P>0.05$) (Figure 4.11).

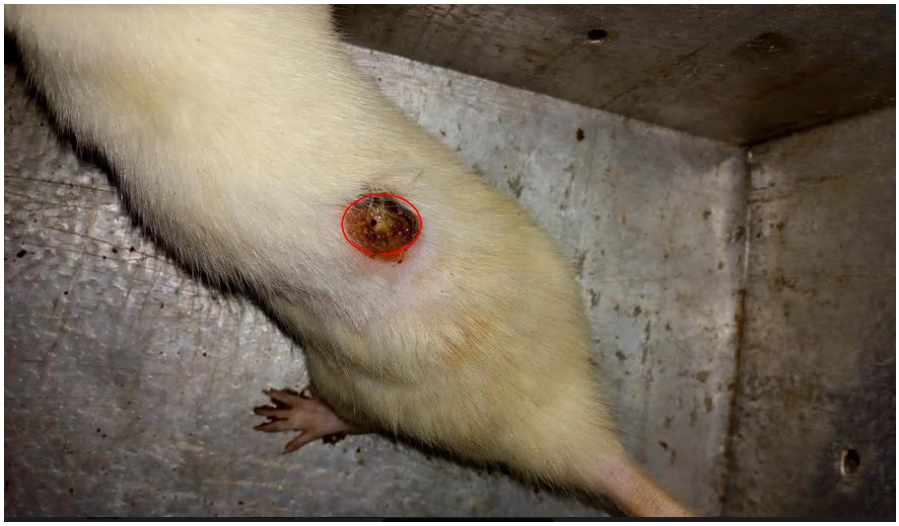


Plate 2: Image of the wound of diabetic control group infected with *Pseudomonas aeruginosa* showing pus.

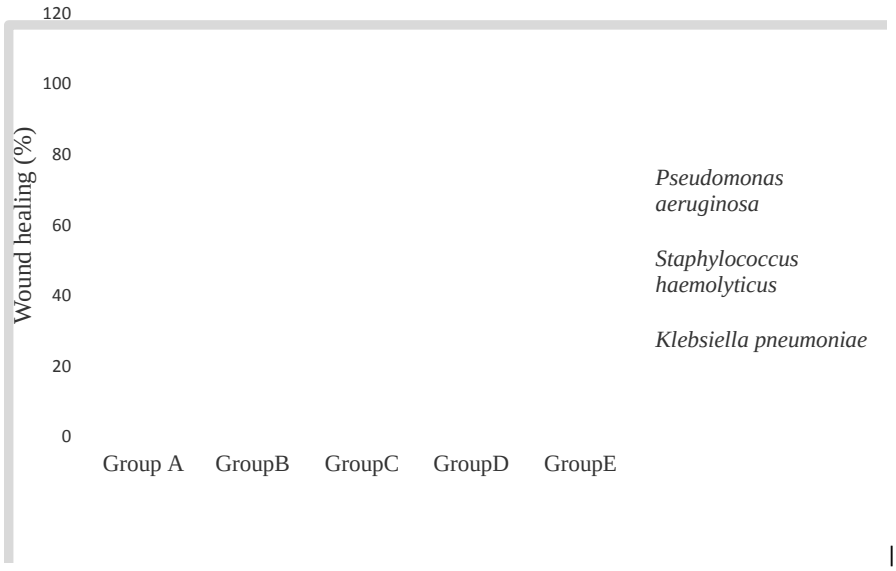


Figure 4.8: Percentage Wound Healing of the Rats on Day 1

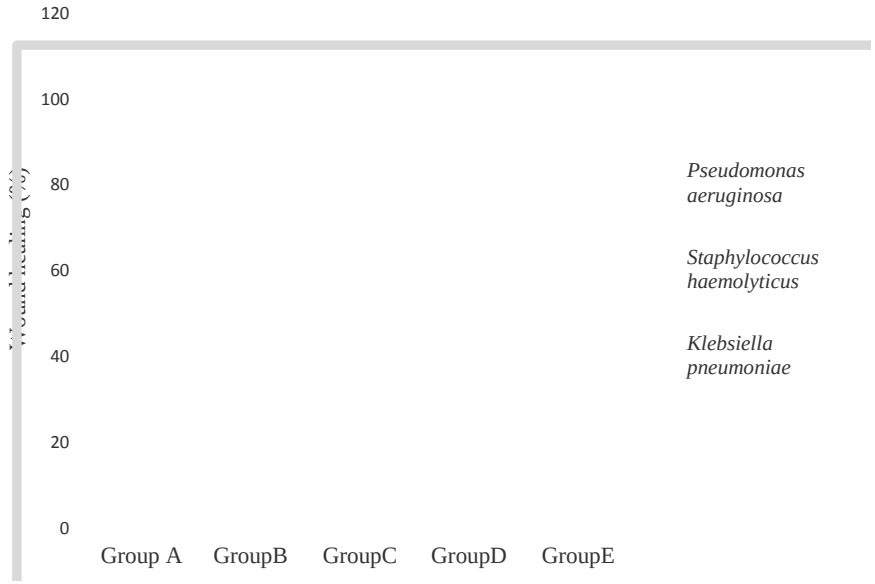


Figure 4.9: Percentage Wound Healing of the Rats on Day 3

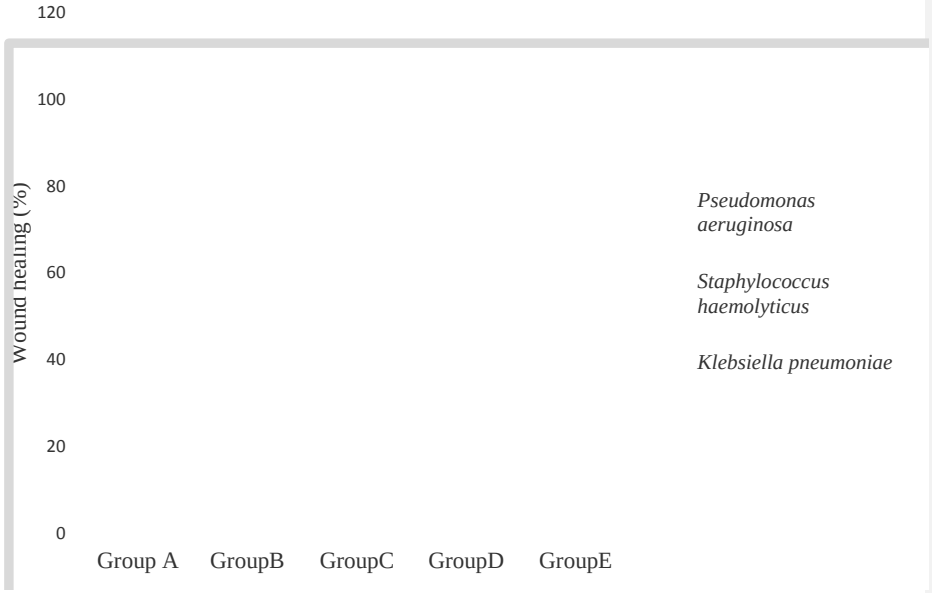


Figure 4.10: Percentage Wound Healing of the Rats on Day 7

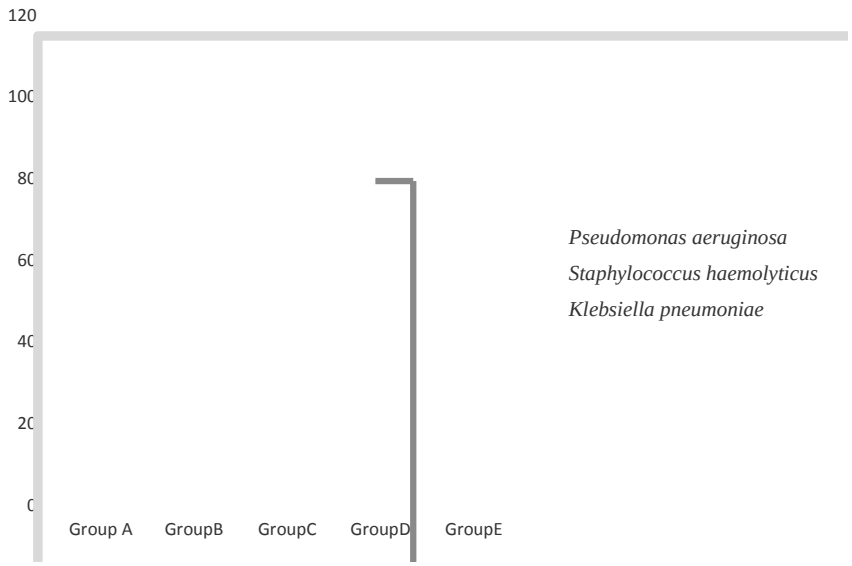
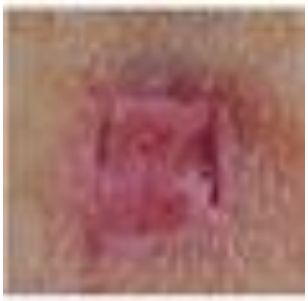


Figure 4.11: Percentage Wound Healing of the Rats on Day 15



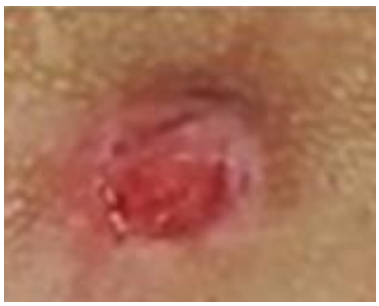
Day 1

Day 3 post wound

Day 7 post wound

Day 15 post wound

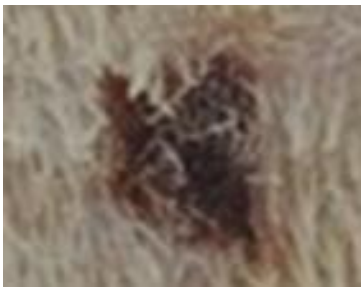
Plate 3: Image of the Post Wound of Normal Control Rat



Day 1

Day 3 post wound

Day 7 post wound



Day 15 post wound

Plate 4: Image of the Post Wound of Diabetic Group Treated with *Lactobacillus plantarum*

4.19. Haematology Report

4.19.1. Total Red Blood Cell Count

The total red blood cell count of the rats for each treatment day are shown in Figures 4.12 to 4.15. Red blood cell count of normal control rats, ranged from 7.04µl to 7.38µl in day 1 (Figure 4.12). The red blood cell count of *Staphylococcus haemolyticus* infected rats in group D and group E were not significant (P>0.05) on day 1. There was a slight increase in red blood cell count of all groups except the diabetic control group on day 3 (Figure 4.13). The treatment group with *Lactobacillus casei* had the highest red blood cell count of 7.21µl in *Pseudomonas aeruginosa* infected rat and 7.27µl in *Klebsiella pneumoniae*-infected rats on day 7 (Figure 4.14) and significantly different (P<0.05) from other treatment groups except for normal control group. The diabetic control rats showed a significant decrease (P<0.05) as compared to normal control rats on day 15. Treatment with *Lactobacillus casei*, *Lactobacillus plantarum* and gentamicin produced a highly significant increase in the lowered blood haemoglobin content of all the infected diabetic rats (Figure 4.15).

The haemoglobin content of all infected animals ranged from 11.70 g/dL to 12.35 g/dL in day 1 (Figure 4.16). The recorded values for haemoglobin in animals infected with *Klebsiella pneumoniae* in group B on day 3 was 12.73g/dL and it was significantly different (P<0.05) from other groups on day 3 (Figure 4.17). The treatment group with *Lactobacillus plantarum* had the highest haemoglobin of 12.33g/dL in *Pseudomonas aeruginosa* infected rat as compared with the normal control rat on day 7 (Figure 4.18). The diabetic rats infected with *Staphylococcus haemolyticus* in group B on day 15 had the lowest haemoglobin value of 11.33g/dL compared with the normal control rats. The normal control rats on day 15 had the highest haemoglobin value of 12.65g/dL and are significantly higher (P< 0.05) from other groups (Figure 4.19).

Comment [O4]:

The packed cell volume of the rats on day 1 showed that the group A(control) had the highest packed cell volume of 36.53% while *Pseudomonas aeruginosa* infected rats in group B had the lowest packed cell volume of 35.08% and it was significantly different ($P< 0.05$) from others (Figure 4.20). There was a gradual decrease in packed cell volume of all the groups in day 3 except normal control group (Figure 4.21). The packed cell volume of *Staphylococcus haemolyticus* and *Klebsiella pneumoniae* infected rats treated with *Lactobacillus casei* was not significantly different ($P>0.05$). The normal control had the highest packed cell volume of 36.71% and was significantly higher from the diabetic control group and the treated group on day 7 (Figure 4.22). The *Pseudomonas aeruginosa* infected rats in group B had the lowest packed cell volume of 32.4% on day 15. The treated group (D and E) exhibited no significant difference in packed cell volume of rats infected with *Pseudomoans aeruginosa* (Figure 4.23).

The mean corpuscular volume (MCV) of the animals showed that the animal infected with *Pseudomonas aeruginosa* in the diabetic control group had the highest volume of 71.4fl on day 1 and is significantly different ($P<0.05$) from *Pseudomonas aeruginosa* infected rats in other groups (Figure 4.24). There was a significant decrease in the levels of mean cell volume of all the infected rats in group B on day 3 (Figure 4.25). The *Lactobacillus casei* treatment group had the highest mean volume of 71.51fl in *Klebsiella pneumoniae* infected rats on day 7 (Figure 4.26). There was no significant difference ($P<0.05$) in the mean cell volume of the animal infected with *Staphylococcus aureus* in group D and E on day 15 (Figure 4.27).

The values counted for mean cell haemoglobin ranged from 17.46pg to 24.48pg. The *Klebsiella pneumoniae* infected rats in group D had the highest mean cell haemoglobin of 21.63pg on day 1 (Figure 4.28). There was no significant difference ($P<0.05$) in the mean cell

haemoglobin count of *Pseudomonas aeruginosa* infected rat in group A, C, D and E in day 3 (Figure 4.29). On day 7, the normal control group had the highest count of 23.37pg while the rats infected with *Pseudomonas aeruginosa* in diabetic control group had the lowest count of 17.46pg (Figure 4.30). There was no significant difference ($P>0.05$) in the mean cell haemoglobin count of rats infected with *Staphylococcus haemolyticus* in all the groups on day 15 except group B (Figure 4.31).

The mean corpuscular haemoglobin concentration (MCHC) are presented in Figure 4.32 to Figure 4.35. The diabetic rat infected with *Pseudomonas aeruginosa* in group D and E, had the highest value of 34.73% on day 1 (Figure 4.32). There was a slight increase in the mean cell haemoglobin concentration of all the rats in each group except group B in day 3 (Figure 4.33). On day 7, treatment group E had the highest haemoglobin concentration of 35.72% in *Pseudomonas aeruginosa* infected rat and is significantly different from others (Figure 4.34). There was no significant difference ($P<0.05$) in mean cell haemoglobin concentration of rats infected with *Klebsiella pneumoniae* following *Lactobacillus casei* and *Lactobacillus plantarum* administration in group C and D respectively in day 15 (Figure 4.35).

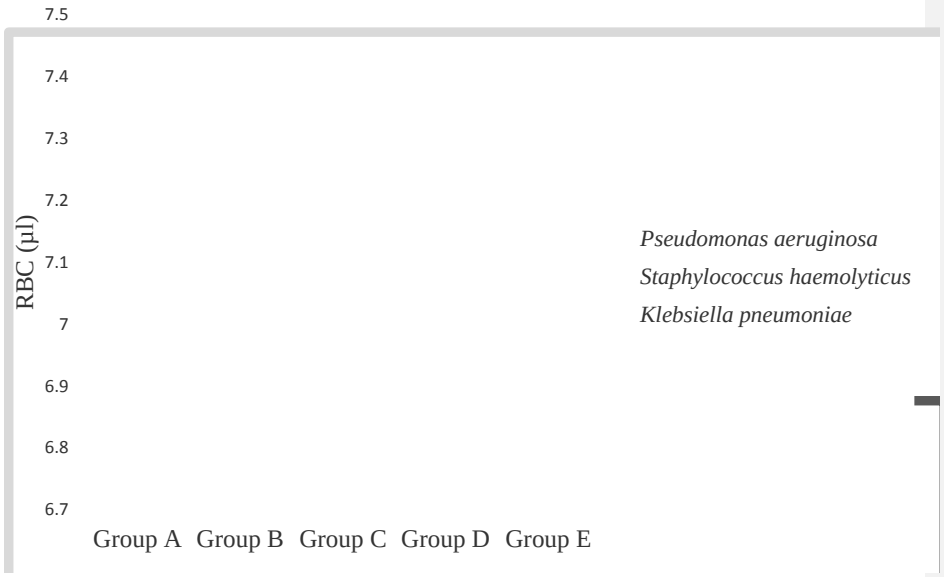


Figure 4.12: The Effect of the *Lactobacillus* on the Red Blood Cell Count on Day 1

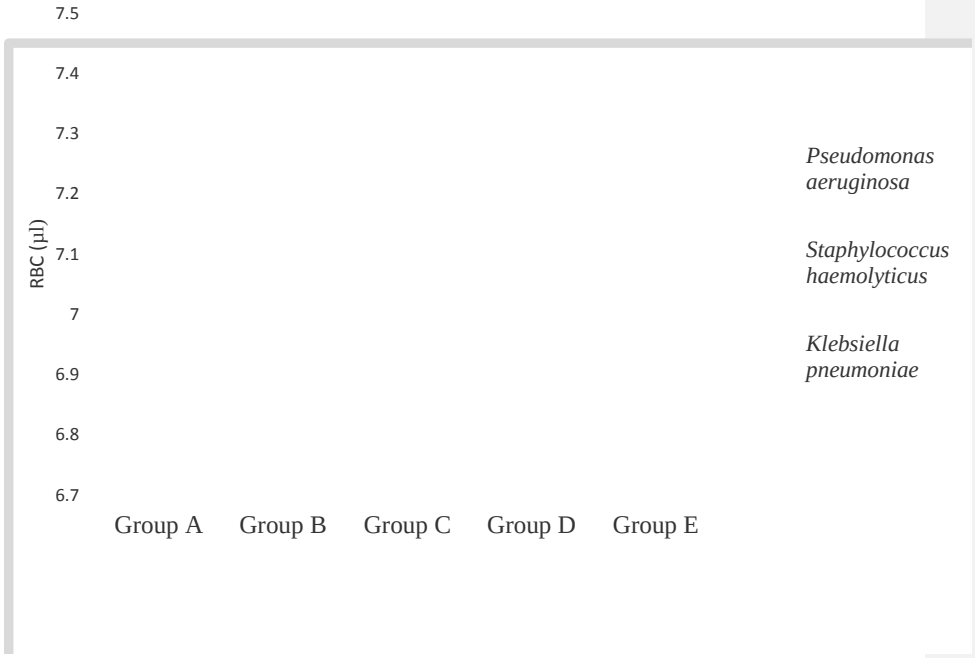


Figure 4.13: The Effect of the *Lactobacillus* on the Red Blood Cell Count on Day 3

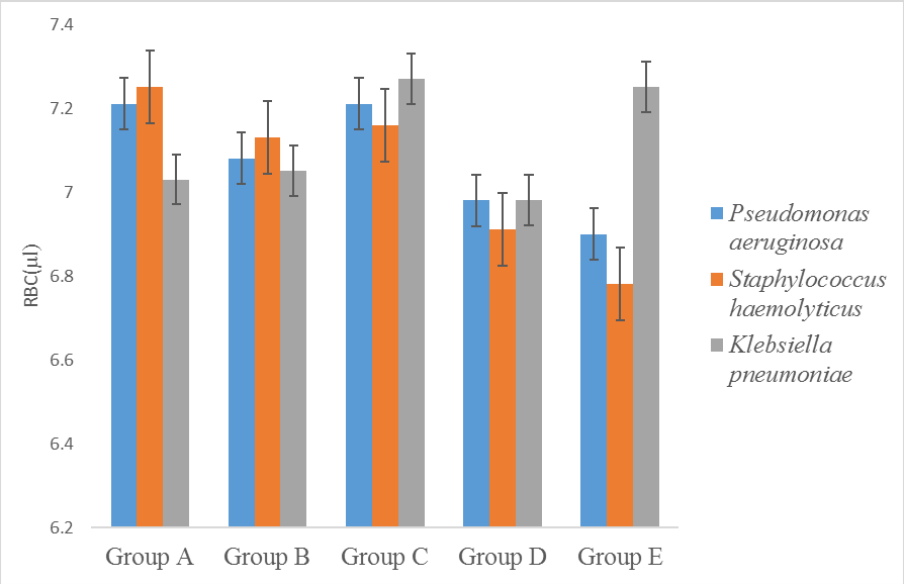


Figure 4.14: The Effect of the *Lactobacillus* on the Red Blood Cell Count on Day 7

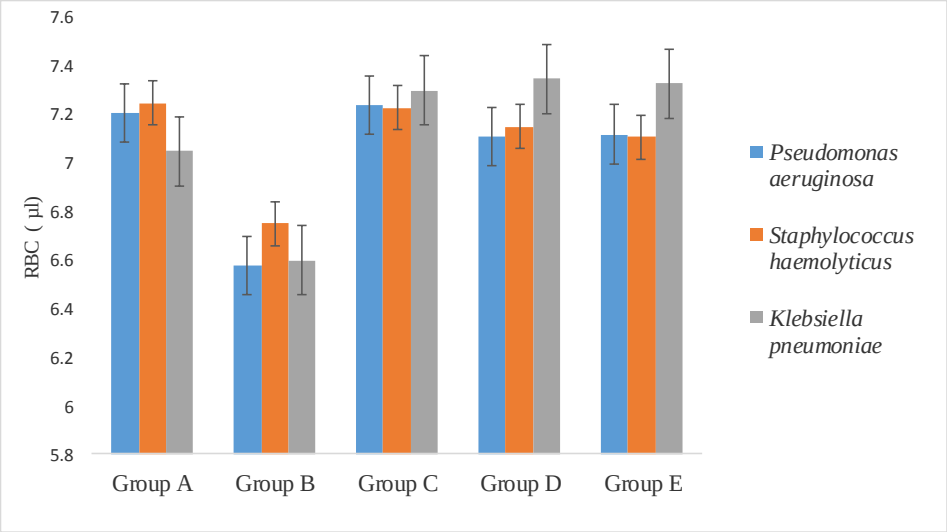


Figure 4.15: The Effect of the *Lactobacillus* on the Red Blood Cell Count on Day 15

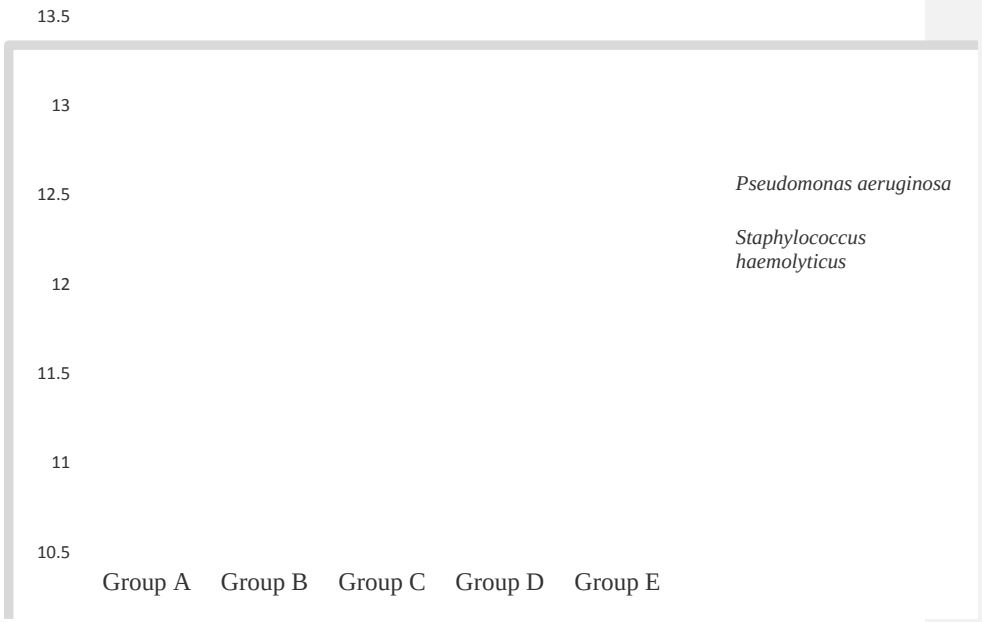


Figure 4.16: The Effect of the *Lactobacillus* on the Haemoglobin Level on Day 1

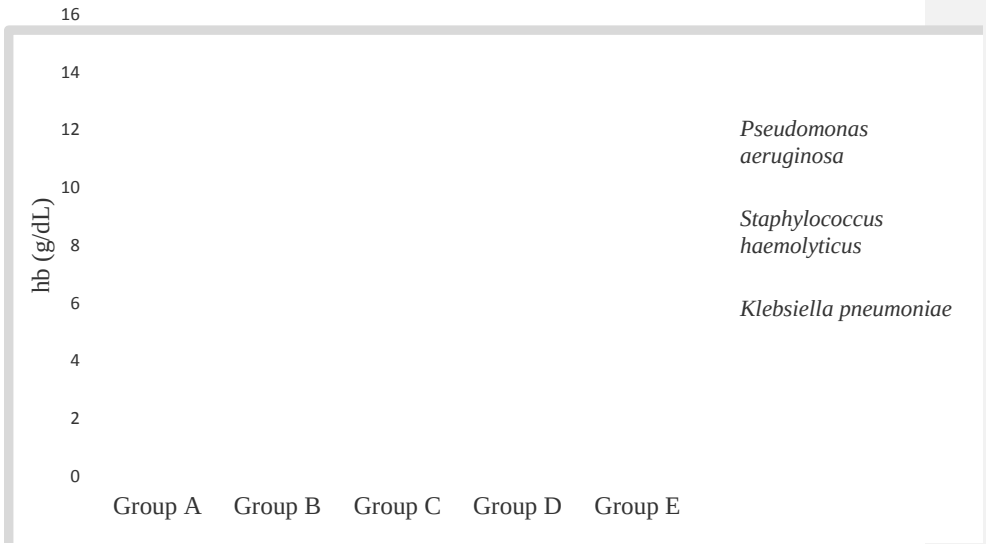


Figure 4.17: The Effect of the *Lactobacillus* on the Haemoglobin Level on Day 3

Figure 4.18: The Effect of the *Lactobacillus* on the Haemoglobin Level on Day 7

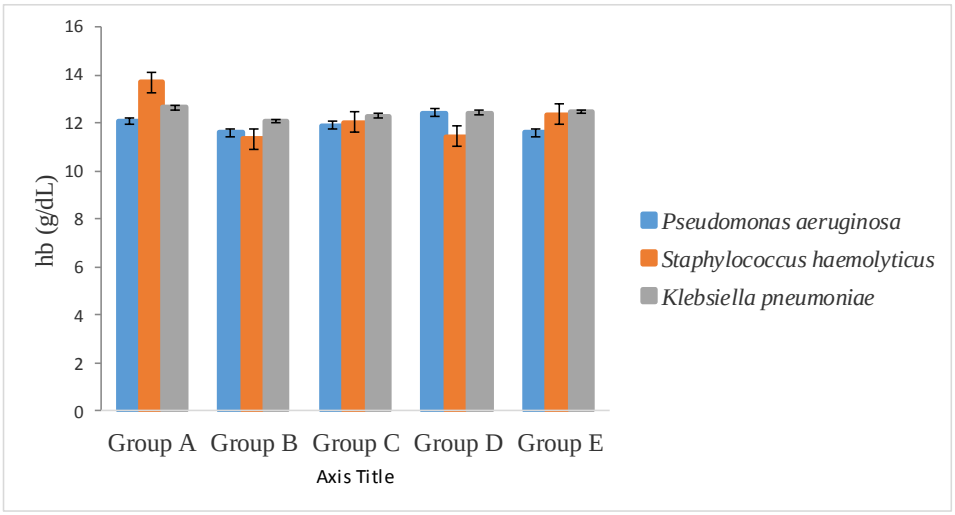


Figure 4.19: The Effect of the *Lactobacillus* on the Haemoglobin Level on Day 15

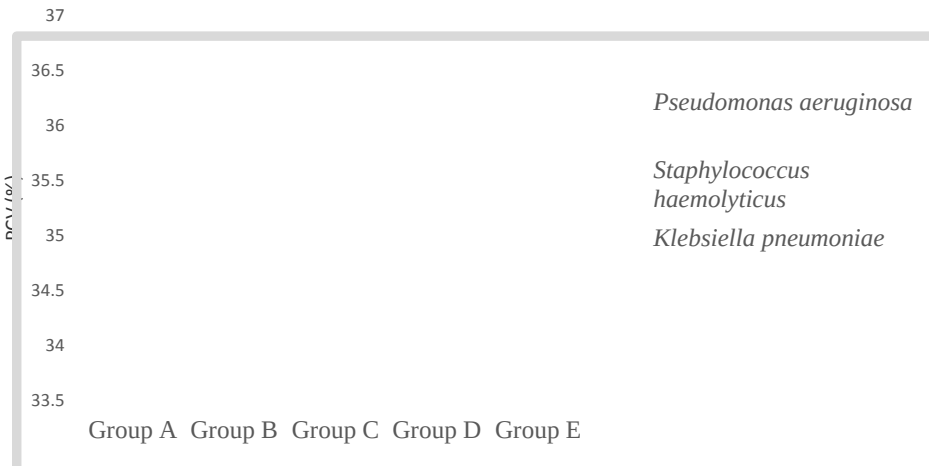


Figure 4.20: The Effect of the *Lactobacillus* on the Packed Cell Volume Level on Day 1

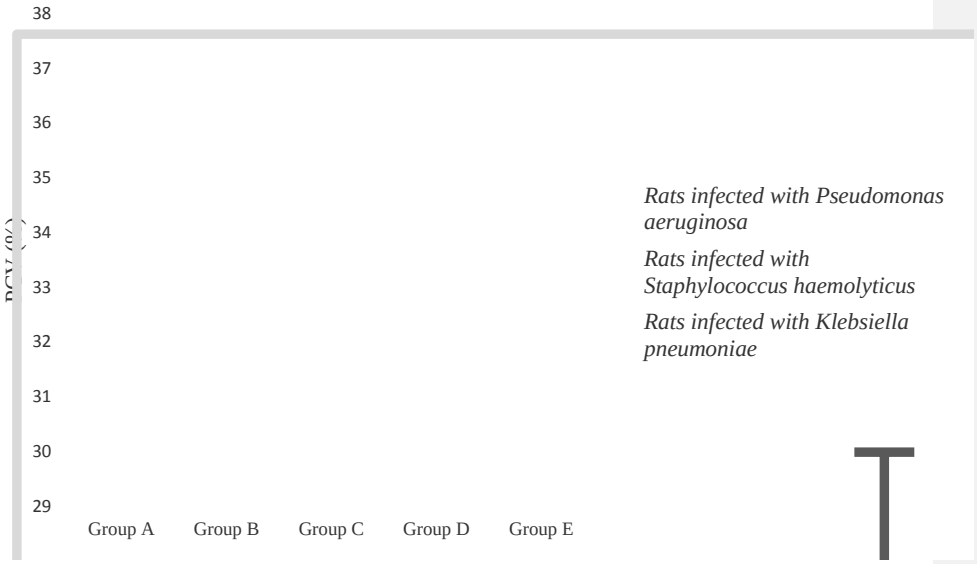


Figure 4.21: The Effect of the *Lactobacillus* on the Packed Cell Volume Level on Day 3

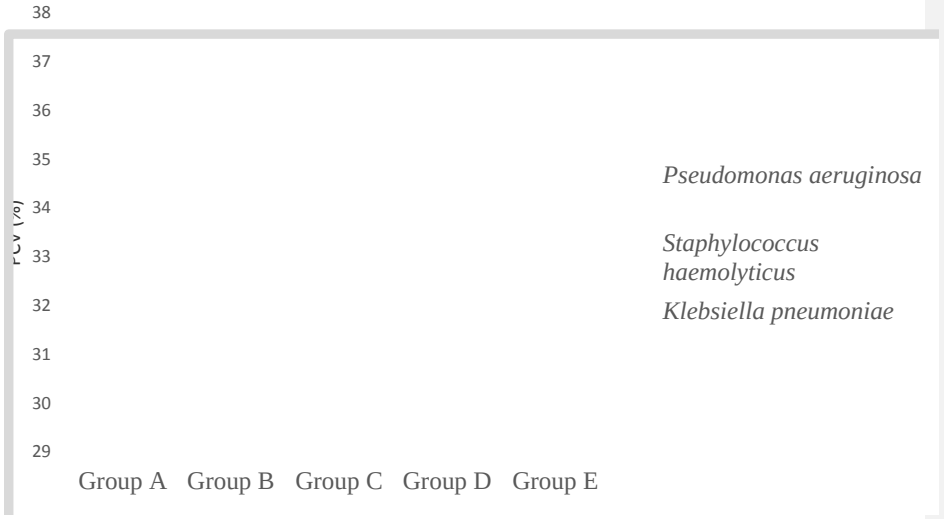


Figure 4.22: The Effect of the *Lactobacillus* on the Packed Cell Volume Level on Day 7

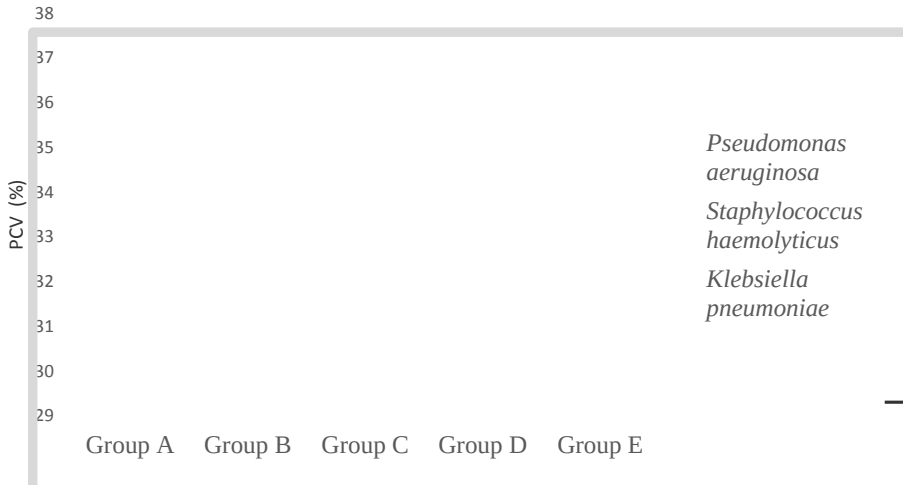


Figure 4.23: The Effect of the *Lactobacillus* Packed Cell Volume Level on Day 15

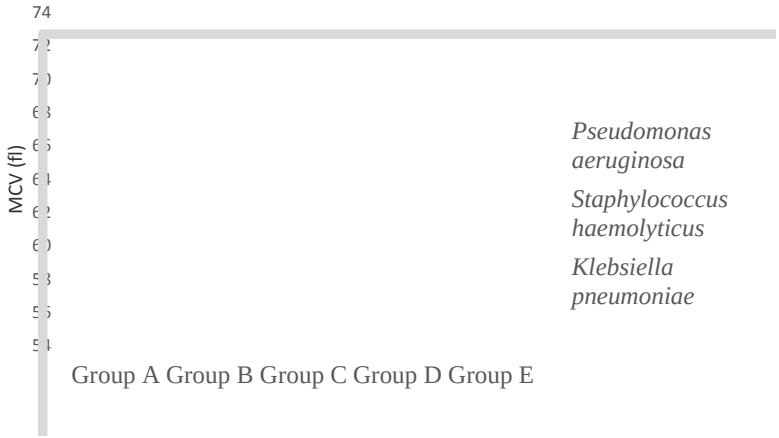


Figure 4.24: The Effect of the *Lactobacillus* on the Mean Cell Volume Level on Day 1

Figure 4.25: The Effect of the *Lactobacillus* on the Mean Cell Volume Level on Day 3

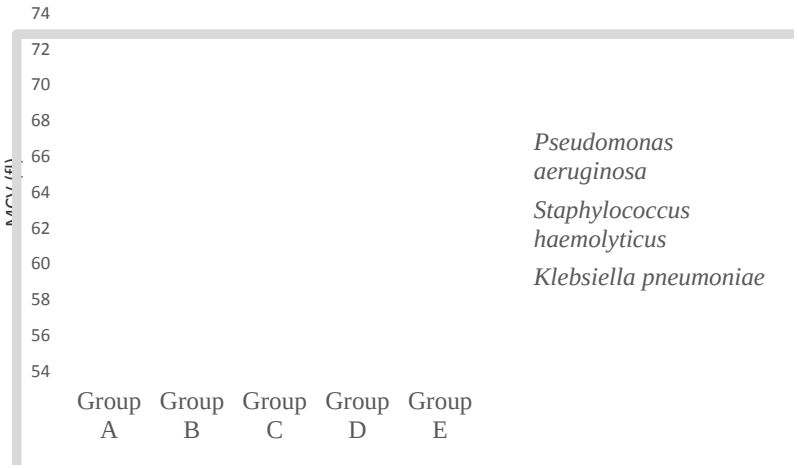


Figure 4.26: The Effect of the *Lactobacillus* on the Mean Cell Volume Level on Day 7

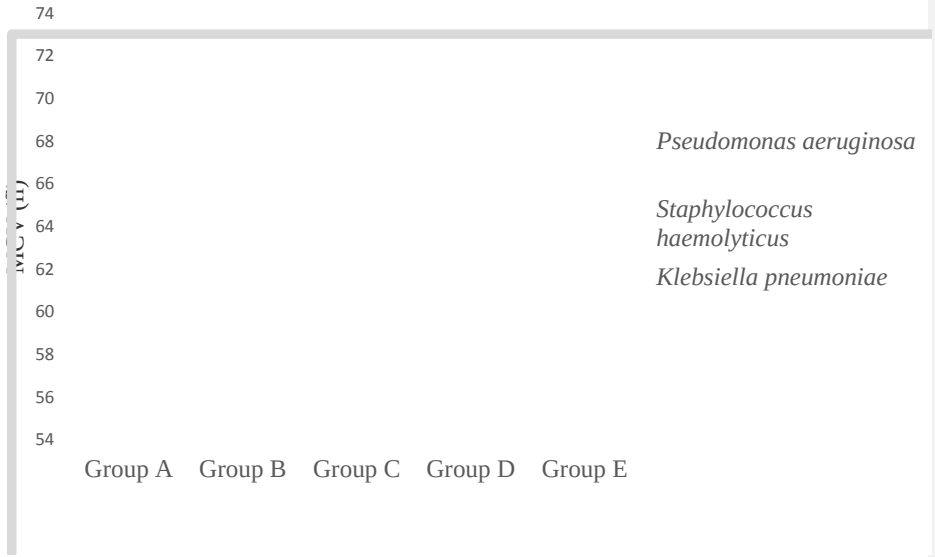


Figure 4.27: The Effect of the *Lactobacillus* on the Mean Cell Volume Level on Day 15

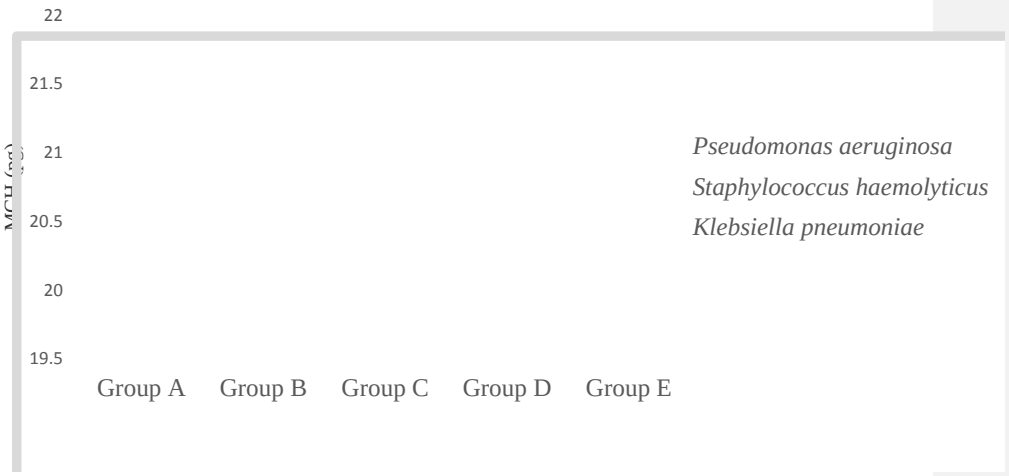


Figure 4.28: The Effect of the *Lactobacillus* on the Mean Cell Haemoglobin Level on Day 1

Figure 4.29: The Effect of the *Lactobacillus* on the Mean Cell Haemoglobin Level on Day 3

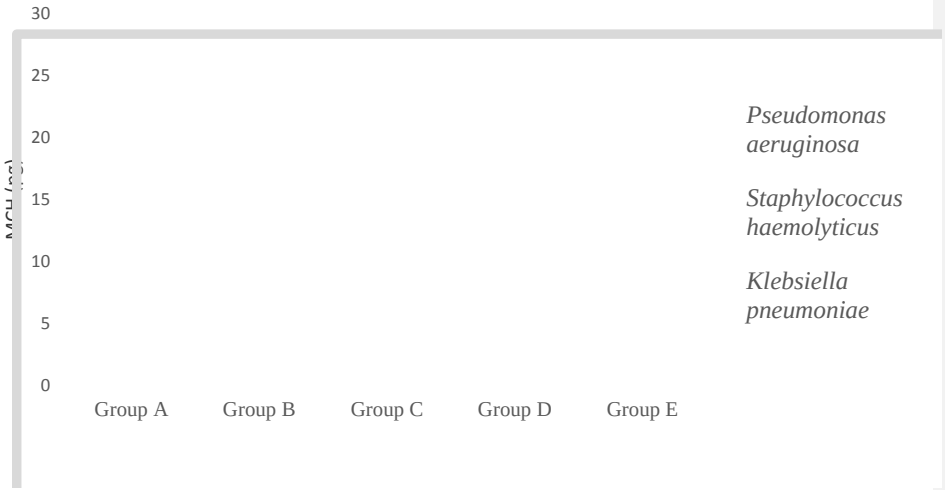


Figure 4.30: The Effect of the *Lactobacillus* on the Mean Cell Haemoglobin Level on Day 7

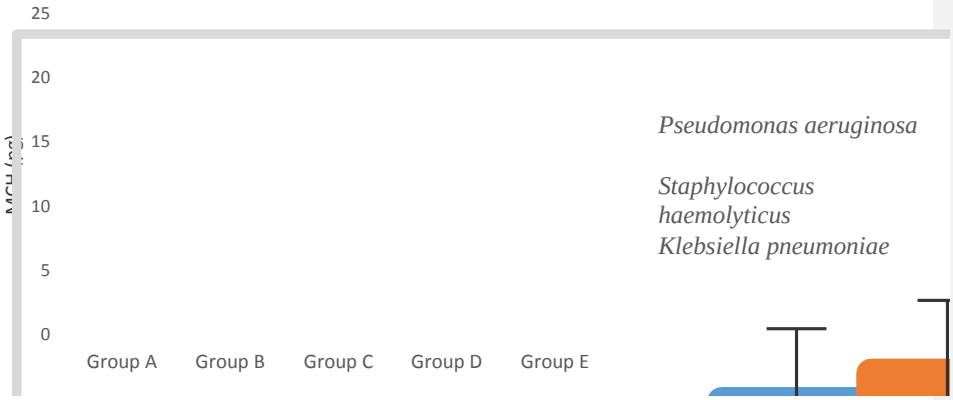


Figure 4.31: The Effect of the *Lactobacillus* on the Mean Cell Haemoglobin Level on Day 15

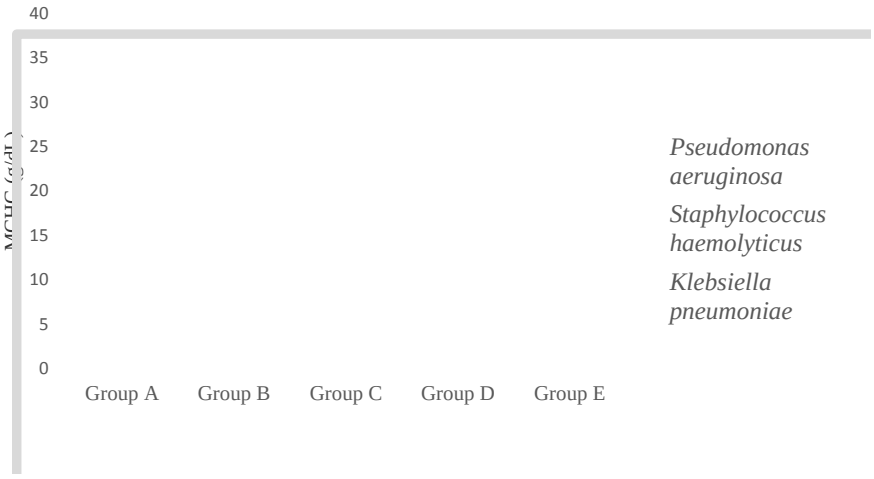


Figure 4.32: The Effect of the *Lactobacillus* on the Mean Corpuscular Haemoglobin Concentration Level on Day 1.

Figure 4.33: The Effect of the *Lactobacillus* on the Mean Corpuscular Haemoglobin Concentration Level on Day 3.

Figure 4.34: The Effect of the *Lactobacillus* on the Mean Corpuscular Haemoglobin Concentration Level on Day 7.

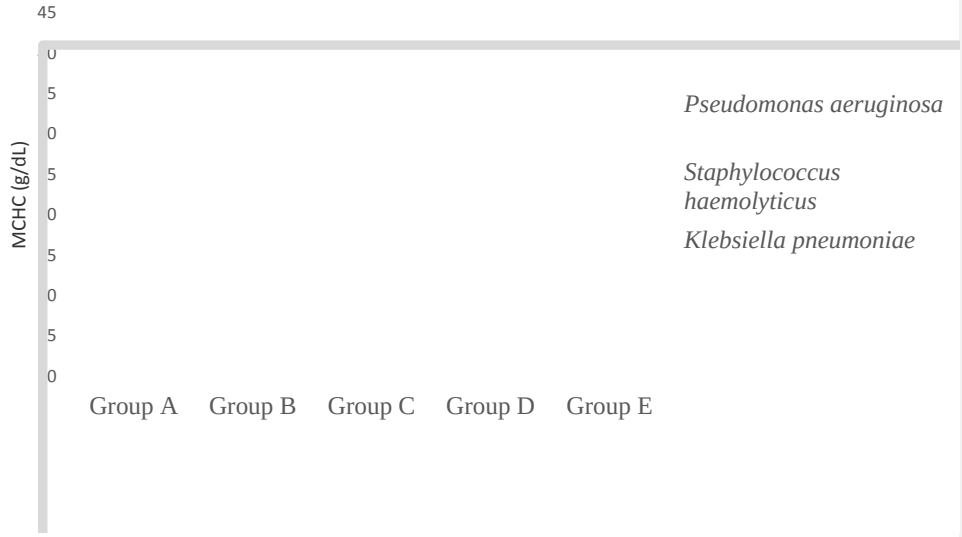


Figure 4.35: The Effect of the *Lactobacillus* on the Mean Corpuscular Haemoglobin Concentration Level on Day 15.

4.19.2. Total White Blood Cell Count

The total white blood cell count is presented in Figure 4.15 to Figure 4.19. White blood cell count in day 1 ranged from 7.31µl to 7.54µl (Figure 4.15). The *Klebsiella pneumoniae* infected rats in group E had the highest white blood cell count of 7.54µl on day 1. The white blood cell count in all the infected rats in diabetic control group had count of 7.50, 7.44 and 7.43µl on day 1 and these increased to 10.68, 11.32 and 10.65µl on day 3. There was no significant difference ($P>0.05$) in the white blood cell count of all the infected rats treated with *Lactobacillus casei* on day 7. The normal control had the lowest count of 7.45µl and is significantly different ($P< 0.05$) from all other groups. On day 15, the diabetic rat infected with *Staphylococcus haemolyticus* in group B had the highest white blood count of 11.21µl (Figure 4.15).

The percentage of neutrophil in rats infected with *Staphylococcus haemolyticus* in group E was 50% and this was significantly different ($P< 0.05$) from the normal control in day 1 (Figure 4.16). There was a significant increase in neutrophil count in all the infected rats in diabetic control group on day 3. Treatment group (*Lactobacillus casei, Lactobacillus plantarum* and gentamicin) showed non-significant change ($P>0.05$) in the percentage of neutrophil in rats infected with *Klebsiella pneumoniae* on day 7. The *Pseudomonas haemolyticus* infected rats had the highest count of 60% and significantly different ($P< 0.05$) from other infected groups including normal control group on day 15.

The normal control group had the highest lymphocytes of 50.33% on day 1 (Figure 4.17). The percentage neutrophil was 50.33% in normal control group on day 1 and on day 3. Thereafter, the difference in the number of neutrophils in the treatment groups were statistically significant when compared to the control group on day 7. On

day 15, the *Pseudomonas haemolyticus*- infected rats in group B had the lowest neutrophil count of 36.6% and it was significantly different ($P< 0.05$) from normal control and other treatment groups.

On days 1, 3, 7 and 15 of the study, the number of monocyte was 1% in all the experimental group and these showed no significant difference ($P>0.05$) (Figure 4.18).

The eosinophil counts are shown in Figure 4.19. There was no significant difference ($P>0.05$) in the percentage of eosinophil count throughout the experiment. The *Staphylococcus haemolyticus*-infected rats in diabetic control group had the eosinophil count of 2.67% on day 7 and on Day 15.

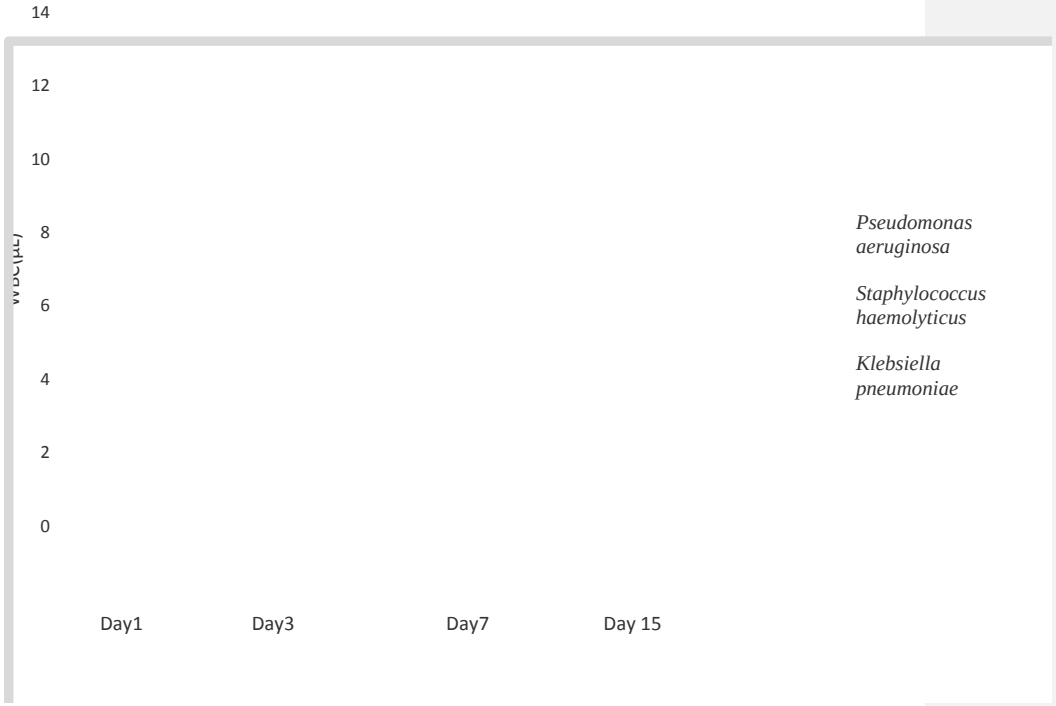


Figure 4.36: The Effect of the *Lactobacillus* on the White Blood Cell Count

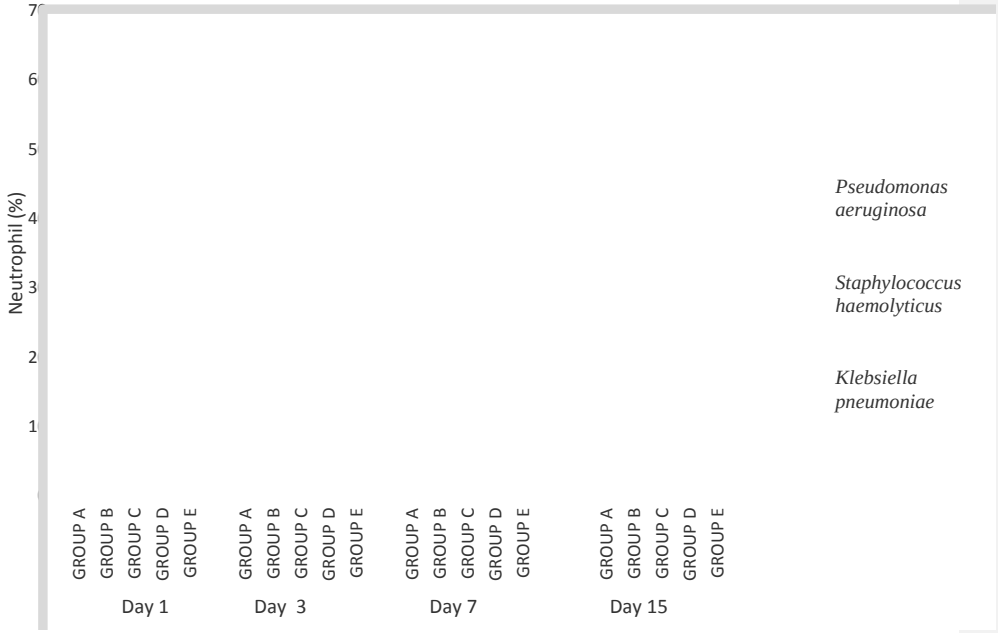


Figure 4.37: The Effect of the *Lactobacillus* on Neutrophil Count

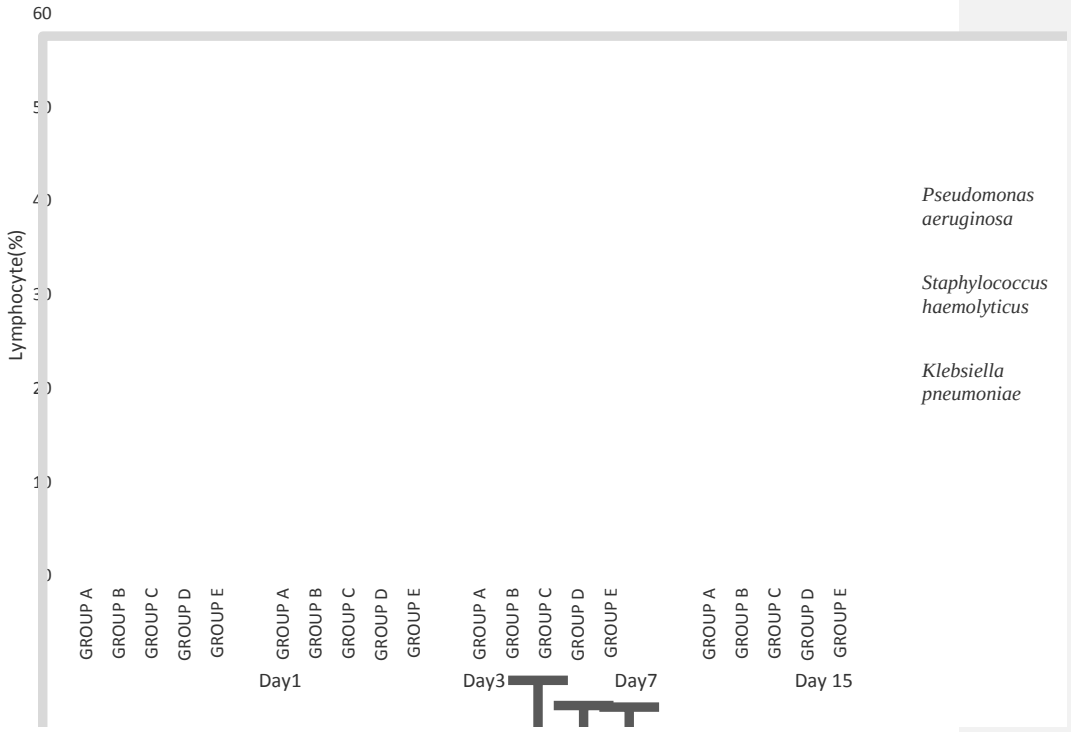


Figure 4.38: The Effect of the *Lactobacillus* on Lymphocyte Count

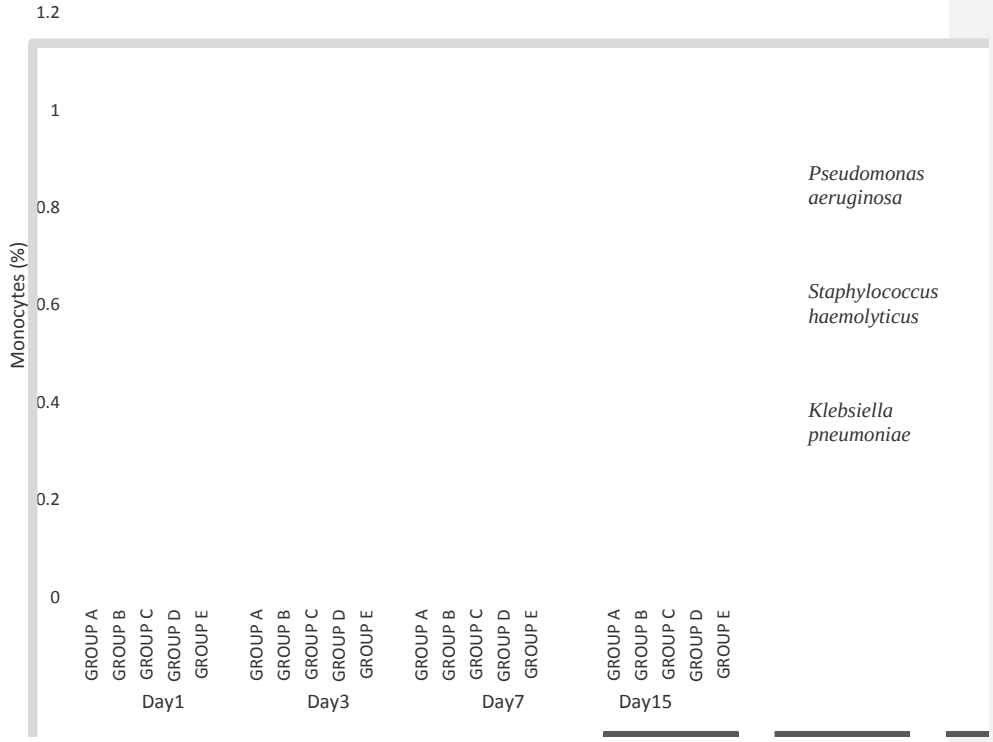


Figure 4.39: The Effect of the *Lactobacillus* on the Monocyte Count

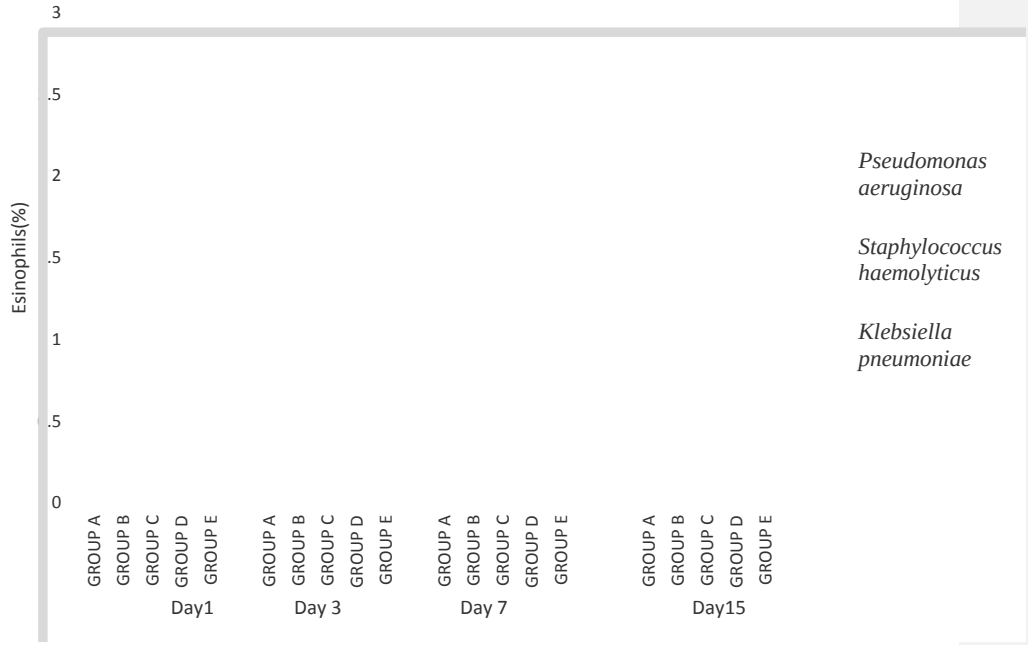


Figure 4.40: The Effect of the *Lactobacillus* on the Eosinophils Count

CHAPTER FIVE

DISCUSSION

The traditional processing of 'kunun-zaki' involves the utilization of millet and sorghum and this makes the product non-dairy drink. The Fermented cereal-based foods have been reported to offer opportunities to include probiotic, prebiotic and fibers in diet of consumers (Martins *et al*., 2013). The paddy rice and sweet potatoes added increased the quality of the products. The production of 'kunun-zaki' without synthetic sugar makes the drink more suitable for diabetic patients.

The recorded pH in this study (Table 4.1) was found to be the same recorded by Akoma and Efiuvwevwere, (2007), while some values recorded were slightly higher than those reported in previous studies (Omowaye and Oluwakunmi, 2015), probably due to cereal types as well as processing conditions used in the studies. Elmahmood and Doughari, (2007), reported the pH value of 'kunun-zaki' to be within the range of 3.5-3.71. Higher pH value of 6.50 was reported in tigernut based 'kunun-zaki' produced by Belewu and Abodunrin, (2008). The acidity of 'kunun-zaki' beverage has been noted to be as result of lactic acid production by some bacteria during fermentation. The low pH values observed in all'kunun-zaki' samples (Appendix VI) could be due to the decomposition of fermentable substrates and sugars by the microorganisms which ferment carbohydrates to produce energy and principally lactic acid. This is in line with what was reported by Agarry *et al*., (2010).

The highest total solids values obtained in sweet potatoes sample were similar to that of Adejuyitan *et al*., (2008) who reported a total solid of 13.42% for 'kunun-zaki' processed from wet milled sorghum. In a study carried out by Ayo *et al.,* (2013), on the production of higher 'kunun-zaki' values of total solids were recorded than those obtained in the present study. The difference may be as a result of the difference in the recipes used during

production and the quantity of water added for reconstitution. However, individual preferences usually determine the amount of water desirable. The time taken for the reconstitution of warm water was shorter than in cold water. This might be because solubility increases with increase in temperature. The higher total solid content had an effect on consumer acceptability as it imparted taste to the beverage.

The titratable acidity values were similar to the observations made by Oluwajoba *et al.*, (2013). High titratable acidity may have resulted from the effect of lactic acid fermentation. They suggested that high what in titratable acidity the sample could be due to the activities of microorganisms especially *Lactobacillus* species, which fermented the carbohydrates to produce energy and principally lactic acid and the high moisture content which was responsible for the activity of microorganisms. Also, the acidity of the samples can be attributed to the added spices and sweetener. The highest specific gravity of the control conforms to the findings of Omowaye and Oluwamukomi (2015), who reported high specific gravity of instant 'kunun-zaki'.

The moisture contents shown in Table 4.2 were slightly higher than the values reported by Olosunde *et al.*, (2014) for 'kunun-zaki' enriched with Moringa seed flour. Ogbonna *et al.*, (2013) reported the lower value for 'kunun-zaki' enriched with soybean. Differences in moisture contents may be due to the methods used in preparing the'kunun-zaki'. 'Kunun-zaki'had low shelf stability due to high moisture content and they readily undergo microbial induced spoilage within two to three days of production (Egbere *et al.*, 2009).

The ash value obtained in this study was higher than the value of ash obtained by Adelekan *et al.*(2013), who reported the ash content of malted soy-'kunun-zaki' to range from 0.14 to

0.43% respectively. The increase in ash content of paddy rice based 'kunun-zaki' was as a result of malting process that was involved.

Pasias *et al*., (2013) and Agarry *et al*., (2010) recorded high protein value in 'kunun-zaki' processed from millet and malted rice. Hotz and Gibson, (2007) reported high protein value in plant–based diets as a result of several traditional household food processing and preparation methods used and this enhanced the bioavailability of micronutrients. The processing includes thermal processing, mechanical processing, soaking, fermentation and germination or malting. The paddy rice contains fiber, essential amino acid, lysine, and gamma-aminobutyric acid. The phenolic compounds are also reported to be more abundant in paddy rice (Tian *et al*., 2008). These nutrients accelerate the metabolism of brain and prevent major diseases such as gastrointestinal cancers, heart disease, high blood pressure, diabetic and beriberi, constipation and Alzheimer's diseases (Hagiwara *et al*., 2008). The low protein content of the control is attributed to the low protein content of millet and sorghum (Achi and Ukwuru, 2015). The nutrient composition of millet and sorghum is influenced by both the environmental factors and genetic (Bhullar and Gruissem, 2013). The processing method such as parboiling and milling influence the variability of rice nutrient content. The most common source of variation is in soil fertility, soil moisture and cultural practices. According to Amusa and Odunbaku, (2009) considerable nutrient losses also occur during conventional processing of 'kunun-zaki'. The high protein content of 'kunun-zaki' with paddy rice is an indication of their usefulness in human diet and as animal feed (Adebayo *et al*., 2010).

Essien *et al*., (2009) reported highest carbohydrate content in 'kunun' processed in the laboratory. The high carbohydrate values observed in 'kunun-zaki' sample blends could be attributed to the high carbohydrate content of sweet potatoes. 'Kunun- zaki' processed with sweet potatoes serves as good source of energy.

The Vitamin C and E contents of the control processed 80-20% millet and sorghum were significantly different ($P< 0.05$) from other samples. This could be as a result of vitamin C being lost during heat processing. The high vitamin C contents in sweet potato based 'kunun-zaki'is in agreement with the findings of Omowaye and Oluwamukomi, (2015) who reported that the vitamin C content of the freshly produced 'kunun' is high. Antia *et al.,* (2008) also reported similar results regarding the composition of sweet potatoes. Sweet potatoes are a nutritious food, low in fat and protein, but rich in carbohydrate. Tubers and leaves of sweet potatoes are good sources of antioxidants, fiber, zinc, potassium, sodium, manganese, calcium, magnesium, iron, and vitamin C and E.

The values of calcium in sweet potatoes based 'kunun-zaki' in (Table 4.3) were in close agreement with the findings of Olosunde *et al.,* (2014) who reported high value in calcium components of 'kunun-zaki'. Calcium works in conjunction with magnesium and phosphorous for building and maintaining strong bones, teeth and metabolism of vitamin D. Calcium also aid in enzyme secretion, fat metabolism, eggshell formation, blood clotting, muscle growth and contraction. It also maintains the healthy heart and facilitates the passage of nutrients in and out of the cell wall (Kevin, 2007). The high value obtained for potassium agreed with the observation of Olufunke and Oluremi, (2015), who observed that potassium was the most predominant mineral in Nigeria agricultural products. Akoma *et al*., (2014) reported a high value for zinc.

The sodium values recorded for the beverage samples were slightly different from those reported by Makinde and Oyeleke, (2012) in kunun zaki and this could be due to the different cereals adopted for their production. Calcium and iron contents were, however were similar to those reported by Adelekan *et al*., (2013). The report of Ogbonna *et al*., (2013) also corroborates the values of magnesium and iron recorded in this study. Minerals are of great importance in diet, as they play important roles in body metabolism. Calcium helps in the

regulation of muscle contractions and transmission of nerve impulses as well as bone and teeth development (Kevin, 2007). Furthermore, potassium is essential for its important role in the synthesis of amino acids and proteins. Moreover, magnesium helps in relaxation of the muscle and in the formation of strong bones and teeth. It also plays fundamental roles in most reactions involving phosphate transfer, believed to be essential in the structural stability of nucleic acid and intestinal absorption while its deficiency can cause severe diarrhea, hypertension and stroke (Fathizadeh *et al*., 2010).

The overall acceptability of taste of 'kunun-zaki' processed with paddy rice 50:50 blend was due to the addition of malted paddy rice. This agrees with the finding of Agarry *et al*., (2010) who reported the production of kunun-zaki with paddy rice to be generally preferred than other samples. The hydrolytic enzymes produced in the grain due to malting aid in digesting the thick slurry by converting the complex carbohydrate to simple sugars resulting in a sweet final product (Solakunmi *et al*., 2013), degrading proteins to simple amino acids and improves the nutritional quality and density of 'kunun zaki' (Efiuvwevwere and Akoma, 2007). It also improves flavour by suppressing the beany flavour of the legumes. Malting altered the chemical composition of rice to a small extent but caused noticeable changes in the quality characteristics (Mohan *et al*., 2010).

The high bacterial counts observed in the 'kunun-zaki' produced with sweet potatoes at 50-50 millet and sorghum (Table 4.5) may be as a result of pH, moisture contents and processing which involves sun drying. The lower count observed in the 'kunun-zaki' samples could be as a result of a strict sanitary measure that was put in place during production: hand gloves were worn, clean potable water was used and also the production area and utensils were properly cleaned and sanitized.

The non-detection of coliforms in the samples could be as a result of good manufacturing and hygiene practices observed during production. Coliforms are majorly of fecal origin and their presence in foods indicates contamination from fecal sources, which is highly undesirable. This is because some coliforms such as *Escherichia coli* can cause diseases such as gastroenteritis, diarrhea, and urinary tract infections (Ayazi *et al*., 2010). Akoma *et al*., (2014) reported the coliform count of laboratory prepared 'kunun-zaki' to be insignificantly low.

The higher lactic acid count of 'kunun-zaki' processed with paddy rice could be as a result of addition of malted rice and also fermentation processes by the mixed microflora of lactic acid bacteria. Lactic acid bacteria and yeast are associated with fermentation of cereal foods given them a sour taste (Sade, 2009). The low values of microbial counts recorded in the 'kunun-zaki' samples could be due to heat treatment (pasteurization) given to the products during production. Similar values were reported by Adeleke *et al*., (2013). The fungal count for both controls was not significantly different ($P < 0.05$). The high fungal count is as a result of high pH value of the sample. The comparably high counts obtained for yeast is an indication of the importance of yeasts alongside the lactic acid bacteria in 'kunun' fermentation as was having been previously reported by Nwachukwu *et al*., (2010). Some yeasts have been reported to be probiotic, hence their presence in 'kunun' will further enhance the status of 'kunun' as a probiotic drink.

The lactic acid bacteria identified in this work are organisms which are naturally present in the human gut. Tthey are the organisms that cause fermentation in 'kunun-zaki' (Adelekan *et al*., 2013; Oluwajoba *et al*., 2013). Lactic acid bacteria (LAB) have been described as Gram positive, catalase negative, cocci or rods non-spore forming bacteria that are aerotolerant, anaerobic or microaerophilic (Endo and Irisawa, 2012). They produce lactic acid as part or major by product from the fermentation of a carbohydrate. Adebayo *et al*., (2010) had earlier

reported the presence of *Bacillus subtilis, Bacillus pumilus, Lactobacillus plantarum, Leuconostos mesenteriodes, Micrococcus spp., Staphyloccus aureus, Streptococcus* sp., *Mucor spp., Rhizopus spp* and *Saccharomyces cerevisiae* in 'kunun' prepared with a combination of sorghum and millet. Adebayo *et al*., (2010) on the other hand, did not isolate lactic acid bacteria and yeast from kunun but other organisms including *Bacillus subtilis, Micrococcus species, Escherichia coli, Staphyloccus aureus, Streptococcus sp., Mucor, Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus* and *Aspergillus, nidulans.* The heavy presence and activities of the lactic acid bacteria are usually responsible for the sour taste resulting from lactic acid production from fermentation of sugars. Probiotics which are acceptable for food or medicinal preparations for humans are those which occur naturally in the intestinal tract of healthy human and in foods (Arora *et al*., 2013). In addition, these bacteria have traditionally been used in the manufacture of the fermented dairy product and generally regarded as safe (Moyane and Jideani, 2013).

The collected pathogenic organisms are endemic in human populations and are regarded as opportunistic bacteria, usually causing infections in children, immunocompromised patients, or patients suffering from the effects of medical surgery (Reddy *et al*., 2012). *Staphylococcus haemolyticus,* can be isolated from the throat or nasal swab samples from approximately one-third of the population and is also commonly found in the skin flora, together with related specie, such as *Staphylococcus aureus* (Ohara-Nemoto *et al*., 2008). Topical infections due to *Staphylococcus haemolyticus* and *Klebsiella pneumoniae* are clinically relevant and cause a variety of serious symptoms, including toxic shock syndrome and skin lesions that can progress to sepsis and systemic shock if they are left untreated (Micheal, 2010).

Enteric *Lactobacilli* are usually able to tolerate pH 3.0 for a few hours, pH 2.0 for several minutes, while viable count will be affected at slightly high acidic pH (Tannock, 2008). This examination gave an indication of the osmotolerance level of the lactic acid strains.

Ibourahema *et al*., (2008) repooted that thatbacterial cells cultured with high salt concentration could show a loss of turgor pressure which would then affect their physiology, enzyme activity, water activity and metabolism. According to Mohd and Tan, (2007) high osmotolerance would be a requirement of lactic acid bacteria strains to be used as commercial strains because when lactic acid is produced by the strain, alkali would be pumped into the broth to prevent an excessive reduction in pH and the free acid would be converted to its salt form, increasing the osmotic pressure on the bacterial cells.

The tolerance of all the isolates to high NaCl concentration (4-9%) further indicates their potential to survive the harsh conditions and bile salt of the intestine. The observed variation in the inhibition of the test pathogens by the lactic acid bacteria is an indication that the organisms possess varying abilities to exert antimicrobial effects on pathogens and this corroborates the report of Azcarate-Peril *et al*., (2008) that antimicrobial activities exerted by lactic acid bacteria are strain specific. In general, tolerance to sodium chloride salts has been considered a condition for colonization and metabolic activity of bacteria in the host intestine (Anadon *et al*., 2008).

The resistance of *Lactobacillus delbruekii* and *Lactobacillus helveticus* may be an indication of their potential to survive the temperature of the human gut since temperature is an important requirement for bacterial growth, and the selected temperature range was chosen to simulate the normal human body temperature. This factor is very important in determining the effectiveness of probiotics since growth and viability during storage and use is one of the important determining factors for the functionality of probiotics. According to Ibourahema *et al*., (2008) the bacterial capability to grow at high temperature is a good characteristic as it could be interpreted as indicating an increased rate of growth and lactic acid population. Lactic acid bacteria are acidophilic which means they are tolerant to low pH. However, this needs to be differentiated from a condition of high concentration of free acids because the

free acids may cause growth inhibitors (Mohd and Tan, 2007). Probiotics bacteria need to survive passage through stomach where the pH can be as low as 1.5 to 2.0 and stay alive for 4 h or more.

The resistance of *Pseudomonas aeruginosa* to ampiclox (Table 4.11) may be due to the synergy between multi-drug efflux system and a type1 AmpC β-lactamase, the production of metallo-β-lactamases (MBL), which can be chromosomally encoded or plasmid-mediated and low outer membrane permeability (Tambekar *et al*., 2009). In fact, the irrational and inappropriate use of antibiotics is responsible for the development of resistance of *Pseudomonas species* to antibiotic monotherapy.

The highest zone of inhibition exhibited by *Lactobacillus plantarum* however, agrees with the findings of Anita *et al*., (2012) who reported *Lactobacillus plantarum* to be most active against *Streptococcus mutans*. The inhibitory activity of lactic acid bacteria has been previously reported and is mainly due to the accumulation of primary metabolites, such as lactic acid, ethanol, carbondioxide and the production of other antimicrobial compounds such as bacteriocins. The production levels and proportion among these compounds, depending on the biochemical properties of the strains used, and physical and chemical conditions of growth (Hoque *et al.*, 2010). Several authors have previously reported that certain probiotic bacteria, such as *Lactobacillus* species exhibit healing and antimicrobial activities (Okereke *et al.*, 2012; Sanders, 2008).

The value of the result obtained for antibacterial effect of *Lactobacillus casei* on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* as shown in Table 4.12, was higher compared to the findings of O'Connor *et al*. (2015) who reported antibacterial activity of *Lactobacillus casei* against *Pseudomonas aeruginosa* and *Klebsiella pneumonia*e to be 8 mm and 7 mm respectively. There are many strains among lactobacilli with documented probiotic

ability, thus they have a more application in prevention of infection. Their inhibitory action is due to the production of lactic acid, bacteriocins or H_2O_2 and diacetyl.

Zone of inhibition also depends on the concentration of the target microorganism plated, the length of activation of lactc acid bacteria, the type and concentration of agar used. It has been demonstrated that interleukin-18, another inflammatory cytokine, inhibits *Pseudomonas aeruginosa* infection (Hoque *et al.*, 2010). Lactic acid bacteria commonly produce bacteriocins which are peptides and have bactericidal activity usually against strains of closely related species (Ambalam *et al*., 2009). Although bacteriocins may enhance survival of lactic acid bacteria in complex ecological systems and interest has focused on prevention of growth of harmful bacteria in the fermentation and preservation of food products. It is more important with respect to probiotics that individual strains may inhibit the growth of or adhesion of pathogenic microorganisms by secreted products, and not merely an effect of acidic pH (Celia *et al*., 2018).

Identification of *Pseudomonas aeruginosa* has traditionally relied on phenotypic methods. This still is the most accurate standard when dealing with typical isolates of *Pseudomonas aeruginosa*. *Staphylococcus haemolyticus* has been associated with septicaemia in neonates and various infections in individuals with compromised host defences and implanted foreign bodies (Podkowik *et al*., 2013). *Staphylococcus haemolyticus* strains have been described frequently causing colonization and infection in hospitalized patients (Micheal, 2010).

In the present study, *Lactobacillus* species isolated from 'kunun-zaki' processed with paddy rice and sweet potatoes drink were identified according to polyphasic taxonomy and was classified as *Lactobacillus casei* and *Lactobacillus plantarum* with high Similarity 99.9% (Appendix 5). Carbohydrate fermentation patterns across the genus *Lactobacillus* have also been shown to be incongruent with the results of DNA-based studies (Boyd *et al*., 2008). In addition, the subjective interpretation of similar carbohydrate fermentation patterns obtained

using the API 50 CHL system may lead to atypical results (Randazzo *et al*., 2009). The lactic acid bacteria isolated are commonly associated with a wide range of African traditional food and beverage fermentations including fufu (fermented cassava), iru (fermented African locust bean), kenkey and ogi (fermented maize), 'kunun-zaki' (fermented millet), ugba (fermented African oil bean), and wara (fermented skimmed cow's milk) (Folarin, 2014). In fact, *Lactobacillus plantarum* is the species most commonly isolated and has been identified as the dominant organism at the end of several natural lactic acid fermentations (Bahrami and Golshan, 2014; Nguyen *et al.*, 2013). Adeyemo and Onilude, (2014) noted the accuracy of the molecular diagnostic method in the ability to rapidly identify *Lactobacillus* from paddy rice silage from genus level to species level using automated systems.

The body weight of the animal obtained before induction of streptozotocin conforms to the findings of Nuzulul *et al.*, (2015) who reported the same value for non streptozotocin induced rats. The slight increase in body weight of animals in group A are consistent with previous studies that the streptozotocin induced diabetic rats showed a significant decrease in body weight at week 2 after streptozotocin induction (Minaiyan *et al*., 2014). The loss of body weight because of induction of streptozotocin was associated with an increase in muscle wasting (gluconeogenesis and glycogenolysis) and the loss of tissue proteins (Emma, 2012; Kato *et al*., 2008). Insulin deficiency can cause a variety of metabolic disorders, including the increased blood glucose level, decrease in protein content and increase of cholesterol as well as triglyceride levels (Tenpe and Yeole, 2009). The loss of weight and decrease in growth rates in the diabetic control rats despite their increased feed intake, is attributed to the fact that streptozotocin induced diabetes is characterized by severe loss in body weight. This reduction is due to lose or degeneration of structural proteins, as the structural proteins are known to be a major contributor to body weights and indicative of the food not being transformed into weight gain or growth attainment.

The observed normal blood glucose level was in accordance with the findings of Somashekar and Sudhakar, (2013) who reported blood glucose level of 71 to 110mg/dL in streptozotocin induced rats. The variation in the blood glucose level might be as a result of body weight of the animal. (Figure 4.6). Blood glucose levels were used to diagnose diabetes. Hyperglycemia in diabetes was caused by insulin secretion deficiency by pancreatic beta cell dysfunction or insulin act resistance in the liver and muscle or combination of both (Simona and Minodora, 2013). Moore *et al.*, (2013) reported blood glucose levels of animal measured after 48 h of streptozotocin administration to be above 200 mg/dL. Diabetes-induced by streptozotocin was characterized by apoptosis of cells of pancreas, attenuation of gene expression of insulin modulating their insulin secretion rate in response to the blood glucose concentration of pancreatic cells (Patel *et al*., 2008). Amir *et al*., (2016) reported a decrease in streptozotocin- induced rats treated with sodium alginate.

Streptozotocin inhibits the biosynthesis and secretion of insulin through interference with the glucose metabolism and oxygen consumption (Shaw *et al*., 2010). Streptozotocin-induced diabetes mellitus (STZ-DM) which is caused by the pancreatic beta cell destruction was similar to type 1 of diabetes in humans. It was characterized by hyperglycemia, glycosuria, polyphagia, hypoinsulinemia, hyperlipidemia and body weight loss. Streptozotocin induced diabetes also shows some complications that include increased susceptibility to infection, cardiovascular disease, retinopathy, changes in angiogenesis, wound healing disorders, decreased expression of growth factors and decreased bone formation (Minaiyan *et al.*, 2014).

The colony counts of infected pathogenic organisms show the successful establishment of a Pseudomonas aeruginosa, *Staphylococcus haemolyticus* and *Klebsiella pneumoniae* in this study.

Rats with streptozotocin-induced diabetes shared diabetic common symptoms which include polyuria, polydipsia and fatigue with increased urination and diarrhea suggesting intestinal dysfunction. The observed behavioural response of the rats are in agreement with the findings of Lee *et al.,* (2012).

The beneficial effects of *Lactobacillus* species on the wound healing (Figure 4.8) are the basis of the rationale for their use as probiotics in the treatment of skin ulcers. Wound healing is a dynamic process, which has three phases, including inflammation, tissue formation, and tissue remodeling that overlap in time. A variety of medical substances can accelerate wound healing. The therapeutic role of probiotics is well reported in cases of ulcerative colitis, Crohn's disease and gastric ulcers (Lam *et al.,* 2007). These probiotics are available as pharmaceutical and food preparations in different countries in the world (Angelakis *et al*., 2011). Bacteria of this genus are able to produce specific substances such as bacteriocins, antibiotics and exopolysaccharide that may accelerate the wound healing process (Liu *et al*., 2010). The production of immune cells, such as neutrophil, macrophage and lymphocytes have been shown to play an important regulatory role in the wound healing and scar formation by initiating an early inflammatory process as well as several other immune responses (Thomas and Kevin, 2016; Zahedi *et al*., 2011).

The increase in red blood cell following administration of *Lactobacillus casei* and *Lactobacillus plantarum* gives an indication that both probiotics can stimulate the formation or secretion of erythropoietin, which stimulates stem cells in the bone marrow to produce red blood cells (Ohlsson and Aher, 2012). The stimulation of this hormone enhances rapid synthesis of red blood cell, which is supported by the improved level of mean cell haemoglobin and mean cell haemoglobin concentration (Abu-Zaiton, 2010). These parameters are used mathematically to define the concentration of haemoglobin and to suggest the restoration of oxygen, carrying capacity of the blood. The decrease in mean cell

haemoglobin values, observed after administration of streptozotocin, is an indication of abnormal hemoglobin synthesis, failure of blood osmoregulation and plasma osmolarity.

The decrease in packed cell volume of all groups except normal control group in day 3 (Figure 4.21) might be as a result of induction of diabetes using streptozotocin. Abnormally low packed cell volume (anemia) may be caused by loss of blood (hemorrhage), the breakdown of red blood cells in circulation (haemolysis), or lack of production of red blood cells by the bone marrow (hypoplasia or aplasia) (Ramsay *et al*., 2008). The degree of anemia in diabetic patients can be associated with a number of factors, including glomerular filtration rate, urinary albumin excretion rate and glycated h (HbA1c) levels (Onat *et al*., 2010). Anemia has been reported to be due to diminished erythropoietin production by failing kidneys and increased nonenzymatic glycosylation of red blood cell membrane proteins.

The peripheral white blood cell count of diabetic rats has been shown to be associated with insulin resistance, type 2 diabetes (Ohshita *et al*., 2009), coronary artery disease, stroke and diabetes micro and macrovascular complications (Tong *et al*., 2010). The profile of the white blood cell count reflects the balance between the rate of granulocyte production and that of white blood cell. Ayman, (2013) reported that diabetes in mice was accompanied by moderate neutrophilic leukocytosis and prolonged circulation times of neutrophils and monocytes and a shortened circulation time of lymphocytes, which increases the susceptibility to infection. The raised leukocyte count may also reflect low-grade inflammation. These immune cells have been shown to play an important regulatory role in the wound healing and scar formation by initiating an early inflammatory process as well as several other immune responses. Neutrophils have a lobulated nucleus. They function as phagocytes and are important in infectious conditions and in inflammation. Increased neutrophil counts (neutrophilia) are caused by inflammation, bacterial infection, acute stress, steroid effects and neoplasia of the granulocytic cell line (Wilgus, 2008). Decreased

neutrophil counts (neutropenia) are caused by viral infections, toxin exposure (including foodborne toxins) and some certain drugs (carbimazole and methimazole). Autoimmune destruction of neutrophils, bone marrow neoplasia not involving the granulocytes and bone marrow aplasia (Ochuko *et al*., 2013).

An increased lymphocyte count (lymphocytosis) may occur for physiologic reasons but significant increases usually indicate leukemia (Oyedemi *et al*., 2011). The reduced levels of white blood cell and lymphocytes in diabetic rat indicate a suppression of the immune system (Onat *et al*., 2010). These cells identify and eliminate pathogens, either by attacking larger pathogens through contact or by phagocytosis. They form part of the innate immune system, which is also an important mediator in the activation of the adaptive immune system. The reduced immunity can contribute to the various complications associated with diabetes mellitus (Ohshita *et al*., 2009).

Increased eosinophil counts (eosinophilia) are caused by allergic/hypersensitivity reactions, parasitism, tissue injury, mast cell tumors and estrus. Decreased eosinophil count (eosinopenia) is always caused by the action of glucocorticoids, either endogenous or therapeutic (Okonkwo and Okoye, 2014).

This lack of cellular proliferation and granulation tissue formation led to delayed wound healing process. Tissues of diabetic rat infected with *Pseudomonas aeruginosa* treated with *Lactobacillus plantarum* showed better wound healing as compared to control. (Lan *et al*., 2008), angiogenesis impairment and loss of phagocytic activity (Guo and DiPietro, 2010). Previous studies demonstrated that fibroblasts isolated from diabetic wounds had lower migratory activity and mitogenic responses, compared with non-diabetic wounds (Xuan *et al*., 2014). These cellular abnormalities impede the formation of granulation tissues and extracellular matrix molecules, resulting in non-healing wounds.

The crucial stages of wound repairing are re-epithelialization which involves the activity of keratinocytes to move across the wound bed for the restoration of epidermal layers. Due to high glucose levels, migration and proliferation of keratinocytes were impaired, resulting in inadequate re-epithelialization (Lan *et al*., 2008).

5.1. CONCLUSION

In the present study, 'kunun-zaki' processed with paddy rice using a mixture of millet and sorghumin the ratio of 50:50% had the highest fiber, ash, protein zinc and iron contents. Secondly, the study has shown that lactic acid bacteria especially *Lactobacillus* species, may be the most important organisms involved in 'kunun-zaki' fermentation. Furthermore, *Lactobacillus plantarum* isolated from 'kunun-zaki' has been shown to be the most effective probiotic, which exerted the highest antimicrobial effects against the tested pathogens. Moreover, the results suggest that both *Lactobacillus casei* and *Lactobacillus plantarum* isolated from 'kunun-zaki' processed with sweet potatoes and paddy rice may be used either as a monoculture to inoculate dairy or non-dairy products or in co-culture with other probiotics for use in the fermentation of sterilized millet and sorghum, thus yielding 'kunun-zaki' with better sensory properties than that prepared by natural fermentation. These lactic acid bacteria may possibly be exploited to produce different varieties of 'kunun-zaki' in the future.

The alterations in the red blood cell, haemoglobin and packed cell volume levels of the diabetic rats showed the occurrence of anemia. The observed increase in these parameters on feeding the rats with the *Lactobacillus casei* and *Lactobacillus plantarum* suggests their potency in the management of the ailment. The partial removal of the epidermal layer of rat's skin allows *Pseudomonas aeruginosa, Staphylococcus haemolyticus* and *Klebsiella pneumoniae* to colonize the skin and this colonization is associated with an inflammatory host

response. Diabetic wounds displayed impairment in re-epithelialization, cell migration and proliferation, as well as granulation tissue formation, leading to deteriorated wound healing process. The results of this study provide scientific evidence, supporting the use of probiotics in the wound- healing process of infected diabetic ulcers. Therefore, it could be used as a novel alternative treatment for diabetic wounds.

5.2. RECOMMENDATION

The studies demonstrated that 'kunun-zaki' processed with sweet potatoes and paddy rice is nutritious and can serves as a potential probiotics drink and that the isolated *Lactobacillus plantarum* was highly effective in enhancing skin ulcer healing. However, further studies would be necessary to elucidate the following;

1). Effect of different packaging materials on the shelf stability of the 'kunun-zaki' processed with thickeners.

2). Effect of different storage conditions (refrigeration, room temperature and freezing) on the shelf stability of the 'kunun-zaki' processed with thickeners.

3). The effect of chemical preservatives on the shelf stability of the 'kunun-zaki' processed with thickeners.

4). The use of flavour and colouring agents to enhance marketability.

5). The effect of combining *Lactobacillus* strains to treat infected wounds in streptozotocin- induced rat.

6). The histopathological studies of the organs of the streptozotocin-induced diabetic rats treated with *Lactobacillus* species.

REFERENCES

Aalaa, M., Malazy, O. T., Sanjari, M., Peimani, M. and Mohajeri-Tehrani, M. (2012). Nurses role in diabetic foot prevention and care; a review. *Journal of Diabetes Metabolism disorder*. 11(1): 24-31.

Abe, S., Nemera, G. S., Angeline, V. N. and Maryke, T. L. (2012). Diversity in starch, protein and mineral composition of sorghum landrace accessions from Ethiopia. *Journal of Crop Science and Biotechnology.* 15(4): 275-280.

Abu-Zaiton, A. S. (2010). Antidiabetic activity of *Ferula asafetida* extract in normal and alloxan induced diabetic rats. *Pakistan Journal of Biological Science*. 13(2): 97-100.

Achi, O. K. and Ukwuru, M. (2015). Cereal-Based fermented foods of Africa as functional. *International Journal of Microbiology and Application*. 2(4): 71-83.

Adebayo, C. O., Adeneye, B. I. and Akpor, O. B. (2014). Assessment of bacterial and fungal spoilage of some Nigerian fermented and unfermented food. *African Journal of Food Science*. 8(3): 140-147.

Adebayo, G. B., Otunda, G. A. and Ajao, T. A. (2010). Physcio-chemical, microbiological and sensory characteristics of kunun prepared from millet, maize and guinea-corn and stored at selected temperatures. *Advanced Journal of Food Science and Technology*. 2(1): 41-46.

Adebiyi, J. A., Obadina, A. O., Adebo, O. A. and Kayitesi, E. (2018). Fermented and malted millet products in Africa: Expedition from traditional /ethnic foods to industrial value-added products. *Journal of Critical Reviews in Food Science and Nutrition*. 58(3): 463-474.

Adejuyitan, J. E. Adelakun, O. E., Olaniyan, S. A. and Popoola, F. I. (2008). Evaluating the quality characteristics of kunun produced from dry-milled sorghum. *African Journal of Biotechnology*. 7(13): 2244-2247.

Adelekan, A. O., Alamu, A. E., Arisa, N. U., Adebayo, Y.O. and Dosa, A. S. (2013). Nutritional, microbiological and sensory characteristics of malted soy-kunun zaki: an improved traditional beverage. *Journal of Advance Microbiology*. 3(4): 389-397.

Adetuyi, A. O. (2007). The nutritive value and antimicrobial property of *Sorghum bicolor*. *Journal of Food Technology*. 2(3): 79-86.

Adeyemo, S. M., Onilude, A. A. and Olugbogi, D. O. (2016). Reduction of Anti-nutritional Factors of Sorghum by Lactic Acid Bacteria Isolated from A*bacha*- an African Fermented Staple. *Frontiers in Science*. 6(1): 25-30.

Adeyemo, S. M. and Onilude, A. A. (2014). Molecular identification of *Lactobacillus plantarum* isolated from fermenting cereals. *International Journal of Biotechnology and Molecular Biology Research*. 5(6): 59-67.

Agarry, O. O., Nkama, I. and Akoma, O. (2010). Production of 'kunun-zaki' (a Nigerian fermented cereal beverage) using starter culture. *International Journal of Microbiology*. 1(2): 018-025.

Akin. M. B., Akin, M. S. and Kirmaci, Z. (2007). Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice cream. *Journal of Food Chemistry*. 104(1): 93-99.

Akoma, O., Agarry, O. O. and Nkama, I. (2013). A study on the production and consumption pattern of Kunun-zaki: A fermented beverage of northern Nigeria. *British Journal Applied Science and Technology*. 3(4): 1220-1227.

Akoma, O., Daniel, A. A., Ajewole, A. E. and Nwodo, P. G. (2014). Quality characteristics of kunun-zaki (A Nigerian fermented cereal beverage) sold within Bida metropolis. *Journal of Agricultural Science*. 3(9): 298-303.

Alam, J., Chandra, S. M. and Mokarrama, M. N. (2015). A comparative analysis of biochemical and hematological parameters in diabetic and non-diabetic adults. *An International Journal of American Mathematical Society.* 2(1): 1-9.

Alavi, A., Sibbald, R. G., Mayer, D., Goodman, L., Botros, M., Armstrong, D. G., Woo, K., Boeni, T., Ayello, E. A. and Kirsner, R. S. (2014). Diabetic foot ulcers; Part 1. Pathophysiology and prevention. *Journal of the American Academy of Dermatology*. 70(1): 21-24.

Amadou, I. M., Gounga, M. and Guo-Wei, L. (2013). 'Millet nutritional composition, some health benefits and processing- A review'. *Emirates Journal of Food and Agriculture*. 25(7): 501-508.

Ambalam, P., Prajapati, J.B., Dave, J. M., Nair, B. M., Ljungh, A. and Vyas, B. R. M. (2009). Isolation and characterization of antimicrobial proteins produced by a potential probiotics strain of human *Lactobacillus rhamnosus*231 and Its effect on selected

human pathogens and food spoilage organisms. *Journal of Microbial Ecology in Health and Disease*. 21(2): 211-220.

Ammann, A. B., Kolle, L. and Brandl, H. (2011). Detection of bacterial endospores in soil by Terbium fluorescence. *International Journal of Microbiology*. 20(7): 1155-1159.

Amir, H., Dwi, P. and Ustadi, M. (2016). Blood glucose level and lipid profile of Streptozotocin-induced diabetes rats treated with sodium alginate from *Sargassum crassifolium*. *Journal of Biological Sciences*. 16(3): 58-64.

Amihud, K. (2009). Sensory evaluation of food flavor. *Journal of Food and Nutrition*. 56(5): 64-69.

Amund, O. D. (2016). Exploring the relationship between exposure to technological and gastrointestinal stress and probiotics functional properties of lactobacilli and bifidobacteria. *Canadian Journal of Microbiology*. 62(9): 715-725.

Amusa, N. A. and Odunbaku, O. A. (2009). Microbiological and nutritional quality of hawked 'Kunun' (A Sorghum Based Non-Alcoholic Beverage) Widely Consumed in Nigeria. *Pakistan Journal of Nutrition*. 8(1): 20-25.

Anadon, A., Larranaga, R. M. and Martinez, A. M. (2008). Probiotic for animal nutrition in the european union, regulation and safety assessment. *Journal of Regulatory Toxicology Pharmacology*. 45(3): 628-633.

Angelakis, E., Million, M., Henry, M. and Raoult, D. (2011). Rapid and accurate bacterial identification in probiotics and yoghurts by MALDITOF mass spectrometry. *Journal of Food Science*. 76(4): 568-572.

Anita, K., Sepideh, H. P. and Maryam, T. E. (2012). Investigation of probiotic chocolate effect on *Streptococcus mutans* growth inhibition. *Jundishapur Journal of Microbiology*. 5(4): 590-597.

AOAC, (2010). Official Methods of Analysis 16th edn. Association of Official Analytical Chemists Washington. D.C. USA.

Arora, M., Sharma, S. and Baldi, A. (2013). Comparative insight of regulatory guidelines for probiotics in USA, India and Malaysia: A critical review. *International Journal of Biotechnology for Wellness Industries*. 2(2): 51-64.

Arun, G. K., Venkatesh, R. S. and Vijaya, G. S. (2010). Nutritional amd rheological properties of sorghum. *International Journal of Food Properties*. 12(1): 55-69.

Ashraf, M., Arshad, M., Siddique, M. and Muhammad, G. (2009). In vitro screening of locally isolated *Lactobacillus* species for probiotic properties. *Pakinstan Vetenary Journal*. 29(4): 186-190.

Ashwani, K., Vidisha, T., Amarjeet, K., Vikas, K. and Kritika, G. (2018). Millet: a solution to agrarian and nutritional challenges. *Journal of Agriculture and Food Security*. 7(4): 31-39.

Atkinson, F. S., Fooster-Powell, K. and Brand-Miller, J. C. (2008). International table of glycemic index and glycemic load values. *Journal of Diabetes Care*. 31(12): 2281-2283.

Awadalkareem, A. M. (2008). Protein, mineral contents and amino acid profile of sorghum flour. *Pakistan Journal of Nutrition.* 7(3): 475-479.

Ayazi, P., Mahyar, A., Jahani, H. H. and Khabiri, S. (2010). Urinary tract infections in children. *Iran Journal of Pediatrician Society.* 2(1): 9-14.

Ayman, M. M. (2013). Hematological alterations in diabetic rats - Role of adipocytokines and effect of citrus flavonoids. *Journal of Experimental and Clinical Science*. 12: 647-657.

Ayo-Omogie, H. N. and Okorie, E. I. (2016). *In vitro* probiotic potential of autochthonous lactic acid bacteria and microbiology of 'kunun' made from mixed grains. *British Microbiology Research Journal*. 14(4): 1-10.

Ayo, J. A. (2013) "Effect of acha (*Digitaria exilis* staph) and millet (*Pennisetum typhodium*) grain on kunun zaki". *British Food Journal*. 106(7): 512-519.

Azcarate-Peril, A. M., Altermann, E., Hoover-Fitzula, R. L., Cano, R. J. and Klaenhammer, T. R. (2008). Identification and inactivation of genetic loci involved with *Lactobacillus acidophilus* acid tolerance. *Journal of Applied Environmental Microbiology*. 70(5): 5315-5322.

Azizpour, K, Bahrambeygi, S. and Mahmoodpour, S. (2009). "History and basic of probiotics". *Research Journal of Biological Sciences*. 4(4): 409-426.

Bahrami, A. P. and Golshan, T. A. (2014). Assessing the bile and acid tolerance and acid production of lactobacilli isolated from traditional sour dough. *Journal of Iranian Biosystem Engeneering.* 45(1): 22-29.

Baliga, M. S., Haniadka, R. and Pereira, M. M. (2011). Update on the chemopreventives effects of ginger and its phytochemicals. *Journal of Critical Reviews in Food Science and Nutrition*. 51(6): 499-523.

Bano, K., Khan, J., Hasina, B., Shahzad, M., Noor A., Jamil, A. A. and Muhammad, A. (2012). Patterns of antibiotics sensitivity of bacterial pathogens among urinary tract infections (UTI) patients in a Pakistan population. *African Journal of Microbiology Research*. 6(2): 414-420.

Bernat, N. N., Chafer, A., Chiralt, A. and Gonzalez-Martinez, C. (2014). Vegetable milks and their fermented derivative products. *International Journal of Food Science*. 3(1): 93-124.

Belewu, M. A. and Abodunrin, O. A. (2008). Preparation of kunun from unexploited rich food Source; Tiger-nut (*Cyperus esculentus*). *Pakistan Journal of Nutrition*. 7(1): 109-111.

Bhullar, N. K. and Gruissem, W. (2013). Nutritional enhancement of rice for human health: The *Journal of Biotechnology Advance*. 31(1): 50-57.

Bollen, C.M. and Beikler, T. (2012). Halitosis: the multidisciplinary approach. *International Journal of Oral Science.* 4(2): 55-63.

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J. C., Gerds, M. L. and Hammes, W. P. (2012). Food fermentations: microorganisms with technological beneficial use. *International Journal of Food Microbiology.* 154(3): 87-97.

Boyd, M. A., Antonio, M. A. D. and Hillier, S. L. (2008). Comparison of API 50 CH strips to whole-chromosomal DNA probes for identification of *Lactobacillus* species. *Journal of Clinical Microbiology.* 43(4): 5309-5311.

Brenner, D. M., Moeller, M. J., Chey, W. D. and Schoenfeld, P. S. (2009). The utility of probiotics in the treatment of irritable bowel syndrome: a systematic review. *America Journal of Gastroenterology*. 104(4): 1033-1049.

Brotman, R. M., Ravel, J., Cone, R. A. and Zenilman, J. M. (2010). Rapid fluctuation of the vaginal microbiota measured by Gram stain analysis. *Journal of Sex Transmission Infection*. 86(4): 297-302.

Brunstrom, M. and Carlberg, B. O. (2016). Effect of antihypertensive treatment at different blood pressure levels in patients with diabetes mellitus: systematic review and meta-analysis. *British Medical Journal.* 24(2): 352: 362.

Butalia, S., Kaplan, G. G., Khokhar, B. and Rabi, D. M. (2016). "Environmental risk factors and Type 1 diabetes: past, present, and future". *Canada Journal of Diabetes.* 40(3): 586-593.

Caroline, L. P. and Camila, S.S. (2017). Mineral content of sorghum genotypes and the influence of water stress. *Journal of Food Science*. 214(1): 400-405.

Célia, C. G., Silva, Sofia, P. M., and Susana, C. R. (2018). Application of bacteriocins and protective cultures in dairy food preservation. *Journal of Frontiers in Microbiology*. 9(4): 594-607.

Cencic, A. and Chingwaru, W. (2010). The role of functional foods, nutraceuticals and food supplements in intestinal health. *Journal of Nutrients*. 2(3): 611-625.

Céspedes, M., Cárdenas, P., Staffolani, M., Ciappini, M. C. and Vinderola, G. (2013). Performance in nondairy drinks of probiotic *Lactobacillus casei* strains usually employed in dairy products. *Journal of Food Science*.78: 756-762.

Chaudhari, P. R. (2018). Rice nutritional and medicinal properties. *Journal of Pharmacognosy and Phytochemistry.* 7(2): 150-156.

Cheesbrough, M. (2009). *District Laboratory Practice in Tropical Countries* part 2. 2nd edn. Cambridge University Press. New York. pp. 295-319. ISBN: 978 0521 67631-1.

Chen, L., Pei, J. H., Kuang, H. M., Chen, Z., Chen Z. W. and Li, H. Z. (2015). Effect of lifestyle intervention in patients with diabetes: A meta- analysis. *Journal of Metabolism*. 64(2): 338-347.

Chiara, D., Marcella, R. and Erica, C. (2017). *Journal of Nutrients*. 9(6): 615-622.

Cichosz, S. L., Fleischer, J., Hoeyem, P., Laugesen, E. and Poulsen, P. L. (2013). Objective measurements of activity patterns in people with newly diagnosed type 2 diabetes demonstrate a sedentary lifestyle. *Journal of Diabetes Medical*. 30(9): 1063-1066.

Claus, W. G. (2007). *Understanding microbes: A laboratory teaching manual*. 1st edn. Freeman custom publishing. New York. pp. 458.

Cuello-Garcia, C. A., Brożek, J. L., Fiocchi, A., Pawankar, R., Yepes-Nuñez, J. J., Terracciano, L., Gandhi, S., Agarwal, A., Zhang, Y. and Schünemann, H. J. (2015). "Probiotics for the prevention of allergy: A systematic review and meta-analysis of randomized controlled trials". *Journal of Allergy Clinical Immunology*. 136(4): 952–961.

Damasceno, G. T., Volpato, Y. K. and Sinzato, O. S. (2011). "Genotoxicity and fetal abnormality in Streptozotocin- induced diabetic rats exposed to cigarette smoke prior to and during pregnancy". *Journal of Experimental and Clinical Endocrinology and Diabetes*. 119(9): 549-553.

De-la Monte, S. M. (2014). "Type 3 diabetes is sporadic Alzheimer′s disease: mini-review". European neuropsychopharmacology. *Journal of the European College of Neuropsychopharmacology.* 24(12): 1954-1960.

De-Moreno, L. A. and Perdigón, G. (2010). The application of probiotic fermented milks in cancer and intestinal inflammation. *British Journal of Nutrition*. 69(4): 421-428.

Devi, P. B., Vijayabharathi, R., Sathyabama, S., Malleshi, N. G. and Priyadarisini, V. B. (2014).Health benefits of finger millet (*Eleusine coracana* L) polyphenols and dietary fiber; a review. *Journal of Foood Science and Technology*. 51(6): 1021-1040.

De-Vrese, M. and Marteau, P. R. (2007). Probiotics and prebiotics: effects on diarrhea. *Journal of Nutrition.* 137(3): 803-811.

Dodd, H., Williams, S., Brown, R. and Venn, B. (2011). Calculating meal glycemic index by using measured and published food values compared with directly measured meal glycemic index. *American Journal of Clinical Nutrition.* 94(4): 992-996.

Dogan, S., Demirer, S., Kepenekci, I., Erkek, B., Kiziltay, A. and Hasirci, N. (2009). Epidermal growth factor containing wound closure enhances wound healing in non-diabetic and diabetic rats. *International Wound Journal.* 6(2): 107-115.

Doleyres, Y. and Lacroix, C. (2008). Technologies with free and immobilised cells for probiotic Bifidobacteria production and protection. *Journal of International Dairy*. 15(2): 973-988.

Dong, J. Y., Szeto, I. M., Makinen, K., Gao, Q., Wang, J., Qin, L.Q. and Zhao, Y. (2013). Effect of probiotics fermented milk on blood [pressure: a meta–analysis of randomized controlled trials. *Britian Journal of Nutrition*. 110(1): 1188-1194.

Durchschein, F., Petritsch, W. and Hammer, H. F. (2016). "Diet therapy for inflammatory bowel diseases: The established and the new". *World Journal of Gastroenterology*. 22(7): 2179-2194.

Egbere, O. J., Pam, K. V., Adesheyan, K. D., Akadir, T. and Oyero, S. K. (2009). Effects of pasteurization on survival patterns of Microorganisms and Vitamin C retention in Kunun-zaki. *African Journal of Biotechnology.* 8(23): 6603-6607.

Elahe, M., Ali, D. and Soudeh, G. (2017). The role of probiotics in cancer treatments. *International Journal of Molecular Cell Medical*. 6(2): 66-76.

Elizabeth, C. V. (2010). Use of probiotics in gastrointestinal disorders: what to recommend? *Journal of Therapy Advance Gastroenterology*. 3(5): 307-319.

Elmahmood, A. M. and Doughari, J. H. (2007). Microbial quality assessment of kunun-zaki beverage sold in Girei town of Adamawa State, Nigeria. *African Journal of Food Science* 8(1): 011-015.

Emam, M. A. (2012). Comparative evaluation of antidiabetic activity of *Rosmarinus officinalis* L. and *Chamomile recutita* in streptozotocin induced diabetic rats. *Journal of Agricultural Biological*. 3(6): 247-252.

Eman, M. and Alfadhli, S. (2015). Gestational diabetes mellitus. *Medical Journal*. 36(4): 399-406.

Endo, A. and Irisawa, T. (2012). Characterization and emended description of *Lactobacillus kunkeei* as a fructohilic lactic acid bacterium. *International Journal of Systemic and Evolutionary Microbiology*. 62(3): 500-504.

Essine, E., Monago, E. E. and Edor, E. (2009). Evaluation of nutritional and Microbiological quality of Kunun (A cereal based non-alcoholic beverage in River State, Nigeria). The *Internet Journal of Nutrition and Wellness.* 10(2): 352-360.

FAO, (2017). World food situation; *http://www.fao.org/worldfoodsituation/csdb/en/*. Accessed 25th July, 2017.

Falagas, M. E., Betsi, G. I. and Athanasiou, S. (2007). Probiotics for treatment of women with bacterial vaginosis. *Clinical Microbiology Infection*. 13(2): 657-664.

Fatema, K., Rahman, F., Sumi, N., Kobura, K. and Ali, L. (2010). Glycemic index of three common varieties of Bangladeshi rice in healthy subjects. *African Journal of Food Science.* 4(8): 531-535.

Fathizadeh, N., Ebrahimi, E., Valiani, M., Tavakoli, N. and Yar, M. H. (2010). Evaluating the effect of magnesium and magnesium plus vitamin B6 supplement on the severity of premenstrual syndrome. *Iranian Journal of Nursing and Midwifery Research.* 15(1): 401-405.

Faustina, D. W. and Cleopatra, A. (2017). Comparative studies on proximate and some mineral composition of selected local rice varieties and imported rice brands in Ghana. *Journal of Agriculture Food Research*. 4(1): 1-7.

Felis, G. E. and Dellaglio, F. (2007). Taxonomy of Lactobacilli and Bifidobacteria. *Journal of Current Issues in Intestinal Microbiology.* 8(3): 44-61.

Fernandes, M. S., Carneiro, F. and Oliveira, C. (2013). Colorectal cancer and RASSF family-a special emphasis on RASSFIA. *International Journal of Cancer*. 132(2): 251-258.

Folarin, A. O. (2014). Safety challenges associated with traditional foods of West Africa *Journal of Food Reviews International.* 30(4): 338-358.

Forbes, B., Daniel, F. S. and Alice, S. W. (2007). *Diagnostic microbiology*. 12th edn. Don Ladig Press. pp. 430. ISBN: 9780323075022.

Frachetti, K. J. and Goldfine, A. B. (2009). "Bariatric surgery for diabetes management". Current Opinion in Endocrinology. *Journal of Diabetes and Obesity.* 16(2): 119-24.

Fraccalvieri, M., Serra, R., Ruka, E., Zingarelli, E., Antoniotti, U., Robbiano, F., Viglione, M., Frisicale, L. and Bruschi, S. (2011). Surgical debridement with VERSAJET: an

analysis of bacteria load of the wound bed pre and post-treatment and skin graft taken. A preliminary pilot study. *International Wound Journal*. 8(2): 155-161.

Ganguly, S., Tan, H. C., Lee, P. C. and Tham, K. W. (2015). "Metabolic bariatric surgery and type 2 diabetes mellitus: an endocrinologist's perspective". *Journal of Biomedical Research*. 29(2): 105-111.

Gardner, D. and Shoback, D. (2017). *Greenspan's Basic and Clinical Endocrinology.* 10th edn, McGraw-Hill Education, New York. pp. 740-844. ISBN: 1259589285.

Gareau, M. G., Sherman, P. M. and Walker, W. A. (2010). Probiotics and the gut microbiota in intestinal health and disease. *Journal of National Review Gastroenterology Hepathology*. 7(9): 503-514.

Garry, G. G., Jeroen, P., Manit, A., Richard, O. D., Matthew, P. D., Janna, D., Timothy, J. F., Jerry, R. J., Louise, C. G.,Carl, M. K., John, E. R., Peter, T. and Kenneth, M. M. (2011).Clinical pharmacokinetics of metformin. *Journal of Clinical Pharmacokinetics*. 50(2): 81-98.

Gijs, D. B., Besten, K. E., Albert, K. G. Koen, V., Dirk-Jan, R. and Barbara, M. B. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Resolution*. 54(9): 2325-2340.

Grimoud, J., Durand, H., Courtin, C., Monsan, P., Ouarné, F. and Theodorou, V. (2010). *In vitro* screening of probiotic lactic acid bacteria and prebiotic glucooligosaccharides to select effective synbiotics. *Journal of Anaerobe*. 5(4): 493-500.

Guarino, A., Guandalini, S., Lo, V. and Andrea, M. D. (2015). Probiotics for Prevention and Treatment of Diarrhea. *Journal of Clinical Gastroenterology*. 49(5): 37-45.

Guo, S. A. and DiPietro, L. A. (2010). Factors affecting wound healing. *Journal of Dental Research*. 89(1): 219-229.

Gupta, V. and Garg, R. (2009). *Probiotics*. *Indian Journal of Medical Microbiology.* 27(3): 202-209.

Habibuddin, M., Daghriri, H. A., Humaira, T., Al-Qahtani, M. S. and Hefzi, A. A. (2008). Antidiabetic effect of alcoholic extract of *Caralluma sinaica* L. on streptozotocin-induced diabetic rabbits. *Journal of Ethnopharmacology*. 117(2): 215-20.

Hagiwara, H., Seki, T. and Ariga, T. (2008). The effect of pregerminated brown rice intake on blood glucose and PAI-1 levels in streptozotocin-induced diabetic rats. *Journal of Bioscience, Biotechnology and Biochemistry*. 68(4): 444-447.

Haller, D., Antoine, J.M., Bengmark, S., Enck, P., Rijkers, G. and Lenoir-Wijnkoop, I. (2010). Guidance for substantiating the evidence for beneficial effects of probiotics: Probiotics in chronic inflammatory bowel disease (IBD) and the functional disorder, irritable bowel syndrome (IBS). *Journal of Nutrition*. 114400(3): 690-700.

Har, H. and Ja, A. S. (2007). Endothelial dysfunction in diabetes mellitus. *Journal of Vascular health and risk management.* 3(6): 853-876.

Hariprassana, K. (2017). *Foxtail millet, Setaria italica (L.) P. Beauv. In: Jagananth PV, editor. Millets and sorghum: biology and genetic improvement*. 3rd edn. Wiley: New York. pp. 112-148.

Hassan, F. H., Golnar, R. and Mohammad, R. F. (2012). Evaluation of wound healing activities of kefir products. *Journal of Burns*. 38(2): 719-723.

Haukioja, A. (2010). Probiotics and oral health. *European Journal of Dentist*. 4(1): 348-355.

Hoque, M. Z., Akter, F., Hossain, K. M., Rahman, M. S. M., Billah, M. M. and Islam, K. M. D. (2010). Isolation, identification and analysis of probiotic properties of *Lactobacillus* species from selected regional yoghurts. *World Journal of Dairy and Food Sciences.* 5(1): 39-46.

Hotz, C. and Gibson, R. S. (2007). Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. *Journal of Nutrition*. 137(1): 1097-1100.

Hu, E. A., Pan, A., Malik, V. and Sun, Q. (2012). "White rice consumption and risk of type 2 diabetes: meta-analysis and systematic review". *Britain Medical Journal.* 126(3): 344-354.

Ibourahema, C., Dauphin, R. D., Jacqueline, D. and Thonart, P. (2008). Characterization of lactic acid bacteria isolated from poultry farm in Senegal. *African Journal of Biotechnology*. 7(5): 2006-2012.

Ibrahim, F. S., Babiker, E. E., Yousif, N. E. and Eltinay, A. H. (2009). Effect of fermentation on biochemical and sensory characteristics of sorghum flour supplemented with whey protein. *Journal of Food Chemistry*. 92(2): 285-292.

Imran, P., Ayesha, R., Muhammad, S. and Muhammad, A. R. (2014). Exploring the Antioxidant Perspective of Sorghum and Millet. *Journal of Food Processing and Preservation*. 39(6): 132-140.

Inzucchi, S. E., Bergenstal, R. M., Buse, J. B., Diamant, M. and Ferrannini, E. (2012). Management of hyperglycaemia in type 2 diabetes: A patient centered approach.

Position statement of the American diabetes association (ADA) and the European association for the study of diabetes (EASD). *Journal of Diabetologia*. 55(6): 1577-1596.

Isolauri, E. (2008). Dietary modification of atopic disease: Use of probiotics in the prevention of topic dermatitis. *Journal of Allergy Asthma*. 4(3): 270-275.

Iwamoto, T., Suzuki, N., Tanabe, K., Takeshita, T. and Hirofuji, T. (2010). Effects of probiotic *Lactobacillus salivarius* WB21 on halitosis and oral health: An open-label pilot trial. Oral Surgery; Oral Pathology; Oral Radiology. *Journal of Endodontology*. 110(2): 201-208.

Jalal, B. Z. and Nasroallah, M. K. (2014). Physiological and pharmaceutical effects of Ginger (*Zingiber officinale*) as a valuable medicinal plants. *European Journal of Experimental Biology*. 4(1): 87-90.

Jeong, J., Junga H., Leea, S., Leea, H., Hwanga, K. T. and Kimb, T. (2010). Anti-oxidant, anti- proliferative and anti-inflammatory activities of the extracts from black raspberry fruits and wine. *Journal of Food Chemistry.* 123(4). 338–344.

Joao, J. M., Clara, I. L., Dolores, P. B. and Andreia, L. P. (2012). A rat model of diabetic wound infection for the evaluation of topical antimicrobial therapies. *Journal of comparative Medicine.* 62(1): 37-48.

Jones, M. L., Martoni, C. J. and Prakash, S. (2013). Oral supplementation with probiotic *L. reuteri* NCIMB 30242 increases mean circulating 25-hydroxyvitamin D: A post hoc analysis of a randomized controlled trial. *Journal of Clinical Endocrinology Metabolism*. 98(5): 2944-2951.

Kang, H. J. and Im, S. H., (2015). Probiotics as an immune modulator. *Journal of Nutrition Science Vitaminology*. 61(1): 103-105.

Kalui, C. M., Mathara, J. M. and Kutima, P. M. (2010). Probiotic potential of spontaneously fermented cereal based foods. *African Journal of Biotechnology*. 9(17): 2490-2498.

Kanmani, P., Kumar, R. S., Yuvaraj, N., Paari, K. A., Pattukumar, V. and Arul, V. (2013). Probiotics and its functionality valuable products-a review. *Journal of Food Science and Nutrition*. 53(4): 641–658.

Karna, B. K. L., Emata, O. C. and Barraquio, V. L. (2007). Lactic acid bacteria and probiotic bacterial from fermented and probiotic dairy products. *Journal of Science Diliman*. 19(2): 23-34.

Kato, A., Minoshima, Y., Yamamoto, J., Adachi, I., Watson, A. and Nash, R. (2008). Protective effects of dietary chamomile tea on diabetic complications. *Journal of Agriculture Food Chemistry.* 56(3): 8206–8211.

Katoh, K. and Frith, M. C. (2012). Adding unaligned sequences into an existing alignment using MAFFT and LAST. *Journal of Bioinformatics*. 28(5): 3144-3146.

Kenny, C. (2014). When hypoglycemia is not obvious: diagnosing and treating under recognized and undisclosed hypoglycemia. *Journal of Primary care diabetes*. 8(1): 3-11.

Kevin, D. C. (2007). Diet, nutrition and bone health. *Journal of Nutrition*. 137(11): 2507-2512.

King, S., Glanville, J., Sanders, M. E., Fitzgerald, A. and Varley, D. (2014). "Effectiveness of probiotics on the duration of illness in healthy children and adults who develop common acute respiratory infectious conditions: a systematic review and meta-analysis". *Britian Journal of Nutrition*. 112(1): 41-54.

Kitabchi, A. E., Umpierrez, G. E., Miles, J. M. and Fisher, J. N. (2009). Hyperglycemic crises in patients with diabetes. *Journal of Diabetes Care*. 32(7): 1335-1343.

Klatt, N. R., Canary, L. A., Sun, X., Vinton, C. L., Funderburg, N. T., Morcock, D. R., Quinones, M., Deming, C. B.and Perkins, M. (2013). Probiotic/prebiotic supplementation of antiretrovirals improves gastrointestinal immunity in SIV-infected macaques. *Journal of Clinical Investigations.* 123(3): 903-907.

Kyu, H. H., Bachman, V. F., Alexander, L. T., Mumford, J. E., Afshin, A., Estep, K., Veerman, L., Delwiche, K., Iannarone, M. L., Moyer, M. L., Cercy, K., Vos, T., Murray, C. J. L. and Forouzanfar, M. H. (2016). "Physical activity and risk of breast cancer, colon cancer, diabetes, ischemic heart disease, and ischemic stroke events: systematic review and dose- response meta-analysis for the Global Burden of Disease Study 2013". *British Medical Journal.* 354(3): 3857-3864.

Lam, E. K., Yu, L., Wong, H. P., Wu, W. K., Shin, V. Y. and Tai, E. K. (2007). Probiotic *Lactobacillus rhamnosus* GG enhances gastric ulcer healing in rats. *European Journal of Pharmacology*. 565(1): 171-179.

Lama, A., Bates, M. P. Covington, A. D., Allen, S. C. H. and Antunes, A. P. M. (2013). Methods of isolation and identification of pathogenic and potential pathogenic bacteria from skins and tannery effluents. *Journal of the American Leather Chemists Association*. 108(2): 48-62.

Lamsal, B. P. and Faubion, J. M. (2009). The beneficial use of cereal components in probiotic foods. *Journal of Food Review International*. 25(2): 103-114.

Lan, C. C., Liu, I. H., Fang, A. H., Wen, C. H. and Wu, C. S. (2008). Hyperglycaemic conditions decrease cultured keratinocyte mobility: implications for impaired wound healing in patients with diabetes. *British Journal of Dermatology*. 159(3): 1103-1115.

Lawlor, D. A., Selvin, E., Stampfer, M., Stehouwer, C. D, Lewington, S., Pennells, L., Thompson, A., Sattar, N., White, I. R., Ray, K. K. and Danesh, J. (2010). "Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies". *The Lancet*. 375(9733): 2215-2222.

Leboffe, M. (2014). *Microbiology Laboratory Theory and Application*. 3rd edn. Englewood, Company Morton Publishing. pp. 105. ISBN: 1617312800.

Lebot, V. (2009). *Tropical root and tuber crops: cassava, sweet potato, yams and aroids. Crop production science in horticulture.* 6th edn. CAB books, CABI, Wallingford, UK. pp. 144-158. ISBN: 978-1-84593- 424-8.

Lee, B. and Bak, Y. (2011). Irritable bowel syndrome, gut microbiota and probiotics. *Journal of Neurogastroenterol Motility*. 17(2): 252-266.

Lee, I. M., Shiroma, E. J., Lobelo, F., Puska, P., Blair, S. N. and Katzmarzyk, P. T. (2012). "Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy". *The Lancet Medical Journal.*380(9838): 219-29.

Lee, Y. K. and Salminen, S. (2009). *Handbook of Probiotics and Prebiotics*. 2nd edn. John Wiley and sons. Hoboken, New Jersey. Canada. pp. 596. ISBN: 978-0-470-13544-0.

Lenzen, S. (2008). "The mechanisms of alloxan and streptozotocin-induced diabetes". *Journal of Diabetologia*. 51(2): 216-226.

Liu, C., Lu, J., Lu, L., Liu, Y., Wang, F. and Xiao, M. (2010). Isolation, structural characterization and immunological activity of an exopolysaccharide produced by *Bacillus licheniformis* 8-37-0-1. *Journal of Bioresource Technology*. 101(14): 5528-33.

Makinde, F., and Oyeleke, O. (2012). Effect of sesame seed addition on the chemical and sensory qualities of sorghum based kunun- zaki drink. *African Journal of Food Science and Technology*. 3*:*204-212.

Malik, V. S., Popkin, B. M., Bray, G. A., Després, J. P. and Hu, F. B. (2010). "Sugar Sweetened Beverages, Obesity, Type 2 Diabetes and Cardiovascular Disease risk". *Journal of Circulation*. 121(11): 1356-1364.

Manuel, G. G., Marta, T. M., Romero, R. J., Pilar, G. M., Sanchez, M. J., Zarzuelo, M. O., Julio,

G. and Juan, D. (2015). Antihypertensive effects of probiotics *Lactobacillus* strains in spontaneously hypertensive rats. *Journal of Molecular Nutrition Research*. 59(11): 2326-2336

.

Maria, R. C., Pala, L. and Mannucci, E. (2013). "Role of insulin in the Type 2 diabetes therapy: past, present and future*". International Journal of Endocrinology and Metabolism*. 11(3): 137-144.

Martín, R., Soberón, N., Vaneechoutte, M., Flórez, A. B., Vázquez, F. and Suárez, J. E. (2008). Characterization of indigenous vaginal lactobacilli from healthy women as probiotic candidates. *International Journal of Microbiology*. 11(3): 261-266.

Martins, E. M., Ramos, A. M., Vanzela, E. S., Stringheta, P. C., Pinto, C. L. and Martins, J. M. (2013). Products of vegetable origin: A new alternative for the consumption of probiotic bacteria. *Food Research International Journal*. 51(2): 764-770.

Maurice, R. M. (2010). *Compositional Analysis of Food.* 3rd edn. Springer press. New York City. pp: 107-109.

McNaught, C. E., Woodcock, N. P., Anderson, A. D. and MacFie, J. (2008). A prospective randomised trial of probiotics in critically ill patients. *Journal of Clinical Nutrition*. 24(4): 211-219.

Meurman, J. H. and Stamatova, I. (2007). Probiotics: contributions to oral health. *Journal of Oral Diseases*. 13(4):443-451.

Michael, O. (2010). *Staphylococcus* colonization of the skin and antimicrobial peptides. *Expert Review Journal of Dermatology*. 5(4): 183-195

Milad, S. (2017). The effects of *Bacillus subtilis* probiotic on cutaneous wound healing in rats. *Journal of Novel Biomedical*. 5(1): 43-47.

Minaiyan, M., Ghannadi, A., Movahedian, A. and Hakim-Elahi, I. (2014). Effect of *Hordeum vulgare L.* (Barley) on blood glucose levels of normal and STZ-induced diabetic rats. *Research in Journal of Pharmaceutical Sciences.* 9(3): 173-178.

Mirjana, M., Milica, Ž., Jelena, A. J., Jovanović, Maja, T., Marija, S., inadinović[a]Jovana, R., Aleksandra, U., Svetlana, D., Nataša, G. and Melita, V. (2017). Oral administration of probiotic *Lactobacillus paraplantarum* BGCG11 attenuates diabetes-induced liver and kidney damage in rats. *Journal of Functional Food*. 38: 427-437.

Moayyedi, P., Ford, A. C. and Talley, N. J. (2010). The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Journal of British Society of Gastroenterology*. 59(3): 325-332.

Mohan, B. H., Malleshi, N. G. and Koseki, T. (2010). Physico-chemical characteristics and non-starch polysaccharide contents of *Indica* and *Japonica* brown rice and their malts. *Journal of Food Science and Technology*. 43(5): 784-791.

Mohanraj, R.1.and Sivasankar, S. (2013). Sweet potato (*Ipomoea batatas* [L.] Lam) a valuable medicinal food. *Journal of Medicinal Food*. 17(7): 733-41.

Mohd A. F. and Tan, I. K. (2007). Isolation of lactic acid bacteria from Malaysian food and assessment of the isolates for industrial potential. *Journal of Bioresources Technology*. 98(7): 1380-1385.

Moree, S. S., Kavishankar, G. B. and Rajesha, J. (2013). Antidiabetic effect of secoisolariciresinol diglucoside in streptozotocin-induced diabetic rats. *Journal of Phytomedicine*. 20(4): 237-245.

Mortazavian, A. M., Khosrokhvar, R., Rastegarand, H. and Mortazaei, G. R. (2010). Effects of dry matter standardization order on biochemical and microbiological characteristics of freshly made probiotic Doogh (Iranian fermented milk drink). *Italy Journal of Food Science.* 22(3): 98-102.

Moro-Garcia, M. A., Alonso-Arias, R., Baitadjieva, M., Fernandez, B. C. Fernandez, M. A., Diaz, R. E., Alonso, S. R., Alvarez, S. M. Saavedra, M. J. and Lopez-Larrea, C. (2013). Oral supplementation with *Lactobacillus delbrueckii* subsp. *bulgaricus* 8481 enhances systemic immunity in elderly subjects. 35(4): 1311-1326.

Moyane, J. N. and Jideani, A. I. O. (2013). The physicochemical and sensory evaluation of commercial sour milk (*amasi*) products. *African Journal of Food Science*. 7(6):56-62.

Muhammad, N. O., Akolade, J. O., Usman, L. A. and Oloyede, O. B. (2012). Haematological parameters of alloxan–induced diabetic rats treated with leaf essential oil of Hoslundia opposite (Vahl). *Journal of Experimental and Clinical Services*. 11(1): 670-676.

Murch, S. H. (2008). Toll of Allergy reduced by probiotics. *Journal of The Lancet.* 357(4): 1057-1059.

Murrray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. I. and Pfaller, M. A. (2007). *Manual of Clinical Microbiology*. 9th edn. ASM press, Washington, DC. pp. 175-280.

Mutegi, E., Sagnard, F., Muraya, M., Kanyenji, B., Rono, B., Mwongera, C., Marangu, C., Kamau, J., Parzies, H., de Villiers, S., Semagn, K., Traoré, P. and Labuschagne, M. (2010). "Ecogeographical distribution of wild, weedy and cultivated *Sorghum bicolor* (L.) Moench in Kenya: implications for conservation and crop-to-wild gene flow". *Journal of Genetic Resources and Crop Evolution*. 57(2): 243-253.

Nele, K., Lisa, M., Hanna, G., Sunil, D., Saroj, B. Eriksson, O. S., Eriksson, S., Roos, S. L., Hong, S. and Ann-Beth, J. (2016). Lactobacilli Reduce *Helicobacter pylori* Attachment to Host Gastric Epithelial Cells by Inhibiting Adhesion Gene Expression. *Journal of Infected Immunology.* 84(5): 1526-1535.

Nguyen, D. T., Van, H. K., Cnockaert, M., De-Brandt, E., Aerts, M. B., Thanh, L. and Vandamme, P. (2013). A description of the lactic acid bacteria microbiota associated with the production of traditional fermented vegetables in Vietnam. *International Journal of Food Microbiolology*. 163(4):19-27.

Nielsen, S. S. (2010). Vitamin C determination by indophenol Method. *In: Food Analysis Laboratory Manual*. 4th edn. Springer International Publishing. Boston. pp. 55-56. ISBN: 978-1-4419-1462-0.

Niers, L., Martin, R., Rijkers, G., Sengers, F., Timmerman, H., Van, U. N., Smidt, H., Kimpen, J. and Hoekstra, M. (2009). The effects of selected probiotic strains on the development of eczema. *Journal of Allergy*. 64(9): 1349-1358.

Nikolic, M., Terzic-Vidojevic, A. and Jovcic, B. (2008). Characterization of lactic acid bacteria isolated from Bukuljac, a homemade goat's milk cheese. *International Journal of Food Microbiology*. 122(12): 162-170.

Nkama, I., Agarry, O. O. and Akoma, O. (2010). Sensory and nutritional quality characteristics of powdered Kunun-zaki; A Nigerian fermented cereal beverage. *African Journal of Food Science*. 4(6): 364-370.

Norman, N. P. and Joseph, H. H. (2007). *Food Science*. 5th edn. CBS Publishes and distributors limited. New Delhi. pp. 24-44. ISBN 978-1-4615-4985-7.

Nuzulul, H., Amandia, D., Permana, S. and Hafiedz, M. (2015). Rat Diabetic Blood Glucose Level Profile with Stratified Dose Streptozotocin (SD-STZ) and Multi Low Dose Streptozotocin (MLD-STZ) Induction Methods. *Journal of Tropical Life Science.* 5(1): 30-34.

Nwachukwu, E. Achi, O. K. and Ijeoma, I. O. (2010). Lactic acid bacteria in fermentation of cereals for the production of indigenous Nigerian foods. *African Journal of Food Science and Technolology*. 1(1): 21-26.

Oak, S. J. and Jha, R. (2018). The effects of probiotics in lactose intolerance: A systematic review. *Journal of Critical Review Food Science and Nutrition*. 9(2): 1-9.

Oakley, B. B., Fiedler, T. L., Marrazzo, J. M. and Fredricks, D. N. (2008). "Diversity of human vaginal bacterial communities and associations with clinically defined bacterial vaginosis." *Applied and Environmental Microbiology*. 74(15): 4898-4909.

Ochuko, L. E., Osaretin, A. T., Ebuehi, F. O., Adeboyejo, M. A. and Gloria, N. E. (2013). Hematological and biochemical changes in diabetic rats fed with fiber-enriched cake. *Journal of Acute Medicine*. 3(2): 39-44.

O'Connor, P. M., Ross, R. P., Hill, C. and Cotter, P. D. (2015). Antimicrobial antagonists against food pathogens: a bacteriocin perspective. *Journal of Current Opinion Food Science.* 2(1): 51–57.

Odebode, S. O., Egeonu, N. and Malschy, O. A. (2008). Promotion of sweet potato for the food industry in Nigeria. *Bulgarian Journal of Agricultural Science*. 14(1): 300-308.

Odom, T. C., Udensi, E. A., Dike, C. O., Ogbuji , C. A., Kanu, A. M. and Aji, R. U. (2012). Comparative studies of ginger (*Zingiber officinale*) and black pepper extracts at different concentrations on the microbial quality of soymilk and kunun- zaki. *African Journal of Biotechnology.* 11(70): 13494-13497.

Ogbonna, A. C., Abuajah, C. I., Akpan, M. F. and Udofia, U. S. (2013). A comparative study of the nutritional values of palmwine and kunun-zaki. *Annals Food Science and Technology.* 14:39-43.

Ohara-Nemoto, Y., Haraga, H., Kimura, S. and Nemoto, T. K. (2008). Occurrence of staphylococci in the oral cavities of healthy adults and nasal oral trafficking of the bacteria. *Journal of Medical Microbiology*. 57(1): 95-99.

Ohlsson, A. and Aher, S. M. (2012). *Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants.* Cochrane Database System Reviewed. 9:CD004863.

Okereke, H. C., Achi, O. K., Ekwenye, U. N. and Orji, F. A. (2012). Antimicrobial properties of probiotic bacteria from various sources. *African Journal of Biotechnology*. 11(39): 9416- 9421.

Okonkwo, P.O. and Okoye, Z. S. (2014). Comparative effects of antidiabetic drug, metformin and deferoxamine, on the hepatotoxic and nephrotoxic side effects of streptozotocin-induced diabetes mellitus in rats. *British Journal of Pharmaceutical Research*. 4(15): 1820-1832.

Olosunde, O. O., Abiodun, O. A., Amanyunose, A. A. and Adepeju, A. B. (2014). Sensory and nutritional characteristics of 'kununzaki' enriched with Moringa (*Moringa oleifera*) seed flour. *American Journal of Experimental Agriculture*. 4(9): 1027-1035.

Olufunke, A. P. and Oluremi, K. O. (2015). Assessment of nutritional and sensory quality of kunun zaki – A Home-made Traditional Nigerian beverage. *Journal of Nutrition and Food Science*. 5(2): 358-465.

Oluwajoba, S. O., Akinyosoye, F. A. and Oyetayo, V. O. (2013). Comparative proximate evaluation of spontaneously fermenting 'kunun-zaki' made from germinated and ungerminated composite cereal grains. *Journal of Food Science and Nutrition*. 1(4): 336-349.

Oluwaseyi, K. A. (2013). Physicochemical characterization of sweet potato starches popularly used in Chinese starch industry. *Journal of Food Hydrocolloids*. 33(2):169-177.

Omemu, A. M., Oyewole, O. B. and Bankole, M. O. (2007). Yeasts and mould associated with ogi-A cereal based weaning food during storage. *Research Journal of Microbiology*. 2(2): 141-148.

Omowaye, O. A. and Oluwamukomi, M. O. (2015). Physical and nutritional composition of instant kunun-zaki powder obtained by three drying methods. *Journal of Research in Sciences*. 1(5): 114-122.

Onat, A. G., Can, H. and Kaya, G. (2010). Hergenc atherogenic index of plasma' (triglyceride/high-density lipoprotein-cholesterol) predicts high blood pressure, diabetes, and vascular events. *Journal of Clinical Lipid*. 4(2): 89-98.

Orel, R. and Trop, T. K. (2014). Intestinal microbiota, probiotics and prebiotics in inflammatory bowel disease. *World Journal Gastroenterology.* 20(4): 11505-11524.

Orthoefer, F. T. (2008). *Rice Bran Oil.* 6th edn. John Wiley and Sons. Printing press. pp. 465. ISBN: 978-0-471-38460-1.

Oyedemi, S. O., Adewusi, E. A., Aiyegoro, D. A. and Akinpelu, O. A. (2011). Antidiabetic and haematological effect of aqueous extract of stem bark of *Afzelia africana* (Smith) on streptozotocin-induced diabetic wistar rats. *Asian Journal of Tropical Biomedical*. 1(6): 353-358.

Pablo, C. B., Lollo, P. N., Morato, C. S., Moura, C. N., Almada, T. L., Felicio, E. A., Esmerino, M. E., Barros, J. A., Farfan, A. S., Santana, R. S., Raices, M. C. and Silva, A. G. (2015). Hypertension parameters are attenuated by the continuous consumption of probiotic minas cheese. *Journal of Food Research International*. 76(3): 611-617.

Panigrahi, P., Braileanu, G. T. and Chen, H. (2007). Probiotic bacteria change *Escherichia coli*- induced gene expression in cultured colonocytes: Implications in intestinal pathophysiology. *World Journal of Gastroenterology.* 13(4): 6370–6378.

Parvez, S., Lee, H. C., Kim, D. S. and Kim, H. Y. (2009) Bile salt hydrolase and chlolesterol removal effect by *Bifidobacterium bifidum* NRRL 1976. *World Journal of Microbiology Biotechnology.* 22(5): 455-459.

Pasias, I. N., Thomaidis, N. S. and Piperaki, E. A. (2013). Determination of total arsenic, total inorganic arsenic and inorganic arsenic species in rice and rice flour by electrothermal atomic absorption spectrometry. *Journal of Microchemical*. 108: 1-6.

Pasquel, F. J. and Umpierrez, G. E. (2014). "Hyperosmolar hyperglycemic state: a historic review of the clinical presentation, diagnosis, and treatment". *Journal of Diabetes Care*. 37(11): 3124-3131.

Patel, A., Shah, N. and Prajapati, J. B. (2014). Clinical application of probiotics in the treatment of *Helicobacter pylori* infection-a brief review. *Journal of Microbiology Immunology Infection*. 47(10): 429-37.

Patel, R., Shervington, A., Pariente, J. A., Martinez-Burgos, M. A., Salido, G. M., Adeghate, E. and Singh, J. (2008). Mechanism of exocrine pancreatic insufficiency in streptozotocin-induced type 1 diabetes mellitus. *Journal of Annual New York Academic Science*. 1084:71-88.

Patrick, O. M. (2012). Lactic acid bacteria in health and disease. *Rwanda Journal of Health Science*. 1:39-50.

Peera, H. and James, V. (2013). Effects of probiotic on gut microbiota mechanisms of intestinal immunomodulation and neuromodulation. *Journal of Theraeutic Advances in Gastroenterology*. 6(1): 39-51.

Pelinescu, D. R., Sasarmaan, E., Chifiriuc, M. C., Staca, I., Nohita, A. M., Avram, I., Serbancea, F. and Dimov, T. V. (2009). Isolation and identification of some

Lactobacillus and *Enterococcus* by a polyphasic taxonomical approach. *Romaniun Biotechnology Letters*. 14(7): 4225-4233.

Pessi, T., Sutas, Y., Hurme, M. and Isolauri, E. (2009). Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* GG. *Journal of Clinical Allergy*. 30:1804-1808.

Peter, J., Bannes, D. M., Drazen, J. M., Rennard, S I. and Thomson, N. C. (2009). *Asthma and COPD: Basic Mechanisms and Clinical Management.* 2nd edn. Academic Press USA. pp. 113-157. ISBN: 978-012-374001-4

Phumudzo, T., Ronald, N., Khayalethu, N. and Fhatuwani, M. (2013). Bacteria species identification getting easier. *African Journal of Biotechnology*. 12(41): 5975-5982.

Picot, J., Jones, J., Colquitt, J. L., Gospodarevskaya, E. Loveman, E., Baxter, L., Clegg, A. J. and (2009). "The clinical effectiveness and cost-effectiveness of bariatric (weight loss) surgery for obesity: a systematic review and economic evaluation". *Journal of Health Technology Assessment.* 41(1): 215-357.

Pignone, M., Alberts, M. J, Colwell, J. A, Cushman, M., Inzucchi, S. E., Mukherjee, D., Rosenson, R. S., Williams, C. D., Wilson, P. W. and Kirkman, M. S. (2010). "Aspirin for primary prevention of cardiovascular events in people with diabetes: a position statement of the American Diabetes Association, a scientific statement of the American Heart Association, and an expert consensus document of the American College of Cardiology Foundation". *Journal of Diabetes Care.* 33(6): 1395-1402.

Pinto, M., Barjas-Castro, M. L. and Nascimento, S. (2013). The new noninvasive occlusion spectroscopy hemoglobin measurement method: a reliable and easy anemia screening test for blood donors. *Journal of Transfusion*. 53(4): 766-769.

Plaza-Diaz, J., Fernandez-Caballero, J. A. and Chueca, N. (2015). Pyrosequencing analysis reveals changes in intestinal microbiota of healthy adults who received a daily dose of immunomodulatory probiotic strains. *Journal of Nutrients*. 7(2): 3999–4015.

Podkowik, M., Park, J. Y., Seo, K. S. Bystron, J. and Bania, J. (2013). Enterotoxigenic potential of coagulase-negative staphylococci. *International Journal of Food Microbiology.* 163(1): 34-40.

Preidis, G. A., Saulnier, D. M. and Blutt, S. E. (2012). Probiotics stimulate enterocyte migration and microbial diversity in the neonatal mouse intestine. *Federation of American Societies for experimental Biology*. 26(3): 1960-1969.

Pundir, R. K., Rana, S., Kashyap, N. and Kaur, A. (2013). Probiotic potential of lactic acid bacteria isolated from food samples an *in vitro* study. *Journal of Applied Pharmceutical Science.* 3(3): 85-93.

Rabot, S., Rafter, J., Rijkers, G., Watzl, B. and Antoine, J. M. (2010). Guidance for substantiating the evidence for beneficial effects of probiotics: Impact of probiotics on digestive system metabolism. *Journal of Nutrition*. 140(3): 677-689.

Ramsay, A. J., Reid, J. C. and Velasco, G. (2008). The type II transmembrane serine protease matriptase-2 - Identification, structural features, enzymology, expression pattern and potential roles. *Frontier Journal of Bioscience.* 13(1): 569-579.

Randazzo, C. L., Restuccia, C., Romano, A. D. and Caggia, C. (2009). *Lactobacillus casei*, dominant in naturally fermented sicilian green olives. *International Journal of Food Microbiology.* 90(8): 9-14.

Ravinder, N., Ashwani, K., Manoj, K., Pradip, V. B., Shalini, J. and Hariom, Y. (2012). Probiotics, their health benefits and applications for developing healthier foods: a review. *Journal of Microbiology*. 334(1): 1-15.

Reddy, M., Gill, S. S. and Wu, W. (2012). "Does this patient have an infection of a chronic wound?". *Journal of the American Medical Association.* 307(6): 605-11.

Reis, J. A., Paula, A. T., Casarotti, S. N., Penna, A. L. B. (2012). Lactic acid bacteria antimicrobial compounds: characteristics and applications. *Journal of Food Engineering Review.* 4(5):124–140.

Rijkers, G. T., Bengmark, S., Enck, P., Haller, D., Herz, U., Kalliomaki, M., Kudo, S., Lenoir-Wijnkoop, I., Mercenier, A., Myllyluoma, E., Rabot, S., Rafter, J., Szajewska, H., Watzl, B., Wells, J., Wolvers, D. and Antoine, J. M. (2010). "Guidance for substantiating the evidence for beneficial effects of probiotics: current status and recommendations for future research". *Journal of Nutrition*. 140(3): 671-678.

Rippe, J. M. and Richard, S. (2010). *Manual of intensive care medicine*. 5th edn. Philadelphia: Wolters Kluwer Health/Lippincott Williams and Wilkins Printing Press. pp. 549. ISBN: 9780781799928.

Ripsin, C. M., Kang, H. and Urban, R. J. (2009). "Management of blood glucose in type 2 diabetes mellitus. *Journal of American Family Physician*. 79(1): 29-36.

Risérus, U., Willett, W. C. and Hu, F. B. (2009). "Dietary fats and prevention of type 2 diabetes". *Journal of Progress in Lipid Research*. 48 (1): 44-51.

Roberfroid, M., Gibson, G. R. and Hoyles, L. (2010). *Prebiotic effects: metabolic and health benefits*. *Britian Journal of Nutrition.* 104(2): 1-63.

Robert, K. M. (2012). *Harper's Illustrated Biochemistry*. 29th edn. New York: McGraw-Hill Medical. pp. 243-256. ISBN: 007176576X.

Robert, L. B. (2010). *Compositional analysis of Food*. 4th edn. Springer International Publishing. Boston. pp. 81-89. ISBN: 978-1-4419-1462-0.

Robert, P. A. and Abish, S. S. (2017). Use of probiotics and oral health. *Journal of Current Oral Health Report*. 4(4): 309-318.

Roger, W. H., Curtis, C. H., Louise, L. Elias, I. S., Mark, P., Elton Z., Frank, S., Niels, J. David, H. W. and Kei, S. (2018). Metformin reduces liver glucose production by inhibition of fructose-1-6-bisphosphatase. *Journal of Nature Medicine*. 24(8): 1395-1406.

Romana-Souza, B., Nascimento, A.P. and Monte-Alto, C. A. (2009). Propranolol improves cutaneous wound healing in streptozotocin- induced diabetic rats. *European Journal of Pharmacology*. 611:77-84.

Ronald, B. P., Landen, W. O. and Ronald, R. E. (2010). *Compositional analysis of Food*. 4th edn. Springer International Publishing. Boston. pp. 189-190.

Rowland, M. K., John, K. J., Mojisola, O. A., Adegbola, O. D., Sarafa, A. A., Bukola, I. K., Adeshola, A. B. and Stephen, O. O. (2017). Effects of addition of different spices on the quality attributes of Tiger-nut milk (kunun-aya) during storage. *Journal of Microbiology, Biotechnology and Food Science*. 7(1): 1-6.

Ruozhi, Z., Nora, G., Jiansu, W., Khuong, L., Chunyang, L., Mohammed, H., Moghadasian, Y. L., Slow, F. B., Apea-Bah, T. B., Zhengfeng, Y. and Garry, X. S. (2018). Isolation of phytotoxic phenols and characterization. *Journal of Agricultural and Food Chemistry*. 66(17): 4512-4520.

Ryan, K. A., Daly, P., Li, Y., Hooton, C. and Otoole, P. W. (2008). Strain-specific inhibition of *Helicobacter pylori* by *Lactobacillus salivarius* and other *Lactobacilli.Journal of Antimicrobial Chemotherapy*. 61(4): 831-834.

Sade, F. O. (2009). Proximate, antinutritional factors and functional properties of processed pearl millet (*Pennisetum glaucum*). *Journal of Food Technology.* 7(3): 92-97.

Saikali, J., Picard, V., Freitas, M. and Holt, P. (2008). Fermented milks, probiotic cultures, and colon cancer. *Journal of Nutritional Cancer*. 49: 14-24.

Saji, S., Kumar, S. and Thomas, V. (2011). "A randomized double blind placebo controlled trial of probiotics in minimal hepatic encephalopathy," *Tropical Gastroenterology*. 32(2): 128-132.

Saleh, A. S. M., Zhang, Q., Chen, J. and Shen, Q. (2013). Millet grains: nutritional quality, processing, and potential health benefits. *Journal of Comparative Review Food Science Food Safety.* 12(2): 281-295.

Sandeep, K. P., Smita, H. P., Manas, R. S., Ramesh, C. R. and Eugenie, K. (2015). Anthocyanin- rich sweet potato (*Ipomomea batatas* L.) Beer: Technology,

biochemical and sensory evaluation. *Journal of Food Processing and Preservation*. 39(6): 225-252.

Sanders, M. E. (2008). Probiotics: definition, sources, selection, and uses. *Journal of Clinical Infectious Diseases*. 46(2): 58-61.

Sarwar, N., Gao, P., Seshasai, S. R., Gobin, R., Kaptoge, S. Di Angelantonio, E. Ingelsson, E. Lawlor, D. A., Selvin, E., Stampfer, M., Stehouwer, C. D., Lewington, S., Pennells, L., Thompson A., Sattar, N., White, I. R., Ray, K. K, and Danesh, J. (2010). "Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies". *The Lancet Journal of Medicine*.375(9733): 2215-2222.

Saunders, S. G., Bocking, A. J. Challis, J. and Reid, G. (2007). "Effect of *Lactobacillus* challenge on *Gardnerella vaginalis* biofilms." *Journal of Colloids and Surfaces*. 55(2): 138-142.

Schulman, A. P., Del Genio, F., Sinha, N. and Rubino, F. (2009). ""Metabolic" surgery for treatment of type 2 diabetes mellitus*"*. *Journal of Endocrine Practice.* 15(6): 624-631.

Scully, C. and Greenman, J. (2008). Halitosis (Breath odour). *Journal of Periodontology*. 48(14): 66-75.

Sekhon, B. S. and Jairath, S. (2010). *Prebiotics, probiotics and synbiotics: an overview*. *Journal of Pharmaceutical Education Research.* 1(1): 13-36.

Selvin, E., Steffes, M. W., Zhu, H., Matsushita, K., Wagenknecht, L., Pankow, J., Coresh, J., and Brancati, F. L. (2010). "Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults". *Northern England Journal of Medical*. 362(9): 800-811.

Sgouras, D., Maragkoudakis, P., Petraki, K., Martinez-Gonzales, B., E. Eriotrou, E., Michopoulos, S., Kalantzopoulos, G., Tsakalidou, E. and Mentis, A. (2008). In vitro and invivo inhibition of *Helicobacter pylori* by *Lactobacillus casei* strain shirota. *Journal of Applied Environment Microbiology*. 70(1): 518-526.

Shafrir, E. (2010). Contribution of animal models to the research of the causes of diabetes. *World Journal of Diabetes*. 1(6): 137-140.

Shah, N. P. (2007). Functional cultures and health benefits. *Journal of International Dairy*. 12(3):1262-1277.

Shah, V. B., Shah, B.S. and Puranik, G. V. (2011). Evaluation of non-cyanide methods for haemoglobin estimation. *Indian Journal of Pathology Microbiology*. 54(4): 764-768.

Shahbazian, H., Yazdanpanah, L. and Latifi, S. M. (2013). Risk assessment of patients with diabeties for foot ulcers according to risk classification consensus of international

working group on diabetes foot (IWGDF). *Pakistan Journal of Medical Science*. 29(3): 730-734.

Shaw, J. E., Sicree, R. A. and Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Journal of Diabetes Research Clinical Practcise*. 87(1): 4-14.

Shirin, A. P. and Jamuna, P. (2010). Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*). *Journal of Medicinal Plants.* 4(24): 2674-2679.

Shoback, D. and David, G. G. (2011). *Greenspan's basic and clinical endocrinology*. 9th edn. New York. McGraw-Hill Medical. pp. 19. ISBN 9780071622431.

Shukla, Y. and Singh, M. (2007). Cancer preventativess properties of ginger: a brief review Journal *of Food Chemistry Toxicology*. 45(5): 683-690.

Sigmon, J. (2008). The starch hydrolysis test. http://www.asmscience.org/content/education/imagegallery/image.3172. Accessed 14th September, 2017.

Simona, C. and Minodora, D. (2013). Diabetes and beta cell function: from mechanisms to evaluation and clinical implications. *Journal of Biochemia Medica.* 23(3): 266-280.

Singh, P. and Raghuvanshi, S. R. (2012). Finger millet for food and nutritional security. *African Journal of Food and Science.* 6(3): 77-84.

Siwaporn, P., Pattanee, W., Richard, F. H. and Rita, W. (2013). Extruded rice grains fortified with zinc, iron and vitamin A increase zinc status of Thai school children when incorporated into a school lunch program. 143(3):362-368.

Snyder, R. J. and Hanft, J. R. (2009). Diabetics foot ulcers- effects on cost and mortality and the advanced-care therapies. *Journal of Ostomy Wound Manage*. 55(11): 28-38.

Soccol, C. R., Vandenberghe, L. P., Spier, M. R., Medeiros, A. B. P., Yamaguishi C. T., De-Dea, L., Pandey, J. A. and Thomaz-Soccol, V. (2010). The potential of probiotics: as a review. *Journal of Food Technolology Biotechnology*. 48 (4): 413-434.

Solange, K. G., Gilbert, D.J. E., Koffi, M. and Bassirou, B. (2014). Review on African traditional cereal beverages. *American Journal of Research Communication*. 2(5): 103-153.

Soltan, M. M., Dallal, S., Zamaniahari, A., Davoodabadi, M. and Rajabi, Z. (2017). Identification and characterization of probiotic lactic acid bacteria isolated from traditional persian pickled vegetables. *Journal of German Society Hygiene Infection Control*. 9(4): 15-23.

Somashekar, B. S. and Sudhakar, P. (2013). Evaluation of *Centella asiatica* leaf extract for wound healing in streptozotocin induced diabetic rats. *Journal of Pharmaceutical, Biological and Chemical.* 4(2): 1082-1090.

Sperottoa, R. A., Ricachenevsky, F. K., de Abreu W. V. and Fettb, J. P. (2012). Iron biofortification in rice: Its a long way to the top. *Journal of Plant Science*. 190(5): 24-39.

Srilakshmi, B. (2015). *Food Science*. 6th edn. New Age International Limited Publisher. New Delhi. pp. 47. ISBN: 978-81-224-3809-3.

Stanton, C., Ross, R. P., Fitzgerald, G. F. andVan, S. D. (2009). Fermented functional foods based on probiotics and their biogenic metabolites. *Current Opinon in Biotechnology*. 16(2):198-203.

Stookey, J. D., Burg, M., Sellmeyer, D. E., Greenleaf, J. E. and Arieff, A. (2007). Aproposed method for assessing plasma hypertonicity in vivo. *European Journal of Clinical Nutrition*. 61: 143-146.

Strozzi, G. P. and Mogna, L. (2008). Quantification of folic acid in human feces after administration of *Bifidobacterium* probiotic strains. *Journal Clinical Gastroenterology*. 42(2): 179-184.

Tajehmiri, A., Daranaki, R. K., Moslem, M. N., Aliabadi, M. A., Lozoumi, Z. and Kolavani, M. H. (2014). Antimicrobial Activity of *Lactobacillus* spp isolated from commercial yoghurts against pathogenic bacteria. *Journal of Pure and Applied Microbiology*. 8(3): 2211-2215.

Takahashi, N., Xiao, J. Z., Miyaji, K. and Iwatsuki, K. (2007). H+-ATPase in the acid tolerance of *Bifidobacterium longum*. *Journal of Milchwissenschaft.* 62: 151-153.

Talliand, A., Motley, T. A., Wunderlich, R. P., Dickerson, J. E., Waycaster, C. and Slade, H. E. (2013). Clinical and economic assessment of diabetic foot ulcer debridement with collagenase: results of a randomized controlled study. *Journal of Clinical Therapy*. 35(): 1805-1820.

Tambekar, D. H. and Bhutada, S. A. (2010). Studies on antimicrobial activity and characteristics of bacteriocins produced by *Lactobacillus* strains isolated from milk of domestic animals. *Internet Journal of Microbiology*. 8(2): 82-88.

Tamime, A. Y., Saarela, M., Sondergaard, A.K., Mistry, V. V. and Shah, N. P. (2008). *Production and maintenance of viability of probiotic microorganisms in dairy products. In: Tamime AY.* 2nd edn. *Probiotic Dairy Products*, Blackwell Publishing Limited. Uk. pp. 39-72.

Tenpe, C. R. and Yeole, P. G. (2009). Comparative evaluation of antidiabetic activity of some marketed polyherbal formulations in alloxan induced diabetic rats. *International Journal of Pharmaceutical Technical Research.* 1(1): 43-49.

Thankachan, P., Rah, J. H. and Thomas, T. (2012). Multiple micronutrient- fortified rice affects physical performance and plsma vitamin B-12 and homocysteine concentrations of Indian school children. *Journal of Nutrition*. 142(5): 1-8.

Thomas, A. W. and Kevin, M. V. (2016). Macrophages in tissue repair, Regeneration, and fibrosis. *Journal of Immunity*. 44(3): 450-462.

Tian, S., Nakamura, K. and Kayahara, H. (2008). Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. *Journal of Agricultural Food Chemistry*. 52(5): 4808-4813.

Tille, P. M. (2014). *Bailey and Scott's diagnostic microbiology*. 13th edn. Mosby, Inc., an affiliate of Elsevier Inc. 3251 Riverport Lane. St. Louis Missouri 63043. pp. 543.

Tomas, M. S., Claudia, O. M., Ocana, V. and Elena Nader-Macias, M. (2008). Production of antimicrobial substances by lactic acid bacteria I: determination of hydrogen peroxide. Methods. *Journal of Molecular Biology*. 268(8): 337–346.

Tong, P. C., Lee, K. F., So, W. Y., Ng, M. H., Chan, W. B., Lo, M. K., Chan, N. N. and Chan, J. C. (2010). White blood cell count is associated with macro- and microvascular complications in chinese patients with type 2 diabetes. *Journal of Diabetes Care*. 27(1): 216-222.

Tortora, G. J., Funke, B. R., and Case, C. L. (2013). *Microbiology: An Introduction.* 11th edn. Pearson Benjamin Cummings publishers. San Francisco New York. pp. 432-457. ISBN: 9780321798541.

Tortora, G. J., Funke, B. R., and Case, C. L. (2010). *Microbiology: An Introduction.* 10th edn. Pearson Benjamin Cummings publishers. San Francisco New York. pp. 135. ISBN: 9780534406530.

Towo, E., Matuschek, E., and Svanberg, U. (2008). Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds, phytate and in vitro accessible iron. *Journal of Food Chemistry*: 94(3): 369-376.

Tripathi, B. and Platel, K. (2011). Iron fortification of finger millet (*Eleucine coracana*) flourwith EDTA and folic acid as co-fortificants. *Journal of Food Chemistry.* 126(2): 537-542.

Tripathi, B., Platel, K. and Srinivasan, K. (2012). Double fortification of sorghum (*Sorghum bicolor L. Moench*) and finger millet (*Eleucine coracana L. Gaertn*) flours with iron and zinc. *Journal of Cereal Science.* 55(5): 195-201.

Tripathy, B. B., Chandalia, H. B. and Das, A. K. (2012). *RSSDI textbook of Diabetes mellitus*.2nd edn. New Delhi: Jaypee Brothers Medical Publishers. pp. 235. ISBN: 9789350254899.

Trivedi, U., Parameswaran, S., Armstrong, A., Burgueno-Vega, D., Griswold, J., Dissanaike, S., Rumbaugh, K. P. (2014). Prevalence of multiple antibiotic resistant infections in diabetic versus nondiabetic wounds. *Journal of Pathogens.* 43(1): 78-81.

Tuomi, T., Santoro, N., Caprio, S., Cai, M., Weng, J. and Groop, L. (2014). "The many faces of diabetes: a disease with increasing heterogeneity". *The Lancet Journal*. 383 (9922): 1084-1094.

Uvere, P. O. and Amazikwu, U.C J. (2011). Processing and evaluation of instant Kunun zaki from millet-cowpea malt and millet-soybean malt. *African Journal of Food Science*. 5(14): 761-768.

Vaghef-Mehrabany, E., Alipour, B., Homayouni-Rad, A., Sharif, S. K., Asghari-Jafarabadi, M. and Zavvari, S. (2014). Probiotic supplementation improves inflammatory status in patients with rheumatoid arthritis. *Journal of Nutrition.* 30(4): 430-435.

Vanderhoof, J. A. (2008). "Probiotics in allergy management". *Journal of Pediatric Gastroenterology and Nutrition.* 47(2): 38-46.

Vanysacker, L., Declerck, S. A., Hellemans, B. and De-Meester, L. (2010). Bacterial community analysis of activated sludge: an evaluation of four commonly used DNA extraction methods. *Journal of Applied Microbiology Biotechnology*. 88(5): 299-307.

Verrotti, A., Scaparrotta A., Olivieri, C. and Chiarelli, F. (2012). "Seizures and type 1 diabetes mellitus: current state of knowledge". *European Journal of Endocrinology*. 167(6): 749-58.

Versalovic, J. (2007). "Probiotics: intestinal gatekeeping, immunomodulation, and hepatic injury," *Journal of Hepatology*. 46(3): 618-621.

Verse, M. and J. Schrezenmeir, J. (2009). Probiotics and non-intestinal infectious conditions. *Britian Journal of Nutrition*. 88(1): 59-66.

Vesterlund, S. (2012). Water activity in dry foods containing live probiotic bacteria. *International Journal of Food Microbiology*. 157(2): 319-321.

Vijan, S. (2010). "Type 2 diabetes". *Annals Journal of Internal Medicine*. 152(5): 31-37.

Vinay, K. and Dinesh, K. (2014). Antioxidant and anti-inflammatory potential of curcumin accelerated the cutaneous wound healing in streptozotocin- induced diabetic rats. *International Journal of Immunopharmacology.* 20(2): 322-330.

Viswanathan, V., Jasmine, J. J., Snehalatha, C. and Ramachandran, A. (2008). "Prevalence of pathogens in diabetic foot infection in south Indian type 2 diabetic patients," *Journal of Association of Physicians of India*. 50(8): 1013–1016.

Vivian, A. and Fonseca, M. D. (2009). Defining and characterizing the progression of type 2 diabetes. *Journal of Diabetes Care*. 32(2): 151-156.

Vong, L., Lorentz, R. J. and Assa, A. (2014). Probiotics *Lactobacillus rhamnousus* inhibits the formation of neutrophil extracellular traps. *Journal of Immunological*. 192(3): 1870- 1877.

Wakil, S. L. and Onilude, A. A. (2011). "Time related total lactic acid bacteria population diversity and dominance in cowpea-fortified fermented cereal-weaning food." *African Journal of Biotechnology.* 10(6): 887-888.

Walencka, E., Rozalska, S. Sadowska, B. and Rozalskab, B. (2008). The influence of *Lactobacillus acidophilus*-derived surfactants on Staphylococcal adhesion and biofilm formation. *Journal of Folia Microbiology.* 53(3): 61-66.

Walker, C. L., Rudan, I., Liu, L., Nair, H., Theodoratou, E. and Bhutta, Z. A. (2013). Global burden of childhood pneumonia and diarrhoea. *Lancet Journal*. 381(9875):1405-1416.

White, B. (2007). Ginger: an overview. *Journal of American Family Physician*. 75(11): 1689-1691.

Wilgus, T. A. (2008). Immune cells in the healing skin wound: influential players at each stage of repair. *Journal of Pharmacology.* 58(2): 112-126.

Willi, C, Bodenmann, P., Ghali, W. A., Faris, P. D. and Cornuz, J. (2007). "Active smoking and the risk of type 2 diabetes: a systematic review and meta-analysis." JAMA: *The Journal of the American Medical Association.* 298(22): 2654-2664.

Wolever, T. M. S. (2013). Is glycemic index (GI) a valid measure of carbohydrate quality? *European Journal of Clinical Nutrition*. 65(5): 522-531.

Wu, H., Esteve, E. and Fredrik, B. (2017). Metformin alters the gut microbiome of individuals with treatment-naïve type 2 diabetes, contributing to the therapeutic effects of the drug. *Journal of Nature Medicine*. 23(5): 850-858.

Xuan, Y.H., Huang, B. B., Tian, H. S., Chi, L. S., Duan, Y. M. and Wang, X. (2014). High-glucose inhibits human fibroblast cell migration in wound healing via repression of bFGF-regulating JNK phosphorylation. *PLoS ONE*. 9(9): 108182.

Yan, F. and Polk, D. B. (2011). Probiotics and immune health. *Journal of Current Opinon Gastroenterology*. 27(5): 496-501.

Yang, J. C., Lu, C.W. and Lin, C. J. (2014). Treatment of *Helicobacter pylori* infection: current status and future concepts. *World Journal Gastroenterology*. 20(4):5283-5293

Yateem, A. Tahani, A., Balba, M. T. and Aldaher, R. (2008). Isolation of lactic acid bacteria with probiotic potential from camel milk. *International Journal of Dairy Science*. 3(4): 194-199.

Zafar, M. and Naqvi, S. (2010). Effects of STZ-induced diabetes on the relative weights of kidney, liver and pancreas in albino rats: a comparative study. *International Journal of Morphology*. 28(3): 135-142.

Zago, M., Fornasari, M. E. and Carminati, D. (2011). Characterization and probiotic potential of *Lactobacillus plantarum* strains isolated from cheeeses. *Journal of Food Microbiology*. 28(5): 1033-1040.

Zahedi, F., Heydari, N. M., Tajabadi, E. M. and Aboutalebi, H. (2011). Comparison of the effects of *Lactobacillus brevis* and *Lactobacillus plantarum* on cutaneous wound healing in rats. *African Journal of Microbiology Research*. 5(24): 4226-4233.

Zhang, M. M., Qian, W., Qin, Y. Y., He, J. and Zhou, Y. H. (2015). *Probiotics in Helicobacter pylori* eradication therapy: a systematic review and meta-analysis. *World Journal of Gastroenterology.* 21(4): 4345-4357.

Zhao, G., Hochwalt, P. C., Usui, M. L., Underwood, R. A., Singh, P. K., James, G. A., Stewart, P. S., Fleckman, P. and Olerud, J. E. (2010). Delayed wound healing in diabetic mice with *Pseudomonas aeruginosa* biofilm challenge: a model for the study of chronic wounds. *Journal of Wound Repair Regeneration.* 18(5): 467-477.

Zhu, X.Y. and Liu, F. (2017). Probiotics as an adjuvant treatment in *Helicobacter pylori* eradication therapy. *Journal of Digestive Diseases*. 18(4): 195-202.

"Oxidase Test and Modified Oxidase Test". *http://web.mst.edu/ ~microbio/Lab _Supplement/Oxidase.html*. Accessed 7th November, 2008.

APPENDIX I

EVALUATION SHEET

Sample code…… Date……..

Sex…….. Panelist…….

Instruction

You have been provided with fifteen (15) coded samples, use the score below to rate the samples according to your degree of likeness or dislike in the score sheet. The score rate is in the following format;

Like extremely= 7 dislike slightly=3

Like very much = 6 dislike very much=2

Like slightly =5 dislike extremely = 1

Neither like nor dislike=4

Attributes	Samples						
KAI	KO	KP	KS	KA	KOI	KPI	KSI
Taste							
Colour							
Flavour							
Mouthfeel							
Overall acceptability							

Please comment on any other attributes noticed on any of the samples that is quite pleasing or displeasing to you.

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Thank you.

APPENDIX II

SEQUENCING

P1 (*Pseudomonas aeruginosa* Sequencing)

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cagattgaacgctggcggcaggcctaacacatgcaagtcgagcggatgaa
gggagcttgctcctggattcagcggcggacGGGTGAGTAATGCCTAGGAA
TCTGCCTGATAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCAT
ACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATG
AGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGAC
GATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACAC
GGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCG
AAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTA
AAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTT
GACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGG
TAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGC
GTAGGTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAA
CTGCATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTC
CTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAG
GCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCA
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGC
CGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACC
GCCTGGGGAGTACGGCcGCAAGGTTAAAACTCAAATGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACC
TTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTT
CGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGA
GATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCA
GCACCTCGGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAA
GGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACAC
GTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAA
TCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGT
GAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGT
TCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCC
AGAAGTAGCTAGTCTAACCGCAAGGGGGACGGTTACCACGGAGTGATTCA
TGACTGGGGTGAAGTctaaaagggggggcccaaaaaatggggtggcccc
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P2 (*Staphylococcus haemolyticus* Sequencing)

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BS 1 806 P3_R
BS 807 807 P3_F
BS 808 826 P3_R
BS 827 827 P3_F
BS 828 828 P3_R
BS 829 831 P3_F
BS 832 836 P3_R
BS 837 843 P3_F
BS 844 844 P3_R

BS 845 1599 P3_F

RD P3_R 853 0 0
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agagcttgctctcgggtgacgagcggcggaCGGGTGAGTAATGTCTGGGA
AtcTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCA
TAATGTCtCAAGACCAAAGtgGGGGACCTTCGGtCCTCATGCCATCAGAT
GTgCCCAgATGGGATTAGCTAGTAGGTGGGGTAAcGGCTCACCTAGGCGA
CGATCCCTAGCTGGTCTGAGAGGATGAcCAGCCACACTGGAACTGAGACA
CGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGC
GCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGT
AAAGcACTTTCAGtgGGGAGGAAGGCGTTgAgGTTAATAtCCTCaGCGAT
TGACGTTACCCgCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCG
GTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCA
CGCAGGCGGTCTgTcAAGTcGGATGTGAAATCCCCGGGCTCAACCTGGGA
ActGCAtTTGAAACTGGcagGCTAGAGTCTTGTAGAGGGGGGTAGAATTC
CAgGTGTAGCGGTGAAATGCGTAGAGATcTGGAGGAATACCGGTGGCGAA
GGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGC
AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGATTTg
GAGGTTGTGCCCT*TGAGG*CGTGGCTTCCGGAGCTAACGCGTTAAATCG
aCC

QA 81 853 1 853

RD P3_F 964 0 0
gAGGTTGTGCCctatGAggacgTGGCTTCCGGAGCTAACGCGTTAAATCG
ACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGA
ACCTTACCTGGTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGC
CTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTG
TGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTG
CCAGCGgTTCGGCCGGGAACTCAAAGGAGACTGCCaGTGATAAACTGGAG
GAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACA
CACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGC
GGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTC
CATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATA
CGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGgggAGTGGGTTG
CAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGA
TTCATGACTGGGGTGaatcacaaggggggacaaaaaaaacgggggaaac
aaggggggtttaagcgggggagagcgtgtgggggggagagggggggcc
cccctccccgggggtgttttccccagaaagtttagcgggggtgtttn
nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn
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QA 15 666 1 799

WA{phrap_params phrap 170302:134812 phrap /data01/bi_result/170302/BI170302-004/FRContig/P3.seq -new_ace phrap version 0.990319}

APPENDIX III

BLASTING

					APPENDIX 111											
								P1 (*Pseudomonas aeruginosa* blast)								
Query				Subject						Score			Identities			Strand
Name	Length	Start	End	Descriptio	Link	AC	Length	Start	End	Bit	Raw	E-value	Match	Total	Pct(%)	
P1_contig_1	1598	1	1473	Pseudom	http://www.ncbi.nlm.nih.gov/nucleotide/861670927?report=genbank&log$=nuclalign&bla	CP012001.	1705	20	1843	2706	1465	0	1472	1475	99	Plus/Plus
P1_contig_1	1598	1	1473	Pseudom	http://www.ncbi.nlm.nih.gov/nucleotide/777183107?report=genbank&log$=nuclalign&bla	LN831024.	1697	17	1941	2706	1465	0	1472	1475	99	Plus/Plus
P1_contig_1	1598	1	1473	Pseudom	http://www.ncbi.nlm.nih.gov/nucleotide/645320483?report=genbank&log$=nuclalign&bla	NR_11767	1527	16	1490	2706	1465	0	1472	1475	99	Plus/Plus
P1_contig_1	1598	1	1469	Pseudom	http://www.ncbi.nlm.nih.gov/nucleotide/636558415?report=genbank&log$=nuclalign&bla	NR_11447	1489	17	1486	2702	1463	0	1468	1470	99	Plus/Plus
P1_contig_1	1598	4	1473	Pseudom	http://www.ncbi.nlm.nih.gov/nucleotide/732170056?report=genbank&log$=nuclalign&bla	LN681564.	1488	1	1472	2700	1462	0	1469	1472	99	Plus/Plus
P1_contig_1	1598	4	1464	Pseudom	http://www.ncbi.nlm.nih.gov/nucleotide/631252401?report=genbank&log$=nuclalign&bla	NR_11359	1461	1	1461	2693	1458	0	1460	1461	99	Plus/Plus
P1_contig_1	1598	4	1461	Pseudom	http://www.ncbi.nlm.nih.gov/nucleotide/914226236?report=genbank&log$=nuclalign&bla	LC069033.	1458	1	1458	2687	1455	0	1457	1458	99	Plus/Plus
P1_contig_1	1598	1	1437	Pseudom	http://www.ncbi.nlm.nih.gov/nucleotide/932249971?report=genbank&log$=nuclalign&bla	KT825518.	1457	20	1457	2643	1431	0	1436	1438	99	Plus/Plus
P1_contig_1	1598	1	1473	Pseudom	http://www.ncbi.nlm.nih.gov/nucleotide/343202811?report=genbank&log$=nuclalign&bla	NR_04328	1531	21	1495	2595	1405	0	1452	1475	98	Plus/Plus
P1_contig_1	1598	1	1473	Pseudom	http://www.ncbi.nlm.nih.gov/nucleotide/219846486?report=genbank&log$=nuclalign&bla	NR_02607	1537	25	1500	2556	1384	0	1450	1480	98	Plus/Plus

						P2 (*Staphylococcus aeruginosa* blast)									
				Subject						Score			Identities			Strand
Query	Length	Start	End	Descriptio	Link	AC	Length	Start	End	Bit	Raw	E-value	Match	Total	Pct(%)	
Name	1515	6	1478	Staphyloc	http://ww	NR_11334	1473	1	1473	2717	1471	0	1472	1473	99	Plus/Plus
P2_contig_1	1515	5	1480	Staphyloc	http://ww	NR_03695	1544	20	1493	2686	1454	0	1470	1477	99	Plus/Plus
P2_contig_1	1515	5	1480	Staphyloc	http://ww	KM873669	1480	4	1479	2667	1444	0	1465	1476	99	Plus/Plus
P2_contig_1	1515	5	1480	Staphyloc	http://ww	NR_11845	1477	1	1476	2665	1443	0	1465	1476	99	Plus/Plus
P2_contig_1	1515	5	1480	Staphyloc	http://ww	NR_13259	1546	23	1498	2662	1441	0	1464	1476	99	Plus/Plus
P2_contig_1	1515	5	1480	Staphyloc	http://ww	AY953148.	1546	23	1498	2662	1441	0	1464	1476	99	Plus/Plus
P2_contig_1	1515	5	1480	Staphyloc	http://ww	NR_11662	1537	21	1497	2660	1440	0	1465	1477	99	Plus/Plus
P2_contig_1	1515	5	1480	Staphyloc	http://ww	NR_03695	1544	20	1493	2641	1430	0	1461	1476	99	Plus/Plus
P2_contig_1	1515	5	1477	Staphyloc	http://ww	NR_02466	1492	20	1492	2638	1428	0	1458	1473	99	Plus/Plus
P2_contig_1	1515	5	1480	Staphyloc	http://ww	AP008934	1657	23	1470	2628	1423	0	1460	1478	99	Plus/Minu

						P3 (*Klebsiella pneumoniae* blast)										
Query				Subject						Score			Identities			Strand
Name	Length	Start	End	Descriptic	Link	AC	Length	Start	End	Bit	Raw	E-value	Match	Total	Pct(%)	
P3_contig_1	1597	1	1465	Klebsiella	http://ww	NR_11768	1530	18	1481	2649	1434	0	1455	1465	99	Plus/Plus
P3_contig_1	1597	1	1465	Klebsiella	http://ww	NR_11768	1530	18	1481	2643	1431	0	1454	1465	99	Plus/Plus
P3_contig_1	1597	1	1465	Klebsiella	http://ww	NR_03679	1534	21	1485	2639	1429	0	1454	1466	99	Plus/Plus
P3_contig_1	1597	1	1465	Klebsiella	http://ww	NR_11768	1530	18	1481	2638	1428	0	1453	1465	99	Plus/Plus
P3_contig_1	1597	1	1465	Klebsiella	http://ww	NR_11768	1524	12	1475	2638	1428	0	1453	1465	99	Plus/Plus
P3_contig_1	1597	1	1465	Klebsiella	http://ww	NR_11370	1465	1	1464	2634	1426	0	1451	1465	99	Plus/Plus
P3_contig_1	1597	1	1463	Klebsiella	http://ww	NR_11324	1462	1	1462	2630	1424	0	1449	1463	99	Plus/Plus
P3_contig_1	1597	1	1465	Klebsiella	http://ww	NR_11768	1530	18	1481	2627	1422	0	1451	1465	99	Plus/Plus
P3_contig_1	1597	3	1455	Klebsiella	http://ww	NR_11927	1452	1	1452	2619	1418	0	1441	1453	99	Plus/Plus
P3_contig_1	1597	6	1455	Klebsiella	http://ww	NR_11200	1449	1	1449	2617	1417	0	1439	1450	99	Plus/Plus

APPENDIX IV

Standard ID

16S rRNA Full Sequencing

macrogen

16S rRNA service report

Order Number : 170211FN-050
Sample name : P1_contig_1

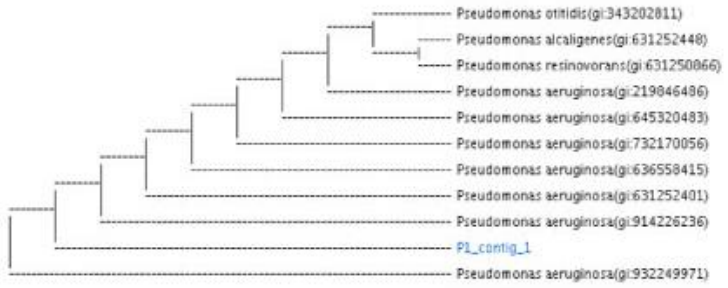
Information

Primer Information

Sequencing Primer Name Primer Sequences	PCR Primer Name Primer Sequences
785F 5' (GGA TTA GAT ACC CTG GTA) 3'	27F 5' (AGA GTT TGA TCM TGG CTC AG) 3
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'	1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
CP012001.1	Pseudomonas aeruginosa	6317050	696960	698434	0	2706	0.0	1472/1475	99

Kingdom	Family	Genus	Species
Bacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas aeruginosa



Characterization

Pseudomonas is a genus of Gram-negative, aerobic gammaproteobacteria, belonging to the family Pseudomonadaceae containing 191 validly described species. The members of the genus demonstrate a great deal of metabolic diversity, and consequently are able to colonize a wide range of niches.

Standard ID

16S rRNA service report

Order Number : 170211FN-050
Sample name : P2_contig_1

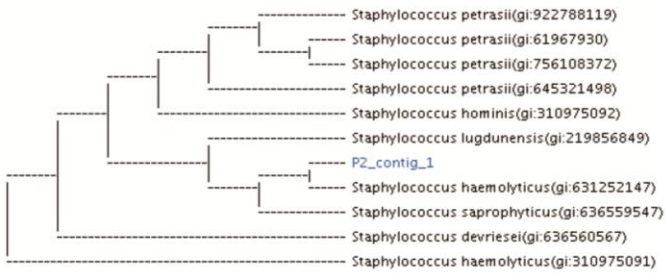
Information

Primer Information

Sequencing Primer Name Primer Sequences	PCR Primer Name Primer Sequences
785F 5' (GGA TTA GAT ACC CTG GTA) 3'	27F 5' (AGA GTT TGA TCM TGG CTC AG) 3
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'	1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
NR_113345.1	Staphylococcus haemolyticus	1473	1	1473	100	2717	0.0	1472/1473	99

Kingdom	Family	Genus	Species
Bacteria	Staphylococcaceae	Staphylococcus	Staphylococcus haemolyticus



Characterization

Staphylococcus is a genus of Gram-positive bacteria. Under the microscope, they appear round (cocci), and form in grape-like clusters. The Staphylococcus genus includes at least 40 species. Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms.

Staphylococcus haemolyticus is a member of the coagulase-negative staphylococci (CoNS). It is part of the skin flora of humans, and its largest populations are usually found at the axillae, perineum, and inguinal areas.

Standard ID

16S rRNA service report

Order Number : 170211FN-049
Sample name : P3_contig_1

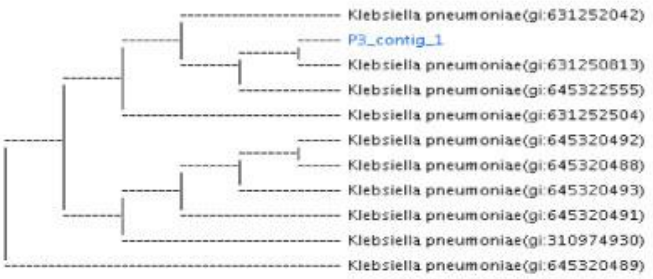
Information

Primer Information

Sequencing Primer Name Primer Sequences	PCR Primer Name Primer Sequences
785F 5' (GGA TTA GAT ACC CTG GTA) 3'	27F 5' (AGA GTT TGA TCM TGG CTC AG) 3
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'	1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
NR_117686.1	Klebsiella pneumoniae	1530	18	1481	95	2649	0.0	1455/1465	99

Kingdom	Family	Genus	Species
Bacteria	Enterobacteriaceae	Klebsiella	Klebsiella pneumoniae



Characterization

Klebsiella is a genus of nonmotile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule. Klebsiella species are routinely found in the human nose, mouth, and gastrointestinal tract as normal flora; however, they can also behave as opportunistic human pathogens. Klebsiella species are known to also infect a variety of other animals, both as normal flora and opportunistic pathogens.

APPENDIX V

Origin and identification of Lactobacilli isolates using API system.

Isolates	Origin of the isolates	API 5O CH identification (% similarity)
L2	Kunun-zaki processed with paddy rice	*Lactobacillus plantarum* (99.9%)
L4	Kunun-zaki processed with sweet potatoes	*Lactobacillus casei*(99.9%)

The percentages following the scientific names of strains represent the similarities from the computer-aided database of the ApiwebTM API 50 CH V5.1 software.

APPENDIX VI

DESCRIPTIVES AND ANOVA FOR THE PHYSICOCHEMICAL PROPERTIES OF KUNUN-ZAKI SAMPLES

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
pH	KO	3	5.5700	.14731	.08505	5.2041	5.9359	5.40	5.66
	KP	3	4.4000	.00000	.00000	4.4000	4.4000	4.40	4.40
	KS	3	4.5967	.00577	.00333	4.5823	4.6110	4.59	4.60
	KA	3	4.3333	.05774	.03333	4.1899	4.4768	4.30	4.40
	KOI	3	5.6700	.02646	.01528	5.6043	5.7357	5.65	5.70
	KPI	3	4.4533	.08505	.04910	4.2421	4.6646	4.39	4.55
	KSI	3	4.6033	.06807	.03930	4.4342	4.7724	4.55	4.68
	KAI	3	4.3500	.08660	.05000	4.1349	4.5651	4.30	4.45
	Total	24	4.7471	.52808	.10779	4.5241	4.9701	4.30	5.70
BRIX	KO	3	5.6500	.05000	.02887	5.5258	5.7742	5.60	5.70
	KP	3	5.6000	.17321	.10000	5.1697	6.0303	5.50	5.80
	KS	3	6.6500	.05000	.02887	6.5258	6.7742	6.60	6.70
	KA	3	5.8600	.05292	.03055	5.7286	5.9914	5.80	5.90
	KOI	3	5.3167	.16073	.09280	4.9174	5.7159	5.20	5.50
	KPI	3	5.2167	.07638	.04410	5.0269	5.4064	5.15	5.30
	KSI	3	6.5333	.10408	.06009	6.2748	6.7919	6.45	6.65
	KAI	3	5.9300	.03464	.02000	5.8439	6.0161	5.89	5.95
	Total	24	5.8446	.50505	.10309	5.6313	6.0578	5.15	6.70
TITRABLEACIDI	KO	3	.5267	.03055	.01764	.4508	.6026	.50	.56

TY	KP	3	.6700	.04583	.02646	.5562	.7838	.62	.71
	KS	3	.6667	.07638	.04410	.4769	.8564	.60	.75
	KA	3	.7500	.01000	.00577	.7252	.7748	.74	.76
	KOI	3	.5267	.03055	.01764	.4508	.6026	.50	.56
	KPI	3	.6700	.04583	.02646	.5562	.7838	.62	.71
	KSI	3	.6667	.07638	.04410	.4769	.8564	.60	.75
	KAI	3	.7500	.01000	.00577	.7252	.7748	.74	.76
	Total	24	.6533	.09111	.01860	.6149	.6918	.50	.76
SPECIFICGRAVITY	KO	3	.9800	.00000	.00000	.9800	.9800	.98	.98
SPECIFICGRAVITY	KO	3	.9800	.00000	.00000	.9800	.9800	.98	.98
	KP	3	.9800	.00000	.00000	.9800	.9800	.98	.98
	KS	3	.9700	.00000	.00000	.9700	.9700	.97	.97
	KA	3	.9867	.00577	.00333	.9723	1.0010	.98	.99
	KOI	3	1.0200	.01000	.00577	.9952	1.0448	1.01	1.03
	KPI	3	.9200	.02000	.01155	.8703	.9697	.90	.94
	KSI	3	.8967	.06807	.03930	.7276	1.0658	.82	.95
	KAI	3	.8400	.01000	.00577	.8152	.8648	.83	.85
	Total	24	.9492	.06021	.01229	.9237	.9746	.82	1.03

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
pH	Between Groups	6.324	7	.903	160.126	.000
	Within Groups	.090	16	.006		
	Total	6.414	23			
BRIX	Between Groups	5.704	7	.815	79.983	.000
	Within Groups	.163	16	.010		
	Total	5.867	23			
TITRABLEACIDITY	Between Groups	.155	7	.022	9.882	.000
	Within Groups	.036	16	.002		
	Total	.191	23			
SPECIFICGRAVITY	Between Groups	.073	7	.010	15.808	.000
	Within Groups	.011	16	.001		
	Total	.083	23			
VISCOSITY	Between Groups	.840	7	.120	48.576	.000
	Within Groups	.040	16	.002		
	Total	.880	23			

APPENDIX VII

DESCRIPTIVES AND ANOVA FOR PROXIMATE COMPOSITION OF KUNUN-ZAKI SAMPLES

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
MOISTURE	KO	3	88.0000	.10000	.05774	87.7516	88.2484	87.90	88.10
	KP	3	86.0667	.05774	.03333	85.9232	86.2101	86.00	86.10
	KS	3	83.6867	.27209	.15709	83.0108	84.3626	83.51	84.00
	KA	3	86.2333	.05774	.03333	86.0899	86.3768	86.20	86.30
	KOI	3	88.3333	.53463	.30867	87.0052	89.6614	88.00	88.95
	KPI	3	87.0000	.00000	.00000	87.0000	87.0000	87.00	87.00
	KSI	3	84.1733	.06807	.03930	84.0042	84.3424	84.12	84.25
	KAI	3	86.2933	.10504	.06064	86.0324	86.5543	86.19	86.40
	Total	24	86.2233	1.58004	.32252	85.5561	86.8905	83.51	88.95
ASH	KO	3	.5833	.02887	.01667	.5116	.6550	.55	.60
	KP	3	4.4500	.13229	.07638	4.1214	4.7786	4.35	4.60
	KS	3	3.4333	.15275	.08819	3.0539	3.8128	3.30	3.60
	KA	3	2.0000	.10000	.05774	1.7516	2.2484	1.90	2.10
	KOI	3	.5700	.14799	.08544	.2024	.9376	.40	.67
	KPI	3	4.3433	.03215	.01856	4.2635	4.4232	4.32	4.38
	KSI	3	3.3467	.13429	.07753	3.0131	3.6803	3.25	3.50
	KAI	3	1.8000	.49244	.28431	.5767	3.0233	1.25	2.20
	Total	24	2.5658	1.49414	.30499	1.9349	3.1968	.40	4.60
FIBER	KO	3	.2600	.00000	.00000	.2600	.2600	.26	.26

	KP	3	.3433	.01528	.00882	.3054	.3813	.33	.36
	KS	3	.2967	.00577	.00333	.2823	.3110	.29	.30
	KA	3	.2667	.01528	.00882	.2287	.3046	.25	.28
	KOI	3	.2367	.00577	.00333	.2223	.2510	.23	.24
	KPI	3	.3200	.02646	.01528	.2543	.3857	.30	.35
	KSI	3	.2833	.01528	.00882	.2454	.3213	.27	.30
	KAI	3	.2600	.02000	.01155	.2103	.3097	.24	.28
	Total	24	.2833	.03595	.00734	.2682	.2985	.23	.36
PROTEIN	KO	3	1.2333	.01528	.00882	1.1954	1.2713	1.22	1.25
	KP	3	1.9000	.13229	.07638	1.5714	2.2286	1.75	1.75
	KS	3	1.5367	.01528	.00882	1.4987	1.5746	1.52	1.55
	KA	3	1.2300	.02646	.01528	1.1643	1.2957	1.20	1.25
	KOI	3	1.1400	.01000	.00577	1.1152	1.1648	1.13	1.15
	KPI	3	1.9000	.13229	.07638	1.5714	2.2286	1.75	2.00
	KSI	3	1.5467	.03055	.01764	1.4708	1.6226	1.52	1.58
	KAI	3	1.3433	.09452	.05457	1.1085	1.5781	1.27	1.45
	Total	24	1.4021	.24306	.04961	1.2994	1.5047	1.13	2.00
CARBOHYDRATE	KO	3	9.0767	.13317	.07688	8.7459	9.4075	8.93	9.19
	KP	3	6.7967	.25716	.14847	6.1578	7.4355	6.61	7.09
	KS	3	9.6467	.47543	.27449	8.4656	10.8277	9.16	10.11
	KA	3	9.1033	.12055	.06960	8.8039	9.4028	8.99	9.23
	KOI	3	8.8833	.62581	.36131	7.3287	10.4379	8.17	9.34
	KPI	3	5.3367	.13317	.07688	5.0059	5.6675	5.19	5.45
	KSI	3	9.1967	.30925	.17854	8.4285	9.9649	8.84	9.39
	KAI	3	9.0933	.31501	.18187	8.3108	9.8759	8.78	9.41

	Total	24	8.3917	1.46387	.29881	7.7735	9.0098	5.19	10.11
FAT	KO	3	.8467	.02309	.01333	.7893	.9040	.82	.86
	KP	3	1.0567	.04041	.02333	.9563	1.1571	1.02	1.10
	KS	3	1.4000	.20000	.11547	.9032	1.8968	1.20	1.60
	KA	3	1.1667	.01155	.00667	1.1380	1.1954	1.16	1.18
	KOI	3	.8367	.01528	.00882	.7987	.8746	.82	.85
	KPI	3	1.1000	.07211	.04163	.9209	1.2791	1.04	1.18
	KSI	3	1.4533	.08505	.04910	1.2421	1.6646	1.39	1.55
	KAI	3	1.2100	.03606	.02082	1.1204	1.2996	1.18	1.25
	Total	24	1.1337	.22704	.04634	1.0379	1.2296	.82	1.60

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
MOISTURE	Between Groups	56.636	7	8.091	165.035	.000
	Within Groups	.784	16	.049		
	Total	57.420	23			
ASH	Between Groups	50.677	7	7.240	172.815	.000
	Within Groups	.670	16	.042		
	Total	51.347	23			
FIBER	Between Groups	.026	7	.004	15.918	.000
	Within Groups	.004	16	.000		
	Total	.030	23			
PROTEIN	Between Groups	1.291	7	.184	43.840	.000
	Within Groups	.067	16	.004		
	Total	1.359	23			
CARBOHYDRATE	Between Groups	47.430	7	6.776	58.369	.000
	Within Groups	1.857	16	.116		
	Total	49.287	23			
FAT	Between Groups	1.073	7	.153	21.795	.000
	Within Groups	.113	16	.007		
	Total	1.186	23			

APPENDIX VIII

DESCRIPTIVE AND ANOVA FOR MINERAL COMPOSITION OF KUNUN-ZAKI SAMPLES.

						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
VAR00002	KO	3	14.4000	.05000	.02887	14.2758	14.5242	14.35	14.45
	KP	3	18.1333	.12583	.07265	17.8208	18.4459	18.00	18.25
	KS	3	18.7833	.02887	.01667	18.7116	18.8550	18.75	18.80
	KA	3	15.2100	.03606	.02082	15.1204	15.2996	15.18	15.25
	KOI	3	13.2000	.05000	.02887	13.0758	13.3242	13.15	13.25
	KPI	3	18.2767	.02517	.01453	18.2142	18.3392	18.25	18.30
	KSI	3	19.7733	.06429	.03712	19.6136	19.9330	19.70	19.82
	KAI	3	13.1167	.10408	.06009	12.8581	13.3752	13.00	13.20
	Total	24	16.3617	2.55470	.52148	15.2829	17.4404	13.00	19.82
VAR00003	KO	3	125.3333	1.52753	.88192	121.5388	129.1279	124.00	127.00
	KP	3	145.6667	1.15470	.66667	142.7982	148.5351	145.00	147.00
	KS	3	154.6667	4.50925	2.60342	143.4651	165.8683	150.00	159.00
	KA	3	131.6667	2.88675	1.66667	124.4956	138.8378	130.00	135.00
	KOI	3	110.3333	4.50925	2.60342	99.1317	121.5349	106.00	115.00
	KPI	3	141.0000	3.60555	2.08167	132.0433	149.9567	138.00	145.00
	KSI	3	140.3333	2.51661	1.45297	134.0817	146.5849	138.00	143.00
	KAI	3	134.3333	5.13160	2.96273	121.5857	147.0809	130.00	140.00
	Total	24	135.4167	13.23341	2.70126	129.8287	141.0046	106.00	159.00
VAR00004	KO	3	184.0000	4.00000	2.30940	174.0634	193.9366	180.00	188.00
	KP	3	220.0000	18.02776	10.40833	175.2166	264.7834	205.00	240.00
	KS	3	245.0000	5.00000	2.88675	232.5793	257.4207	240.00	250.00
	KA	3	202.0000	7.21110	4.16333	184.0866	219.9134	196.00	210.00
	KOI	3	189.0000	3.60555	2.08167	180.0433	197.9567	185.00	192.00
	KPI	3	195.6667	3.21455	1.85592	187.6813	203.6521	192.00	198.00
	KSI	3	226.6667	2.88675	1.66667	219.4956	233.8378	225.00	230.00
	KAI	3	183.3333	2.88675	1.66667	176.1622	190.5044	180.00	185.00
	Total	24	205.7083	22.43344	4.57921	196.2355	215.1811	180.00	250.00
VAR00005	KO	3	2.2500	.25000	.14434	1.6290	2.8710	2.00	2.50
	KP	3	3.3167	.07638	.04410	3.1269	3.5064	3.25	3.40
	KS	3	1.8133	.03215	.01856	1.7335	1.8932	1.79	1.85

	KA	3	1.9233	.02517	.01453	1.8608	1.9858	1.90	1.95
	KOI	3	1.8267	.11676	.06741	1.5366	2.1167	1.70	1.93
	KPI	3	3.2367	.10263	.05925	2.9817	3.4916	3.15	3.35
	KSI	3	1.8867	.02309	.01333	1.8293	1.9440	1.86	1.90
	KAI	3	1.8500	.06083	.03512	1.6989	2.0011	1.78	1.89
	Total	24	2.2629	.61969	.12649	2.0012	2.5246	1.70	3.40
VAR00006	KO	3	2.7733	.05859	.03383	2.6278	2.9189	2.73	2.84
	KP	3	3.9100	.05292	.03055	3.7786	4.0414	3.85	3.95
	KS	3	2.9400	.11136	.06429	2.6634	3.2166	2.82	3.04
	KA	3	2.7333	.10408	.06009	2.4748	2.9919	2.65	2.85
	KOI	3	1.9167	1.66158	.95931	-2.2109	6.0442	.00	2.95
	KPI	3	3.5600	.03606	.02082	3.4704	3.6496	3.53	3.60
	KSI	3	2.8433	.02082	.01202	2.7916	2.8950	2.82	2.86
	KAI	3	2.8500	.07000	.04041	2.6761	3.0239	2.80	2.93
	Total	24	2.9408	.75109	.15332	2.6237	3.2580	.00	3.95
VAR00007	KO	3	21.0667	.04726	.02728	20.9493	21.1841	21.03	21.12
	KP	3	22.2667	.08505	.04910	22.0554	22.4779	22.18	22.35
	KS	3	23.6733	.01528	.00882	23.6354	23.7113	23.66	23.69
	KA	3	22.6400	.06557	.03786	22.4771	22.8029	22.57	22.70
	KOI	3	21.1767	.02517	.01453	21.1142	21.2392	21.15	21.20
	KPI	3	22.4900	.03606	.02082	22.4004	22.5796	22.45	22.52
	KSI	3	24.1100	.01000	.00577	24.0852	24.1348	24.10	24.12
	KAI	3	22.7233	.09292	.05364	22.4925	22.9541	22.62	22.80
	Total	24	22.5183	1.01589	.20737	22.0894	22.9473	21.03	24.12

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
VAR00002	Between Groups	150.032	7	21.433	4445.936	.000
	Within Groups	.077	16	.005		
	Total	150.109	23			
VAR00003	Between Groups	3831.167	7	547.310	44.527	.000
	Within Groups	196.667	16	12.292		
	Total	4027.833	23			
VAR00004	Between Groups	10658.958	7	1522.708	26.598	.000
	Within Groups	916.000	16	57.250		
	Total	11574.958	23			
VAR00005	Between Groups	8.636	7	1.234	100.298	.000
	Within Groups	.197	16	.012		
	Total	8.832	23			
VAR00006	Between Groups	7.381	7	1.054	3.016	.032
	Within Groups	5.594	16	.350		
	Total	12.975	23			
VAR00007	Between Groups	23.687	7	3.384	1097.486	.000
	Within Groups	.049	16	.003		
	Total	23.737	23			

APPENDIX IX

DESCRIPTIVE AND ANOVA FOR SENSORY ATTRIBUTES OF KUNUN-ZAKI SAMPLES

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
TASTE	KO	3	5.8400	.03606	.02082	5.7504	5.9296	5.80	5.87
	KP	3	6.3833	.01528	.00882	6.3454	6.4213	6.37	6.40
	KS	3	5.8167	.04726	.02728	5.6993	5.9341	5.78	5.87
	KA	3	6.5367	.04041	.02333	6.4363	6.6371	6.50	6.58
	KOI	3	5.8433	.03512	.02028	5.7561	5.9306	5.81	5.88
	KPI	3	6.3433	.04041	.02333	6.2429	6.4437	6.30	6.38
	KSI	3	5.7067	.16010	.09244	5.3089	6.1044	5.55	5.87
	KAI	3	5.6433	.04041	.02333	5.5429	5.7437	5.60	5.68
	Total	24	6.0142	.33724	.06884	5.8718	6.1566	5.55	6.58
COLOUR	KO	3	6.1200	.01000	.00577	6.0952	6.1448	6.11	6.13
	KP	3	5.6667	.06506	.03756	5.5050	5.8283	5.60	5.73
	KS	3	5.2067	.06028	.03480	5.0569	5.3564	5.15	5.27
	KA	3	5.6267	.04933	.02848	5.5041	5.7492	5.57	5.66
	KOI	3	6.3433	.04041	.02333	6.2429	6.4437	6.30	6.38
	KPI	3	5.3167	.07767	.04485	5.1237	5.5096	5.23	5.38
	KSI	3	6.3000	.05000	.02887	6.1758	6.4242	6.25	6.35
	KAI	3	5.8567	.04933	.02848	5.7341	5.9792	5.80	5.89
	Total	24	5.8046	.41170	.08404	5.6307	5.9784	5.15	6.38
	KO	3	5.0933	.08327	.04807	4.8865	5.3002	5.00	5.16
MOUTHFEEL									
	KP	3	6.7700	.19975	.11533	6.2738	7.2662	6.54	6.90
	KS	3	5.4867	.01528	.00882	5.4487	5.5246	5.47	5.50
	KA	3	6.5700	.12124	.07000	6.2688	6.8712	6.46	6.70
	KOI	3	5.0767	.02517	.01453	5.0142	5.1392	5.05	5.10
	KPI	3	5.7133	.05859	.03383	5.5678	5.8589	5.67	5.78
	KSI	3	5.4033	.04509	.02603	5.2913	5.5153	5.36	5.45
	KAI	3	6.3000	.07000	.04041	6.1261	6.4739	6.23	6.37
	Total	24	5.8017	.63772	.13017	5.5324	6.0710	5.00	6.90
FLAVOUR	KO	3	5.0967	.01528	.00882	5.0587	5.1346	5.08	5.11
	KP	3	6.3533	.02517	.01453	6.2908	6.4158	6.33	6.38

	KS	3	5.7733	.03055	.01764	5.6974	5.8492	5.74	5.80
	KA	3	6.4567	.13577	.07839	6.1194	6.7939	6.33	6.60
	KOI	3	5.1867	.02082	.01202	5.1350	5.2384	5.17	5.21
	KPI	3	5.7100	.13892	.08021	5.3649	6.0551	5.55	5.80
	KSI	3	5.5333	.07638	.04410	5.3436	5.7231	5.45	5.60
	KAI	3	6.3633	.07767	.04485	6.1704	6.5563	6.30	6.45
	Total	24	5.8092	.51576	.10528	5.5914	6.0270	5.08	6.60
ACCEPTABILITY	KO	3	5.3500	.10440	.06028	5.0906	5.6094	5.23	5.42
	KP	3	6.3533	.04163	.02404	6.2499	6.4568	6.32	6.40
	KS	3	5.3700	.01000	.00577	5.3452	5.3948	5.36	5.38
	KA	3	6.4200	.02000	.01155	6.3703	6.4697	6.40	6.44
	KOI	3	5.1400	.16462	.09504	4.7311	5.5489	5.04	5.33
	KPI	3	6.0300	.02646	.01528	5.9643	6.0957	6.00	6.05
	KSI	3	5.4833	.01528	.00882	5.4454	5.5213	5.47	5.50
	KAI	3	6.0700	.04000	.02309	5.9706	6.1694	6.03	6.11
	Total	24	5.7771	.47967	.09791	5.5745	5.9796	5.04	6.44

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TASTE	Between Groups	2.545	7	.364	81.846	.000
	Within Groups	.071	16	.004		
	Total	2.616	23			
COLOUR	Between Groups	3.852	7	.550	191.423	.000
	Within Groups	.046	16	.003		
	Total	3.898	23			
MOUTHFEEL	Between Groups	9.208	7	1.315	144.622	.000
	Within Groups	.146	16	.009		
	Total	9.354	23			
FLAVOUR	Between Groups	6.015	7	.859	132.612	.000
	Within Groups	.104	16	.006		
	Total	6.118	23			
ACCEPTABILITY	Between Groups	5.206	7	.744	139.130	.000
	Within Groups	.086	16	.005		
	Total	5.292	23			

APPENDIX X

DESCRIPTIVE AND ANOVA FOR ANTIMICROBIAL ASSAY

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Pseudomonas	L1	3	9.7133	.05508	.03180	9.5765	9.8501	9.65	9.75
	L2	3	13.5667	.07638	.04410	13.3769	13.7564	13.50	13.65
	L3	3	8.6900	.03606	.02082	8.6004	8.7796	8.65	8.72
	L4	3	13.8633	.03215	.01856	13.7835	13.9432	13.84	13.90
	L5	3	9.4567	.04041	.02333	9.3563	9.5571	9.42	9.50
	L6	3	5.1467	.02082	.01202	5.0950	5.1984	5.13	5.17
	L7	3	8.4533	.04933	.02848	8.3308	8.5759	8.42	8.51
	L8	3	7.6333	.06658	.03844	7.4679	7.7987	7.56	7.69
	Total	24	9.5654	2.79263	.57004	8.3862	10.7446	5.13	13.90
Staphylococcus	L1	3	9.4233	.06429	.03712	9.2636	9.5830	9.35	9.47
	L2	3	14.0267	.02517	.01453	13.9642	14.0892	14.00	14.05
	L3	3	8.4567	.05508	.03180	8.3199	8.5935	8.42	8.52
	L4	3	14.2500	.03000	.01732	14.1755	14.3245	14.22	14.28
	L5	3	8.1400	.01000	.00577	8.1152	8.1648	8.13	8.15
	L6	3	9.2267	.01155	.00667	9.1980	9.2554	9.22	9.24
	L7	3	7.5733	.07572	.04372	7.3852	7.7614	7.52	7.66
	L8	3	7.1933	.01528	.00882	7.1554	7.2313	7.18	7.21
	Total	24	9.7863	2.66521	.54403	8.6608	10.9117	7.18	14.28
Klebsiella	L1	3	8.4600	.02000	.01155	8.4103	8.5097	8.44	8.48
	L2	3	11.8333	.02309	.01333	11.7760	11.8907	11.82	11.86
	L3	3	8.7967	.03512	.02028	8.7094	8.8839	8.76	8.83
	L4	3	13.1900	.02646	.01528	13.1243	13.2557	13.16	13.21
	L5	3	7.2467	.03512	.02028	7.1594	7.3339	7.21	7.28
	L6	3	7.0433	.06028	.03480	6.8936	7.1931	6.98	7.10
	L7	3	6.8167	.03512	.02028	6.7294	6.9039	6.78	6.85
	L8	3	6.9833	.10408	.06009	6.7248	7.2419	6.90	7.10
	Total	24	8.7963	2.32195	.47397	7.8158	9.7767	6.78	13.21

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Pseudomonas	Between Groups	179.332	7	25.619	10179.652	.000
	Within Groups	.040	16	.003		
	Total	179.372	23			
Staphylococcus	Between Groups	163.348	7	23.335	12529.056	.000
	Within Groups	.030	16	.002		
	Total	163.377	23			
Klebsiella	Between Groups	123.964	7	17.709	7155.207	.000
	Within Groups	.040	16	.002		
	Total	124.004	23			