DETERMINATION OF THE MICROBIOLOGICAL AND SAFETY PROFILES OF *KULIKULI* FERMENTED WITH LACTOBACILLUS PLANTARUM

BY

TAUHEED, Faridah Laraba
MTech/SLS/2018/8351

DEPARTMENT OF MICROBIOLOGY SCHOOL OF LIFE SCIENCES
FEDERAL UNIVERSITY OF TECHNOLOGY MINNA,
NIGER STATE

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ABSTRACT

Fermentation of legumes is attracting the attention of researchers and industry in relation to the development of healthier, tasty, and technologically adapted products. The study focused on the effect of fermentation on the microbiological and safety profiles (nutritional, antinutitional and physicochemical composition as well as the haematological and histopathological effect in rats) of fermented *Kulikuli* with *Lactobacillus plantarum*. *Lactobacillus plantarum* were isolated from Corn steep liquor and was subsequently used to ferment *Kulikuli* samples. The microbial load and the nutritional composition of the fermented *Kulikuli* samples were determined using standard methods. The antinutritional quality and physicochemical properties were also examined using the method of the Association of the Official Analytical Chemist (AOAC). The blood of anesthetized albino rats was collected using the cardiac puncture method, and serum biochemical and, haematological parameters, and histopathology of organs of rats were examined after been fed with the samples for 28 days. The bacterial counts of the fermented *Kulikuli* samples ranged from 0.0 - 2.0±0.00 x 10^4 CFU/g (Unfermented *Kulikuli* (UK), fermented *Kulikuli* (K), fermented *Kulikuli* and *Lactobacillus plantarum* (KL) had no bacterial growth while fermented *Kulikuli* all-purpose flour and *Lactobacillus plantarum* (KFL) had the highest count of 2.0±0.00 x 10^4 CFU/g. The fungal counts also ranged from 0.0 - 2.0±0.00 x 10^4 CFU/g (Unfermented *Kulikuli* (UK), fermented *Kulikuli* (K), fermented *Kulikuli* and chia flour (KC) and fermented *Kulikuli* all-purpose flour and *Lactobacillus plantarum* (KFL) had zero counts and fermented *Kulikuli* and *Lactobacillus plantarum* (KL) had the highest fungal count of 2.0±0.00 x 10^4 CFU/g). *Aspergillus niger, A. fumigatus, A. flavus, A. terreus, Klebsiella pneumoniae, Staphylococcus aureus* and species of *Bacillus, Enterobacter, Micrococcus, Proteus* and *Tricophyton* were isolated. The moisture, protein, ash, fibre, lipid and the carbohydrate contents of the *Kulikuli* samples ranged from 2.71±0.08 - 4.60±0.14%, 22.53±0.25 - 31.21±0.49%, 3.47±0.09 - 4.53±0.05%,0.25±0.05 - 0.86±0.05%, 30.64±0.40 - 35.33±0.45% and 27.56±0.64 - 36.33±0.03% respectively. The antinutritional composition of the *Kulikuli* samples for tannins ranged from (16.65 - 18.58 mg/100g), saponins (19.84 - 37.12 mg/100g), phytates (12.60 - 84.25 mg/100g), cyanides (3.60 - 513.57 mg/100g) and oxalates (0.03 - 0.15mg/100g). The range of free fatty acid, peroxide and the iodine values for the fermented *Kulikuli* samples were (6.75 – 10.32) mgKOH/g, (2.17 – 8.75) meq/kg and 66.55g/100g – 75.23g/100g respectively. The haematological parameters (haemoglobin, packed cell volume (PCV), white blood cells (WBC), red blood cells (RBC), platelets, mean corpuscular volume, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin) of the rats fed with fermented *Kulikuli* with *Lactobacillus plantarum* were within the normal range. The safety of the *Kulikuli* supplemented diet was further verified with the help of histopathological studies which showed no abnormalities. The results from this study revealed that fermented *Kulikuli* with *Lactobacillus plantarum* is relatively safe for human consumption.

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ABBREVIATIONS

µ/L- microlitre

µmol/L- micromole per litre

ALT - Alanine transaminase

ANOVA- Analysis of Variance

AST - Aspartate transaminase

CFU/g- Colony forming unit per gramme

CRD- Completely randomised design

CTAB - Cetyl trimethylammonium bromide

EDTA -Ethylenediaminetetraacetic acid

FAO – Food and Agricultural Organization

g/dl- gramme per decilitre

g/mL- grams per millilitre

Hb- Haemoglobin,

HDL-C- High density lipoprotein cholesterol,

ICMSF- International Commission on Microbiological Specifications for Foods K- Fermented *Kulikuli*

KC- Fermented *Kulikuli* and chia flour

KCL- Fermented *Kulikuli,* chia flour and *Lactobacillus plantarum*

KF- Fermented *Kulikuli* and all- purpose flour

KFL- Fermented *Kulikuli*, all- purpose flour and *Lactobacillus plantarum*

KL- Fermented *Kulikuli* and *Lactobacillus plantarum*

L.G.A- Local Government Area

LDL-C- low density lipoprotein cholesterol MAC -MacConkey Agar

MCHC-Mean corpuscular haemoglobin concentration

MCH-Mean corpuscular haemoglobin

MCV-Mean corpuscular volume

mg/dL - milligramme per decilitre

Mg/Kgbw- milligramme per kilogram of body weight

MR- Methyl red

MRS agar- De Mann Rogosa Sharpe MRS agar

NA-Nutrient Agar

PCR -Polymerase chain reaction PCV - Packed cell volume

PLT-Platelet count RBC-Red blood cells

ROW- Relative organ weights

S.E.M- Standard Error of Mean

SDA- Sabouraud Dextrose Agar

SDS -Sodium Dodecyl Sulfate

SPSS- Statistical Package of the Social Sciences

VP -Voges Proskauer

WBC- White blood cells

WHO- World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Arachis hypogaea l (Groundnut) is an important leguminous crop cultivated in Nigeria and other tropical countries. It is rich in protein (22-30%), and oil (35-56%) (Balogun *et al.*, 2020) and contains appreciable amounts of minerals (phosphorus, calcium, magnesium and potassium) and vitamins (E, K and B). The difference in the nutritional composition may be due to the soil type, season and region of cultivation (Hajjarpoor *et al.*, 2021; Witcombe, 2021). This annual plant is generally distributed in the tropical, sub-tropical and temperate regions and represents the second most important legume in the world based on total production after soybean (Vendramini, 2020). The main producing countries are China, India, Nigeria, the United States, Indonesia and Sudan (Semba *et al.*, 2021; Naseh *et al.*, 2023).

Kulikuli (Peanut cake) is the byproduct of oil extraction from groundnut fried in oil. It is also used as a groundnut-based snack native to the coasts of West Africa (Achimugu and Okolo, 2020)**.** *Kulikuli* is a popular Nigerian snack which can be eaten either alone or with garri flakes, custard, pap, koko, fura, or kamu. It is also used in making suya and kilishi (beef jerky) just like its parent material (groundnut) it is rich in protein and crude fat (Balogun *et al.*, 2020; Dauda *et al.*, 2020). Apart from being a part of the diet of most age ranges, *Kulikuli* is mostly consumed by middle aged and younger persons, particularly students and is classified as street food which satisfies the essential need of the urban population by being affordable and available, it is also used as a major ingredient in the production of poultry feed formulation (Omari *et al.*, 2020).

Kulikuli has a long shelf life, and good taste, which makes it a good product for

fortification and nutritional improvement (Akinsola *et al.*, 2020; Oluwamukomi *et al.*, 2021). Groundnuts provide considerable amounts of mineral elements as supplements to meet the dietary requirements of humans and farm animals. Besides, groundnut is a good source of vitamin E, magnesium, fibre, polyphenols, vitamin B-6, proteins and unsaturated fats. Recent studies have indicated that groundnuts have the potential to improve age-related impairments in cardiometabolic health and cognitive function that increases with ageing (Coates *et al.*, 2020). The presence of anti-nutrients such as tannin, phytic acid, and trypsin inhibitors necessitates its processing to enhance adequate nutritional benefits.

Fermentation has been known to preserve food with modification of the physicochemical properties and impact the raw material's functional quality. Functional foods have been a topic of considerable interest in the food and nutrition industry for years (Monteiro *et al*., 2023), they can be defined as any food to which a constituent(s) has been added during its processing stage to provide benefits. These constituent(s) can be a known nutrient (e.g., calcium to contribute to bone health), a non-nutrient (e.g., probiotic bacteria to improve gastrointestinal health), or a herb (e.g., Ginseng known to improve alertness). The American Dietetic Association (ADA) referred to 'functional foods' as 'whole, fortified, enriched or enhanced food that should be consumed regularly.

These foods contain ingredients that aid specific body functions and improve health and well-being. The functional food microorganisms include lactic acid bacteria such as *Lactobacillus*, *Lactococcus*, *Leuconostoc* etc. Lactic acid bacteria (LAB) have played a multifunctional role in food, agricultural, and clinical applications (Ayivi *et al.*, 2020; Daba *et al.*, 2021). The use of LAB in food fermentation is one of the oldest food-preserving techniques. Up to the 20th century, food fermentation remained an unregulated process while the discovery and characterization of LAB has changed the views on food

fermentation. Properties such as nutritional, environmental, and adhesional adaptations have provided LAB with the ability to adapt to different environments (Mendoza *et al.*, 2023).

Lactic acid bacteria have made highly remarkable contributions not only in dairy products but also in other fermented foods like fermented fruits, fermented grain products and vegetables. Recent studies have shown the ability of some strains of lactic acid bacteria isolated from Sourdough to hydrolyze some proteins in wheat including albumins, gliadins and globulins (Wang *et al.*, 2021). In addition, LAB are recurrent inhabitants of human mucosal surfaces such as the oral cavity, vagina, and gastrointestinal tract (Zeise *et al.*, 2021). The metabolic activities of LAB are analogous to the production of many beneficial compounds such as organic acids, polyols, exopolysaccharides and antimicrobial compounds. The many benefits of LAB span from enhancing the shelf life and safety of foods, improving food textures, and contributing to the nutritional value of food products through the removal or reduction of anti-nutrients without changing of pleasant sensory profile (Difonzo *et al.*, 2021; Raj *et al.*, 2021).

The production of groundnut, the raw material for *Kulikuli* production has been hampered following contamination by fungi species (Adamu, 2021) such as *Aspergillus, Pennicilium* and *Fusarium*, especially *Aspergillus flavus* which secretes aflatoxins and causes cancer in man. It has also been reported that the incidence of *Aspergillus flavus* is enhanced through broken shells during harvest, and kernel splitting during processing (Cervini *et al.*, 2021). The expression of mycotoxins on food materials is known to vary depending on the presence of other microorganisms. For example, when *Aspergillus parasiticus* was grown in the presence of some bacteria such as *Streptococcus lactis* and *Lactobacillius casei*, aflatoxin production was reduced (Diguță *et al.*, 2020; Guan *et al.*, 2021)For a nation like Nigeria to have a healthy population, it is pertinent that a

relationship between Nutrition, food and health should be reinforced. This can be done by making use of the available resources to meet the ever-growing nutritional requirements of a Nation. Common legumes like groundnut, cowpea and beans are cheaper than animal proteins (eggs, meat and milk) and easier to access (Anzani *et al.*, 2020; Oyeyinka *et al.*, 2021).

1.2 Statement of the Research Problem

Kulikuli is consumed in many West African countries and the data available on this indigenous snack is limited in terms of its safety and nutritional status (Aasa *et al.*, 2023). The scarcity of data may also be because *Kulikuli* is still regarded as a protein source for low-income earners and is not considered a major food even though it has been reported to serve as a major protein supplement due to its high crude protein (Bature, 2021). According to Food Standards Agency, nearly 900,000 cases of food poisoning occur yearly which has led to a high rate of morbidity and mortality due to food-borne illnesses (Bowen *et al.*, 2023).

Lipid oxidation is also another cause of decreased shell life, adverse taste, loss of nutrients and creation of undesirable smell during prolonged storage of peanut products (Pateiro *et al.*, 2021; Rossi-Márquez *et al.*, 2021). Oxidation of the lipid fraction of peanut and peanut products is one of the main causes of decomposition due to its high level of fatty acid unsaturation (López *et al.*, 2022). Hence the need for the development of preservative methods for peanut products to prolong its shelf life.

Adequate nutrition is an important element in the prevention of many civilization-related diseases such as diabetes, cardiovascular disease and obesity (Oyedeji *et al.*, 2021; Kawęcka and Pasternak, 2022). This malnutrition can be combated by fortifying basic foods that are consumed daily, such as *Kulikuli* which is cheap and readily available (Oluwamukomi *et al.*, 2021). Hence the need for adequate investigation to determine the

best means of enhancing this cheap peanut product to reduce the number of malnourished children and women in Nigeria.

Health-conscious consumers are increasingly seeking functional foods to control and improve their health and well-being. The field of functional foods, however, is in its infancy. Claims about the health benefits of functional foods must be based on sound scientific criteria (Baker *et al.,* 2022).

Healthy food can make a significant contribution to health and well-being, but busy consumers may not have time to access their optimum diet. Functional foods can provide ingredients that improve health in a convenient way (Kaur *et al.,* 2023). Increased urbanization is linked to a lifestyle in which our daily routine requires less physical activity and greater access to energy- intensive food (Yan *et al.,* 2023).

1.3 Aim and Objectives of the Study

The aim of this study was to determine the microbiological and safety profiles of *Kulikuli* fermented with *Lactobacillus plantarum*

The objectives of the study were to:

i. isolate *Lactobacillus plantarum* from Corn steep liquor

ii. determine the microbial load of *Kulikuli* samples

iii. determine the nutritional and antinutritional qualities of the *Kulikuli* samples.

iv. determine the physicochemical properties of the fermented *Kulikuli*

v. evaluate the safety test of the fermented *Kulikuli* in rats

1.4 Justification for the Study

Groundnuts and its derivative (*Kulikuli*) when compared with other nuts contain more plant protein than most nuts (Awuchi *et al.*, 2021). However, it is still considered a poor man's food due to this availability and affordability even though in recent times it is gradually becoming a popular export snack to other parts of the world. The acceptability of *Kulikuli* among all age groups has made it a good product for fortification and nutritional improvement (Oluwamukomi *et al.*, 2021). *Kulikuli* contains a considerable number of mineral elements (calcium, magnesium, iron) which helps in the fulfilment of the dietary requirements of humans and farm animals (Awuchi *et al.*, 2021).

Fermented foods play an important role in providing food security, enhancing livelihoods, and improving the nutrition and social well-being of the people (Ikegwu *et al.*, 2023). Fermentation leads to improved food preservation (Rivero-Pino *et al.*, 2023), food quality, and an increased range of edible food products. An enhancement of the nutritive value by an increase of essential nutrients or reduced levels of toxicants in food remains a benefit of the fermentation process. An increasingly important health-promoting role is ascribed to bioactive food components, which are nutritional components or non-nutritional compounds naturally found in the raw material or formed in the product in the course of technological processing, which could enhance, inhibit or modify physiological and metabolic functions of the organism (García *et al.*, 2023). Some bioactive compounds include, e.g., polyphenols, carotenoids, phytoestrogens, sterols, stanols, vitamins, dietary fibre, fatty acids, probiotics, prebiotics, and bioactive peptides (Cichońska and Ziarno, 2022). For the development of functional foods, the gastrointestinal tract is an obvious goal as it acts as an interface between diet and all other metabolic functions. The appropriate balance of healthy bacteria to prevent harmful bacteria invasions depend on

the GI function. Ingredients for modifying the composition and metabolism of the gut microflora are among the most promising areas for functional food development. They include probiotics, prebiotics and synbiotics (mixtures of probiotics and prebiotics).

Proper and balanced nutrition is based on both quality and quantity of food consumption to meet the energy and nutritional needs without causing any health risks. Unfortunately, the modern diet is only rich in quantity but not quality and to appease hunger is different from feeding. The quality deficiency of modern foods stems from the need to provide food to as many people as possible and quickly (Albahri *et al.*, 2023). In recent years, consumers' awareness of these tendencies, has gradually begun to return to green, in the highest sense of the term, without sacrificing comfort, which is essential in modern times. Thus, the functional foods already produced are naturally rich in preserved elements, sometimes with the addition of vitamins or active substances necessary to regain and maintain good health (Ikegwu *et al.*, 2023).

In view of the health-promoting properties of food in recent years, studies have been focused on products of plant origin, one of such plants is *Salvia hispanica*, commonly called chia (Younis *et al.*, 2021). Chia is a herbaceous plant that has also been used for medicinal purposes for thousands of years. Currently, chia seeds are consumed as ingredients or additions to many foodstuffs: baked products, muesli, dairy drinks, fruit smoothies or salads (Bekhit *et al.*, 2022), and are ascribed as very nutritive due to its high fibre and polyunsaturated fats content (Rodríguez-Lara *et al.*, 2021).

The desire for possible fortification of *Kulikuli* using chia seed and *Lactobacillus planatarum* from a cheap and available source (Corn steep liquor) necessitated this research.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Arachis hypogaea l* (Groundnut)

Peanut or "groundnut" is a legume crop grown mainly for its edible seeds (Lawal *et al.*, 2023). In western Nigeria (Yoruba land) peanut is called "epa", in Eastern Nigeria (Igboland) as "ntu oka" and in Northern Nigeria (Hausa land) as "gyada". Peanut (*Arachis hypogaea*) is technically considered a pea and belongs to the family (*Fabaceae*) of bean/legume. Although a legume; it is generally included amongst the oilseeds due to its high oil content. It is widely grown in the tropics and subtropics, is important to small and large commercial producers. Peanuts are rich in protein, oil and fibre (Tachie *et al.*, 2023). Apart from oil, peanuts are widely used for the production of peanut butter, confectionaries, roasted peanuts, snack products, extenders in meat product formulation, soups and desserts (Nabi *et al.*, 2023).

Groundnut occupies an important position in the economy of developing nations. China leads in the production of peanuts, having a share of about 45% of overall world production, whereas India has a 16% share and the United States of America has 5% (Balasubramanian *et al*., 2020). The crop was introduced in Nigeria in the 16th century and an estimated value of 1.4 million hectares of land has been used for its cultivation (Omorodion and Odu, 2022).

There are thousands of peanut cultivars around the world. Certain cultivar groups are preferred for particular uses because of differences in flavour, oil content, size, shape, and disease resistance. For many uses, the different cultivars are interchangeable, however, the most popular cultivars are spanish, runner, malagache, espanola, kresting,virginia and

valencia (Karatay *et al.*, 2020). Most peanuts marked in the shell are of the virginia type, along with some valencias selected for large size and the attractive appearance of the shell.

Figure 2.1: Peanut Seeds (Source: Morsi, (2023))

Spanish peanuts are used mostly for peanut candy, salted nuts, and peanut butter (Sithole *et al.*, 2022). Malagache, espanola, kresting are mostly found in Nigeria (Musa, 2020). Peanuts are consumed all over the world in a wide variety of forms most of which are traditional cuisine. It has notably been the source of elimination of malnutrition amongst the population in many African countries in recent years (Desire *et al.*, 2021). Protein, fats, and fibre are the major components of peanuts. All these components are present in their most beneficial forms. The protein is plant-based, the fat is unsaturated, and the fibre is a complex carbohydrate which is proven to be the best for human nutrition (Soni *et al.*, 2022). Table 2.1 shows the classification of peanuts

2.1.1 History of peanuts

The history of peanuts dates back to the times of the ancient Incas of Peru (Biwer *et al.,* 2022). They were the first to cultivate wild peanuts and offered them to the sun god as part of their religious ceremonials. They used to refer to peanuts as *ynchic.* The modern history of peanut popularization began with the civil war of the 1860s in America. George Washington Carver is known as the "father of the peanut industry'' as he developed more than three hundred products from peanuts (Arya *et al.*, 2016).

Table 2.1 Classification of *Arachis hypogea l*

Taxonomic group	Plant
Kingdom	Plantae
Class	Tracheophytes
Order	Fabales
Family	Fabaceae
Sub family	Faboideae
Genus	*Arachis*
Species	*hypogaea*

Source: Khan *et al.* (2023)

2.2 Local Uses of Groundnut

There are several traditional food products made from peanuts in Nigeria. These fall under different categories based on the processing methods and other ingredients added during the processing. Some of these peanut foods include peanut butter, peanut oil, peanut brittle, *Kulikuli,* etc.

2.2.1 Peanut flour

This is obtained by grinding peanuts into a fine powder, which can be used to make soups, stews, sauces, confectionaries, puddings and bakery products (Nachay and Malochleb, 2018).

2.2.2 Peanut butter

Peanut butter is one of the major food products made from peanuts. It is a nutrient and energy-dense food, rich in unsaturated lipids, protein, fibre etc. (Bonku and Yu, 2020) It's appealing flavour, the convenience of use and excellent shelf life contributes to its popularity. The peanuts used for commercial peanut butter are first sorted and roasted using pan roasting or the traditional roaster until it is well roasted to develop flavour. The roasted peanuts are then air–blast and allowed to cool. After cooling, the peanuts are brushed to remove the skin, kernel, dust, moulds and other foreign materials present on the exterior. Processed peanuts are ground and packaged in plastic or glass containers. It is ensured that the peanut paste does not come into contact with water to prolong its shelf life (Opoku-Boahen *et al.*, 2013; Raigar and Mishra, 2022).

2.3.3 Peanut brittle

Peanut brittle or candied roasted nuts is a local snack consisting of flat broken pieces of hard sugar candy embedded with nuts which are usually less than 1cm in width. The peanuts are sorted, roasted, dehulled and allowed to cool. The peanuts are then pounded in a mortar into a coarse mass or rough powdery texture. The sugar is melted, poured

over the coarse mass and mixed until it becomes stiff. The mixture is then rolled into flat, kite shapes or any preferred shape for consumption or arranged in a glass framed box for sale (Abdulrahaman *et al.*, 2014).

2.2.4 *Kulikuli*

Kulikuli is the residue obtained after the extraction of oil from groundnut paste (Emelike and Akusu, 2018). It is a well-known food item that contributes to an overall dietary protein intake for a large segment of the population especially school children and young adults. It is prepared by sorting, roasting, dehulling and winnowing peanuts. The roasted peanuts are then milled into a smooth paste and water is added to it in bits whiles simultaneously kneading until the texture of the paste toughens and the oil begins to separate. After the oil is collected, the cake that is left is what is used to prepare the *Kulikuli*. Powdered pepper, salt and other spices are added to the peanut cake and fried in oil until it becomes crispy. They can then be consumed or arranged in a glass box for sale (Achimugu and Okolo, 2020).

2.2.5 Dankwa

Dankwa (Powdered soft cookies) is prepared with corn, peanuts, sugar, salt, pepper and other spices. First of all, the corn and peanuts are sorted and roasted together. The peanuts are then dehulled and allowed to cool. The corn and spices are milled together. Subsequently, the dehulled peanuts, sugar and salt are added to the milled corn and spices and milled. The mixture is then moulded into spherical shapes and packaged for sale (Ekpa *et al.*, 2019).

2.2.6 Yaji

Yaji is a mixture of different spices and additives for seasoning foods (Adenugba and Fapohunda, 2022). Generally, yaji can be made up of several spices depending on the type of yaji required and these spices include; ginger, garlic, cloves, red pepper, black

pepper and peanut (Memudu and Duru, 2021). According to History, the word Yaji was named after a 14th century Hausa ruler called Yaji, which means the 'Hot one' (Ugbogu *et al.*, 2018). *Yaji* is made by grinding *Kulikuli* into a fine powder, after which the other ingredients (ginger, garlic, cloves, red pepper, black pepper and seasoning) are added (Ugbogu *et al.*, 2018).

2.2.7 Peanut oil

Peanut oil is a vegetable oil derived from peanuts, which became popular due to the shortage of other oils during World War II (Rizzo, 2022). It is high in mono-saturated fat (oleic acid or omega 9). It also contains high amounts of linoleic acid (a type of omega-9 fatty acid) making it a popular choice for high cooking. It is rich in Vitamin E, an antioxidant that has many health benefits (Idrissi *et al.*, 2022). It is processed by sorting, roasting, dehulling and winnowing peanuts. This is followed by milling the peanuts into a smooth paste. Cold water is then added to the peanut paste immediately after milling and kneaded until the oil separates (Abdulrahaman *et al.*, 2014).

2.3 Lactic Acid Bacteria

There are over 200 strains of lactic acid bacteria. Only those that are useful for processing food can be considered "lactic acid starter culture". Lactic acid starter cultures are also used as probiotic food supplements to help maintain a healthy gut microbiome (Dahiya and Nigam, 2022). They can therefore provide several health benefits by helping to digest food, boosting the immune system, improving digestive health and regulating the gut flora.

2.4 Fermentation of Legumes Using Lactic Acid Bacteria (LAB)

The growing interest in the lactic acid fermentation of legumes is clear from the increasing number of scientific studies about the effects of lactic acid fermentation on the nutritional, physicochemical, and sensorial properties of various sources of legume

protein ingredients, including soybeans, chickpeas, lupins, peas, faba beans, lentils, beans, peanuts etc. Lactic acid fermentation can be applied to legume seed, legume flour or protein-enriched ingredients. However, the efficiency and functionality of lactic acid fermentation depends greatly on the type of LAB strain, the fermentation technique, the type of legume, the composition of the protein ingredient, and slightly on the genetic variety (Elechi and Nwiyi, 2022; Emkani *et al.*, 2022). Figure 2.2 outlines the effects of lactic acid fermentation on legume protein.

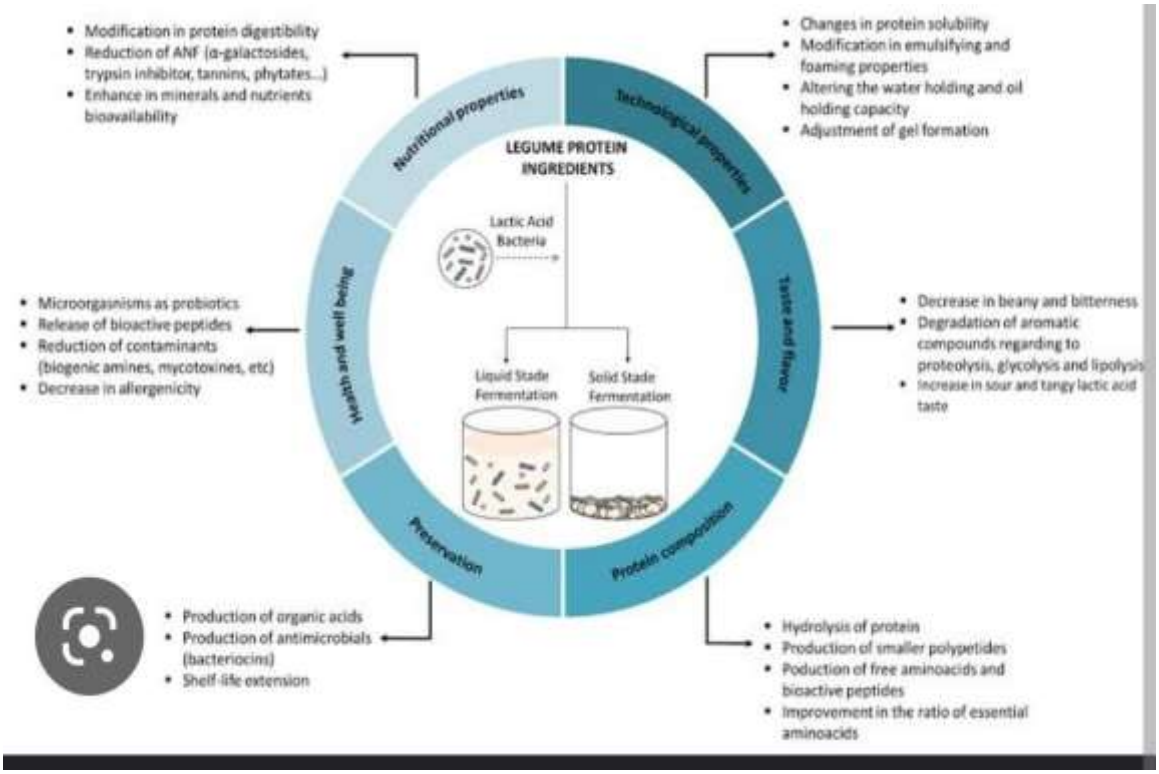


Figure 2.2: Schematic Image Depicting the Effect of Lactic Acid Fermentation on Legume Protein (Emkani *et al*., 2022).

2.5 The Effects of Applied Fermentation Techniques on Legume Products

The selection of the fermentation technique is one of the most important parameters defining the properties of fermented legumes. Solid- and liquid-state fermentation (SSF and LSF, respectively) are two common techniques that have been used for food preservation and the improvement of the nutritional quality of legume ingredients. The SSF is defined as fermentation involving solids in the absence or near-absence of free

water (Filipe *et al.*, 2023). In this case, the substrate must possess enough moisture to support the growth and metabolism of microorganisms. Hence, the microorganisms grow between inter-particle spaces that are surrounded by a gaseous phase. The use of SSF is favourable from an environmental and economic point of view compared to LSF since it requires less energy and water. In LSF, also known as submerged fermentation (SmF), the fermenting microorganisms grow in liquid containing nutrients (Al-Dalali *et al.*, 2022). Studies have shown that both SSF and LSF can lead to diverse modifications in protein functional properties and nutritional qualities. Limón *et al.* (2015) studied the effect of both LSF and SSF using *Lactiplantibacillus plantarum* on bioactive compounds of kidney bean seeds, and the results showed that both methods were suitable for obtaining a water-soluble protein fraction. However, it seems that the amount of peptide released during LSF of kidney bean flour was higher compared to the SSF of cracked kidney beans. The same researchers showed that some protein bands disappeared in the electrophoresis pattern of SSF compared to LSF. However, in their study, SSF presented a higher content of soluble phenolic compounds and higher antioxidant activity compared to LSF. In fact, increasing the content of total phenolic compounds and decreasing the content of antinutritional compounds in the SSF of cracked grains compared to the LSF of flour has been discussed in many studies. To name but a few, Bartkiene *et al.* (2015) studied the fermentation of lupine seed and soy beans with *Lactobacillus sakei*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus*; On the contrary, Torino *et al.* (2013) conducted a study of both LSF and SSF of lentil seeds using *Lb. plantarum* and showed that the water-soluble fraction of lentil protein possessed higher antioxidant activity in the case of LSF compared to SSF. They also showed that the SSF protein had slightly fewer free amino groups compared to LSF, which means that the SSF group showed higher proteolytic activity compared to LSF (Emkani *et al.*, 2022; Lu *et al.*, 2022).

2.6 Effect of Fermentation on Legume Protein Composition

Lactic acid fermentation modifies the content and composition of proteins due to the presence of enzymes and other components such as acids. Microbial proteases can break peptide bonds and produce new polypeptides or even free amino acids (Wang *et al.*, 2023). The LAB have a complex proteinase system, which is composed of extracellular protease, which initiates the degradation of protein into peptides; the peptide transporter; and intracellular proteases, which degrade peptides into shorter oligopeptides and free amino acids. Organic acids such as lactic acid and acetic acid may disrupt the ionic interaction between protein side chains that stabilize the secondary structure, and thus, the presence of acid may lead to the loss of the secondary and tertiary structures of the protein .Ma *et al.* (2017) observed that fermented pea flour with a mixed culture containing *Streptococcus thermophiles*, *Lb. bulgaricus*, and *Lb. acidophilus* exhibited severe damage in the legume cell wall structure compared to non-fermented flours, as determined through the use of scanning electron microscopy. These changes led to smaller peaks in the differential scanning calorimetry (DSC) thermograms of fermented samples, indicating less energy for the breakage of intermolecular bonds within protein bodies to achieve protein denaturation. Native soy protein is more resistant to fermentation with different species of *Lactobacillus* and *P. pentosaceus* than denatured protein (Chan *et al.*, 2023). When hydrolysis conditions are adequate, fermentation may even modify the free amino acid profile.

Changes in the amino acid profile seem to be dependent on the kind of legume and microorganism used. In the fermentation of pea-protein-enriched flours with *Lb. plantarum* (Cabuk *et al.*, 2018), all amino acid mass fractions (mg aa/100 g sample) increased with fermentation time, except for arginine and tryptophan, which remained similar. Sozer *et al.* (2019) reported an improvement in the ratio of essential amino acids

in faba beans after fermentation. The hydrolysis of legume protein differs between LAB strains (Raveschot *et al.*, 2018). García-Gurrola *et al.* (2023) observed that enzymes from twelve different lactic acid bacteria from *Lacticaseibacillus paracasei*, *Lb. fermentum*, *Lactobacillus lactis*, *Lb. plantarum*, *Lb. helveticus*, *Lb. reuteri*, and *P. pentosaceus* hydrolyzed soy protein fractions (7S β-conglycinin, 11S glycinin) from protein isolates to different extents. In a study on soy milk fermentation by *Lb. plantarum* (Rui *et al.*, 2019), it was observed that both α and β subunits of conglycinin were likely to be degraded by almost all of the strains. However, glycinin was less preferable for almost all of the strains. Emkani *et al.* (2022) observed differences in the polypeptide profiles of pea globulin and albumin fractions when using a novel pea protein extraction method, assisted by lactic acid fermentation, compared with a traditional extraction method.

These differences, probably related to the proteolytic activity of bacteria, induced changes in the thermal denaturation properties measured by DSC through modification of the polypeptide composition and conformation. Moreover, differences in the polypeptide profiles of the initial legume proteins may determine their sensitivity during fermentation. This could be the reason that differences were observed in the proteolytic effects of lactic acid fermentation with *Leuconostoc mesenteroides*, *Lb. plantarum*, and *Lb. brevis* co-cultures when comparing flours from different lupine cultivars (Shah *et al.*, 2023). The degree of proteolysis also depends on the legume type, as observed in the fermentation of different legume flours (yellow and red lentils, white and black beans, chickpeas, and peas) with nine selected LAB strains of different species (*Lb. plantarum, P. acidilactici, Lc. mesenteroides, Lactobacillus rossiae,* and *Lb. brevis*) (De Pasquale *et al.*, 2020). The highest proteolysis in their study corresponded to white beans, followed by chickpeas and black beans.

Comparing the concentrations of free amino acid in various legumes, the authors also

showed that, except for red and yellow lentils, which had low amino acid contents before and after fermentation, the highest and lowest increase in the total free amino acids corresponded to peas and chickpeas, respectively. Microorganisms were able to decompose medium-molecular-weight and low- molecular-weight polypeptides in studies of lactic acid fermentation of pea (David-Troncoso *et al.*, 2022), lupine (Schlege *et al.*, 2019) and mung bean flour with *S. thermophilus* and different species of *Lactobacillus*, respectively. Studies on the effect of fermentation on protein content have shown different results, depending on the legume substrate and the lactic acid bacteria used for fermentation.

Changes in the protein structure and hydrolysis of proteins up to the formation of amino acids may decrease the extraction yield of proteins, as indicated in a study of faba bean flour fermentation with different LAB strains (Xu *et al.*, 2019). Other authors, however, did not observe a diminution in protein content after fermentation, indicating that fermentation only changes the molecular size of proteins, such as in the fermentation of lyophilized chickpea and faba bean flours with a yoghurt starter (*Lb. bulgaricus* and *S. thermophilus*) (Chandra-Hioe *et al.*, 2016) Other authors have indicated an increase in the protein content after fermentation. In a study of the fermentation of pea- protein-enriched flour using *Lb. plantarum*, the authors observed an increase in the percentage of protein, fat, and ashes, presumably due to an increase in the bacterial biomass and the loss of carbohydrates during the fermentation process (Cabuk *et al.*, 2018).

2.7 Effect of Lactic Acid Fermentation on Nutritional Properties of Legume Protein

One of the most widely studied aspects of the lactic acid fermentation of legumes is the impact on their nutritional properties. Aspects such as protein digestibility, antinutritional factors, antioxidant capacity, and allergenicity have been considered.

2.7.1 Protein digestibility

Protein digestibility can be defined as how well protein is hydrolyzed by humans (Sozer *et al.*,2019). Considering the fact that fermentation favours the release of protein components, an increase in *vitro* protein digestibility after fermentation is expected.

Certain authors have also suggested that the reduction of antinutritional compounds (e.g., phenolics and tannins) due to fermentation would limit protein crosslinking, making proteins more susceptible to proteolytic attack (Chandra-Hioe *et al.,* 2016; Cabuk *et al.,* 2018). The improvement of protein digestibility after lactic acid fermentation has been studied, as in the case of yellow field pea flour with a lactic acid mixed culture (Ma *et al.*, 2017) and soy flours fermented using different species of lactic acid bacteria (*Lb. sakei, P. acidilactici*, and *P. pentosaceus*) (Bartkiene *et al.,* 2015). However, other authors have not observed an obvious trend regarding *in vitro* protein digestibility after fermentation, such as in the fermentation of pea protein with *Lb. plantarum* (Cabuk *et al.*, 2018). Different behaviours have been observed regarding protein digestibility depending on the kind of legume and even the legume cultivar (Emkani *et al.,* 2022).

2.7.2 Antinutritional compounds

Legume seeds contain certain components, classified as antinutritional factors or non-nutritive compounds, that negatively affect the nutritional quality of legume ingredients (Alrosan *et al.,* 2022). Examples of these antinutritional factors (ANFs) are α-galactosides; phenolic compounds including tannins, trypsin, and chymotrypsin inhibitors; phytic acid, saponins, isoflavones and biogenic amines (Xing *et al.*, 2020). Certain fermenting microorganisms, such as *Streptococcus* sp., *Leuconostoc* sp., and *Lactobacillus* sp., show α-galactosidase activity, which gives them the ability to transform α-galactosides into absorbable mono- and disaccharides (Harlé *et al.*, 2020). Sourdough fermentation of legumes such as chickpeas fermented with *Pediococcus*

strains (Xing *et al.*, 2020); yellow and red lentil, white and black bean, chickpea, and pea flours fermented with *Lb. plantarum* and *Lb. brevis* (De Pasquale *et al.*, 2020) and faba beans fermented with *P. pentosaceus* (Coda *et al.*, 2017) were found to exhibit decreased concentrations of raffinose in the legumes. However, the diminution depended on the kind of legume, as indicated in a study of fermentation of different varieties of chickpeas, lentils, and peas using *Lb. plantarum* and *Lb. brevis* (Knez *et al.*, 2023).

In general, lactic acid fermentation has been found to increase the total phenolic compounds (TPC) of legumes, with differences depending on the kind of legume, the type of LAB bacteria, and the fermentation process. Indeed, the increase in TPC related to the release of these components from the cell wall of the plant tissue could occur due to structural degradation or due to enzymatic conversion during fermentation. De Pasquale *et al.*, (2020) compared the TPC values of four different legume flours—yellow and red lentils, white and black beans, chickpeas, and peas fermented by *Lb. plantarum*, *P. acidilactici*, *Lc. mesenteroides*, *Lb. rossiae*, and *Lb. brevis*. Their results showed that the TPC content was higher for fermented samples compared to the unfermented ones. However, the amount of TPC differed between different fermented legumes. The highest and lowest TPC values belonged to chickpeas and red lentils, respectively.

The degradation of tannins due to fermentation has been indicated in pea flour with a mixed lactic acid bacteria culture containing *S. themophilus*, *Lb. bulgaricus*, and *Lb. acidophilus* (Ma *et al.*, 2017); in red and yellow lentil, white and black bean, chickpea, and pea flours with *Lb. plantarum* and in faba beans with *Lb. plantarum.* An increase in the total tannin levels has been observed in the first five hours of fermentation of pea protein concentrate with *Lb. plantarum* (Cabuk *et al.*, 2018), but it decreases afterwards. The initial increase could be caused by the same factors that affected the increase in the phenolic content. The hydrolysis of condensed tannins follows different pathways,

which involve enzymes such as decarboxylases and oxygenases (Curiel *et al.*, 2015). In consequence, the tannase activity present in fermenting microorganisms was responsible for the degradation of tannins. Phytic acid decreases protein digestibility because it binds with enzymes such as proteases and amylases. Additionally, phytic acid forms complexes with certain minerals, such as calcium, copper, magnesium, iron, manganese, zinc, and amino group derivatives in protein moieties, and thus decreases their absorption in the gastrointestinal tract (Sharma *et al.*, 2021). For people with high daily pulse consumption, this can result in anaemia due to iron deficiency. Some LABs can degrade phytic acid by producing a phytase enzyme. For instance, phytic acid decreased during the fermentation of soymilk with *S. thermophilus*, *Lactobacillus fermentum*, *Lb. plantarum*, *Lacticaseibacillus casei*, *Lb. bulgaricus*, and *Lb. acidophilus (*Nguyen and Vu, 2022). Microorganism-inherent phytases dissociate non-soluble organic complexes with minerals. Studies have indicated that the effect on phytates was closely dependent on the microbial strain (Emkani *et al.*, 2022). It has also been observed that phytic acid degradation is pH-dependent. The optimal pH for most phytases ranges between 4.0 and 6.0. Saponins are a class of glucosides found mainly in plants. They are generally characterized by their bitter taste and by their ability to affect membrane integrity (Doyle *et al.*, 2023). This ability has been associated with both deleterious and beneficial effects on human health.

Saponins reduce nutrient absorption due to the complexation of vitamins or the inhibition of digestive enzymes (Samtiya *et al.*, 2020). However, soybean saponins show health-promoting benefits, such as the prevention of hypercholesterolemia, the suppression of colon cancer cell proliferation, and the anti-peroxidation of lipids. It seems that lactic acid fermentation leads to the reduction in saponin from fermented legumes, as indicated in a study of soymilk fermented with *S. thermophilus* (Emkani *et al.*, 2022) and in a study of

the fermentation of soy flour with *Lb. plantarum.*

2.7.3 Antioxidant activity

The antioxidant capacity of legumes is also modified by fermentation because of changes in antioxidant components such as vitamin C, tocopherol, and glutathione. Furthermore, some of the active peptides and amino acids formed during proteolysis have antioxidant activity (Ma *et al.*, 2017). Antioxidant activity during fermentation has also been studied by observing changes in antioxidant compounds. Vitamin C is an effective antioxidant that acts both directly via a reaction with aqueous peroxyl radicals and indirectly by restoring the antioxidant properties of fat-soluble vitamin E. Studies have shown that fermentation reduces the content of vitamin C in cowpea flour and lupine seeds (Jafarpour and Hashemi, 2023). Fermentation has also been shown to affect tocopherol content and, consequently, vitamin E content. Vitamin E is the most important lipophilic radical-scavenging antioxidant ever studied (Baldi *et al.*, 2023). The effect of fermentation on tocopherol and vitamin E depends on the kind of legume, the kind of microorganism, and even the kind of fermentation process used (Singh *et al.*, 2020). In the fermentation of soybean seeds and flour with *Lb. plantarum*, vitamin E activity decreased as a result of a sharp diminution in α- tocopherol and a slight diminution in β- and γ-tocopherol. The δ-Tocopherol, however, increased sharply (Singh *et al.*, 2020). In lupine seed fermentation, the authors in (Emkani *et al.*, 2022) indicated that α-tocopherol increased, whereas γ and δ decreased, during fermentation with *Lb. plantarum* or with autochthonous microflora. In consequence, vitamin E levels notably decreased.

2.7.4 Other vitamins and bioactive compounds

Apart from antinutritional compounds and antioxidant vitamins, fermentation may also affect the levels of other bioactive compounds. Results have indicated that, in general, natural fermentation reduces the pigment content, such as the anthocyanin content, of

indigenous Nigerian legumes seeds (cowpea, Bambara nut, red bean, pigeon pea, African yam bean seed, African oil bean seed, and groundnut) (James *et al.*, 2020). This decrease could be related to the adsorption mechanism between the fermenting flora and anthocyanins (Rasheed *et al.,* 2022). Decreases in carotenoid and flavonoid contents during fermentation have also been observed (James *et al.*, 2020). It has also been demonstrated that the fermentation of legume flours such as faba bean, soybean, and lupine flours with *Levilactobacillus brevis* (formerly *Lb. brevis*) noticeably increased the amount of vitamin B12 (Xie *et al.*, 2021). The scientific use of the term "vitamin B12" is usually confined to cyanocobalamin. The B12 is naturally present in foods of animal origin. In foods of vegetal origin, B12 can be found only after fermentation or fortification (Kumar *et al.*, 2023a).

2.7.5 Allergenicity

Legume allergies are some of the most common food-related allergies (Anzani *et al.,* 2020). A food allergy is defined as an adverse reaction of the human immune system to an otherwise harmless food component (Moore *et al.,* 2017). The main allergens in legumes are proteins (Licandro *et al.,* 2020), due to the presence of certain proteins responsible for adverse reactions, or due to the poor absorption of legume proteins in the gut. In consequence, modifications in protein structure and content could reduce legume allergenicity. As previously indicated legume fermentation partially hydrolyzes proteins and improves digestibility, and thus, fermentation would decrease the allergenicity of legumes. The fermentation of soybean meal with a mixture of *Lb. casei*, yeast, and *B. subtilis* degraded major protein allergens due to hydrolysis during fermentation (Yang *et al.,* 2018). These authors also observed a reduction in allergenicity measured via the diminution in *in vitro* immunoglobulin E (IgE)-binding capacity and via milder damage to rat intestines. In consequence, fermentation offers an interesting opportunity to produce

hypoallergenic food products from legumes (Emkani *et al.*, 2022).

2.8 Effect of Fermentation on the Functional Properties of Legume Protein

Food can be considered functional if it is satisfactorily demonstrated to beneficially affect one or more target functions in the body beyond its adequate nutritional effects, improve well-being and health, or reduce the risk of disease (Colombo *et al.,* 2020). According to this definition, fermented legumes could be considered functional foods as a result of their effects relating to the reduction in antinutritional compounds, the increase in antioxidant activity and protein digestibility. Furthermore, certain fermenting microorganisms are considered probiotics, that is live microorganisms that, when administrated in adequate amounts, confer their own health benefits to the host. The consumption of fermented legumes has been associated with health benefits such as anti-obesity, antihypertensive, antiallergic, antimicrobial, and antioxidant effects, as well as the prevention of heart disease, cancer, gastrointestinal disorders, diabetes, and osteoporosis.

Sanjukta and Rai (2016), in their study on the potential health benefits of bioactive peptides produced during soybean fermentation, indicated that bioactive peptides are either produced by protein hydrolysis during fermentation or released by fermenting microorganisms. The characteristics of these bioactive peptides depend on the specific microbial strains and the initial proteins. Limón *et al.,* (2015) compared the bioactive peptides in kidney bean protein obtained via LSF and SSF with *Lb. plantarum* and showed a higher potential of antihypertensive activity due to the presence of high angiotensin l-converting enzyme (ACE)-inhibitory activity and the content of γ-aminobutyric acid (GABA). That study suggested that *Lb. plantarum* had a higher capacity for the production of bioactive peptides compared to *B. subtilis*. This indicates that the effect was directly related to the kind of microorganism. In consequence, the increase in the consumption of fermented legumes may increase the health and quality of life of a significant proportion

of the population (Gänzle *et al.*, 2020).

2.9 Binding Agents

2.9.1 Wheat flour

Wheat is the most important stable food crop for more than one-third of the world population and contributes more calories and proteins to the world diet than any other cereal crop (Ben-Hassen and El Bilali, 2022). It is nutritious, easy to store, transport and can be processed into various types of food. Wheat is considered a good source of protein, minerals, B-group vitamins and dietary fibre (Siddiqui *et al.*, 2022), although the environmental conditions can affect the nutritional composition of wheat grains with its essential coating of bran, vitamins and minerals; it is an excellent health- building food. Wheat flour is used to prepare bread, produce biscuits, confectionary products, noodles and vital wheat gluten or seitan (Kure *et al.*, 2021). Wheat is also used as animal feed, for ethanol production, for brewing of wheat beer, wheat-based raw material for cosmetics, as wheat protein in meat substitutes and to make wheat straw composites. The many faces of wheat-wheat berries, wheat bran, grouts, and cracked wheat, offer a good source of fibre and reduce the risk of colon cancer. Wheat also lowers the level of estrogen in the blood which reduces the risk of breast and prostate cancers (Marima *et al.*, 2022).

2.9.2 Nutritional contents

Globally, there is no doubt that the number of people who rely on wheat for a substantial part of their diet amounts to several billions. Therefore, the nutritional importance of wheat proteins should not be underestimated, particularly in less developed countries where bread, noodles and other products such as couscous may provide a substantial proportion of the diet.

(i) Wheat provides nearly 55% of carbohydrates and 20% of food calories. It contains carbohydrates (78.10%), protein (14.70%), fat (2.10%), minerals (2.10%) and

considerable proportions of vitamins (thiamine and vitamin-B) and minerals (zinc, iron) (Bello *et al.*, 2020).

(ii) Wheat is also a good source of trace minerals like selenium and magnesium, which are essential to good health (Bulut *et al.,* 2022; Jaiswal *et al.,* 2022).

(iii) Wheat grains are also rich in pantothenic acid, riboflavin and some minerals, sugars etc. The barn, which consists of pericarp testa and aleurone, is also a dietary source of fibre, potassium, phosphorus, magnesium, calcium, and niacin in small quantities. The kernel of wheat is a storehouse of nutrients essential to the human diet (Dwivedi *et al.,* 2022; Iqbal *et al.*, 2022).

2.9.3 Types of wheat flours and their uses

2.9.3.1 *All-purpose flour*

All-purpose flour is the finely ground endosperm of the wheat kernel separated from the bran and germ during the milling process. All-purpose flour is made from hard wheat or a combination of soft and hard wheat from which the home baker can make a complete range of satisfactory baked products such as yeast breads, cakes, cookies, pastries and noodles (Kanojia *et al.*, 2018).

2.9.3.2 *Bread flour*

Bread flour is made from the endosperm of the wheat kernel, and is milled primarily for commercial bakers but is also available at retail outlets. Although similar to all-purpose flour, it has greater gluten strength and is generally used for yeast bread (Sarwar *et al.*, 2023).

2.9.3.3 *Self-rising flour*

Self-rising flour is all-purpose flour with salt and leavening added. One cup of self-rising flour contains one and a half teaspoons of baking powder and 1/2 teaspoon salt. Self-

rising flour can be substituted for all-purpose flour in a recipe by reducing salt and baking powder according to those proportions (Gardner, 2022).

2.9.3.4 *Whole wheat flour*

Whole-wheat flour is a course-textured flour ground from the entire wheat kernel and thus contains the bran, germ and endosperm (Tyl and Marti,2022). The presence of bran reduces gluten development. Baked products made from whole-wheat flour tend to be heavier and denser than those made from white flour.

2.9.3.5 Cake flour

Cake flour is made from milled soft wheat, suitable for cakes, cookies, crackers and pastries (Gómez, 2022). It is low in protein and gluten.

2.9.3.6 *Pastry flour*

Pastry flour is made from ground soft, low-gluten wheat (Iqbal *et al.,* 2022). Comparable in protein but lower in starch than cake flour.

2.9.3.7 *Gluten flour*

This flour is used by bakers in combination with flours having a low protein content because it improves the baking quality and produces gluten bread of high protein content (Šmídová and Rysová, 2022).

2.9.3.8 *Semolina*

Semolina is made from coarsely ground endosperm of durum wheat. High in protein and is used in the production of high-quality pasta products (Ikbal *et al.*, 2022).

2.9.3.9 *Durum flour*

This is a by-product of semolina production. It is used to make commercial noodles (Iqbal *et al.*, 2022).

2.9.3.10 *Farina*

Farina is made from coarsely ground endosperm of hard wheat. It is a prime ingredient in many U.S. breakfast kinds of cereal. Also used in the production of inexpensive pasta which is very low in saturated fat. It contains little to no cholesterol; it is very low in sodium and sugar. Farina is high in dietary fibre, manganese and phosphorus (Ikbal *et al.*, 2022).

2.10 Chia Seed

The word "Chia" is derived from Aztec word "chian" meaning oily (Motyka *et al.*, 2022). The word "chia" creates a part of the name of the present Mexican State Chiapas, where chia is produced in the highest volume (Soria-Barreto *et al.*, 2023). Chia (*Salvia hispanica* L.) is an annual herbaceous plant that belongs to the *Lamiaceae* family (Abdel Ghani *et al.*, 2023). Table 2.2 shows the classification of chia seeds. Chia is a desert plant that was cultivated for centuries by the Aztecs of ancient Mexico. Chia seeds were a very important crop of the Aztec people, along with corn, as they incorporated these seeds into their daily diet (Masood, 2022). Chia seeds were eaten in Aztec epoch alone or blended with cereals, in whole or milled into flour.

Chia is an annual herb growing up to 1.75 metres (5 feet 9 inches) tall, with opposite leaves that are 4–8 cm long and 3–5 cm wide (Makokha *et al.*, 2023). Its flowers are purple or white and are produced in numerous clusters in a spike at the end of each stem. The seeds are small ovals with a diameter of around 1 mm (1⁄32 in). They are mottle-coloured, with brown, grey, black, and white. The seeds are hydrophilic, absorbing up to 12 times their weight in liquid when soaked. While soaking, the seeds develop a mucilaginous coating that gives chia-based beverages a distinctive gelatinous texture (Nyingi and Mburu, 2021).

Figure 2.3: Chia Plants, Flowers and Seeds (a) Purple and White Flowers, (b) Seeds from different Commercial Lines**.** Source: Hernández-Pérez *et al*. (2020)

Table 2.2: Classification of *Salvia hispanica*

Taxonomic group	Plant
Kingdom	Plantae
Class	Tracheophytes
Order	Lamiales
Family	Lamiaceae
Genus	*Salvia*
Species	*hispanica*

Source: Nyingi and Mburu (2021).

It is now widely cultivated and commercialized for its (omega) ω-3 alpha-linolenic acid (ALA) content and antioxidant properties. It is characterized by high contents of polyunsaturated fatty acids, mainly α-linolenic acid (ALA), which accounts for approximately 60% of all fatty acids (Islam *et al.,* 2023). Linoleic, oleic and palmitic acids are found in lower amounts. Furthermore, chia is a good source of omega-3 for commercial food applications because it can be added straight to products without flavour alterations, commonly required in products containing fish oil. Other oil seeds such as flax, with high concentrations of ALA, contain anti-nutritional and vitamin antagonistic factors, however, Chia seeds are not reported to contain any anti-nutritional factors (Khursheed *et al.*, 2023). Chia seed is a good source of dietary fibre, containing about 5 percent soluble fibre which appears as clear mucilage when it is placed in water. The seeds are ground into meals and then processed into bread, biscuits, muffins, and cakes (Mondor, 2023).

The seed composition which on average varies from (30-33%) fat, (6%) moisture, (15-25 %) protein, (18-30 %) fibre and (26-41 %) carbohydrate makes chia seeds an important ingredient in food as well as medicine (gar, 2022). Analyses of the amino acid composition confirmed the presence of 10 exogenous amino acids, among which, the greatest contents were arginine, leucine, phenylalanine, valine and lysine. Proteins in chia seeds are also rich in endogenous amino acids, mainly glutamic and aspartic acids, alanine, serine and glycine (Motyka *et al.*, 2023; Salem *et al.*, 2023).

The presence of fatty acids in chia seeds is crucial for health, antimicrobial, and antioxidant activity (Khursheed *et al.*, 2023). Most species from the genus Salvia have horticultural and homoeopathic importance as good sources of natural constituents like flavonoids such as myricetin, quercetin and kaempferol, and polyphenols chlorogenic and caffeic acid (Nyingi and Mburu, 2021). Chia seeds contain functional purposes that help the digestive system, enhance skin health, support strong bones and muscles, and help reduce heart illnesses, among other diseases (Islam *et al.*, 2023).

2.10.1 Application of chia seeds

Several studies have been done on chia seeds in the food industry, chia seed has been applied in various forms such as whole, oil, ground, or gel. Chia seed intake is recommended to be a 48% daily allowance (Motyka *et al.*, 2022). Chia seeds are added as supplements into food products such as pasta, cereals, snacks, biscuits, or cakes (Khursheed *et al.*, 2023). They can be used as substitutes due to their hydrophilic characteristics for fats and eggs in baked items. Butter can be fortified with Chia to increase its nutritional value (Maldonado *et al.*, 2023).

Chia mucilage from chia seeds is utilized as a functional coating with enhanced functional characteristics, serving as a stabilizer, emulsifier, suspending agent, binder, or adhesive due to its viscosity and water-holding capabilities (Mujtaba *et al.*, 2023).

Chia mucilage is the source of polysaccharides and is used to produce polymer blends for consumable coating and films. Chia gum contains a fat content of 26.2%, and if fat extraction is conducted, two parts are obtained: gum with fat and defatted gum. The defatted gum contains high protein, carbohydrates, and ash contents (Nassef *et al.*, 2023). Chia seeds are packed with essential omega-3 fatty acids, dietary fibre, protein, antioxidants, and minerals such as calcium (Aamer *et al.*, 2023). These nutrients give it a range of benefits, such as boosting weight loss to controlling blood sugar levels. They also support bone and tooth health, boost metabolism, and reduce inflammation. Chia seeds are rich in antioxidants, which are not only good for the cells, but for the skin as well as help slow down the signs of ageing (Khalid *et al.*, 2023). The antioxidants in chia seeds have also been found to reduce the risk of cancer and heart disease.

Chia is a crucial ingredient for the development of functional food products (Dinçoğlu and Yeşildemir, 2019). The bioactive components found in chia seeds may be used to fortify food, besides being used as a vehicle for increasing the number or amount of vital nutrient intake. The formulation of new functional foods with the incorporation of chia seeds is a promising and innovative way of protecting and delivering omega-3 fatty acids into a range of food products (Islam *et al.*, 2023; Khalid *et al.*, 2023 and Khursheed *et al.*, 2023).

Due to the presence of a-linolenic acid, Chia seeds are crucial for the formation of certain biochemicals including thromboxane and leukotrienes, that are linked to the human body's vital physiological functions (Duchnik *et al.*, 2023). Also, Chia seeds' fatty acid has been observed to be capable of blocking sodium and calcium channel dysfunctions, averting possible hypertension. During pregnancy, Chia seed consumption has been found to help in the fetus' brain and retina development (Borasio *et al.*, 2023; Rizzo *et al.*, 2023).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in the capital city of Niger state (Minna), in the North central part of Nigeria. The samples were purchased from Kure market, Minna Niger State respectively. Minna is situated amid latitude 9◦36'55" N to 37'13" N and flanked by longitude: 6◦32'0" E to 6◦ 31'40" E. The map of Niger State showing the study is presented in Figure 3.1.

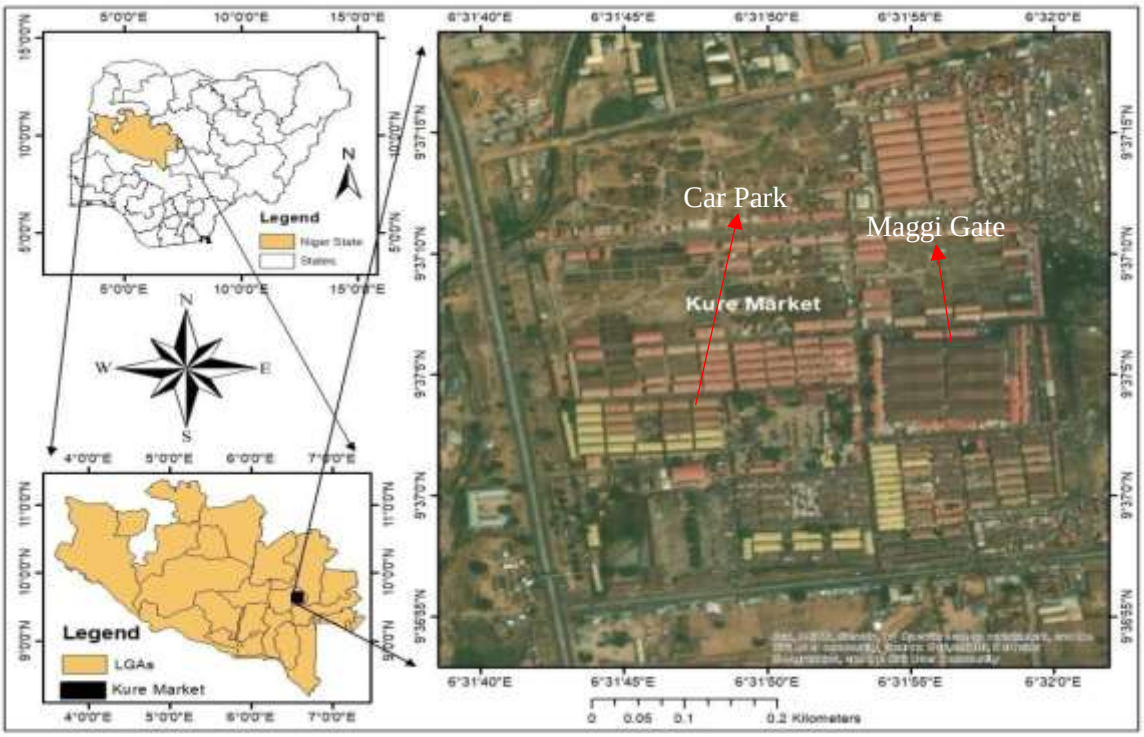


Figure 3.1: A Map showing Kure Market, Minna Niger state
(Source: Geography Department, Federal University of Technology, Minna)

3.2 Collection and Identification of Groundnuts

Groundnut seeds and ready-to-eat *Kulilkuli* (Plates I and II) were purchased from Kure Market, Chanchaga, Local Government Area, Niger State, Nigeria in August, 2020. The samples (groundnut seeds and *Kulilkuli*) were transferred to sterile containers and transported to the Centre for Genetic Engineering (STEP-B), Federal University of

Technology, Minna, Niger State for further analyses. The groundnuts were authenticated by an ethnobotanist from the Department of Plant Biology, Federal University of Technology in Minna, Niger State as *Arachis hypogea* L (Fabaceae). Vouchers specimen were deposited in the Herbarium Unit of the Department.

Plate I: *Arachis hypogaea* L (Groundnut seeds) (Source: Fieldwork)

Plate II: *Kulikuli* from the market (Source: Fieldwork)

3.3 Isolation of Bacteria from Corn Steep Liquor

The method of Karigidi and Olaiya (2020) was employed in the preparation of Corn steep liquor. Ten grams (10g) of washed white corn grains were soaked in hot water for 72 hours. It was milled using a blender and filtered using a clean muslin cloth. The filtrate was allowed to settle and the supernatant (corn steep liquor) was collected and used for the isolation of *Lactobacillus* species. Pour plate technique was employed for the isolation of *Lactobacillus* species. One millilitre (1 ml) of the corn steep liquor sample was dispensed in 10 ml of water and swirled for homogeneity. A tenfold serial dilution was performed from the sample homogenate and 1 ml aliquot of 10^{-7} ,10^{-8} and 10^{-9} of the diluents were inoculated on De Mann Rogosa Sharpe (MRS) agar. The inoculated plates were incubated at 37°C for 72 hours in an anaerobic jar and suspected LAB colonies (whitish elevated dry colonies) were then subcultured on De Mann Rogosa Sharpe (MRS) agar to obtain a pure culture. The purified isolates were maintained on MRS Agar slants as stock cultures at -4°C (Orji *et al.*, 2020).

3.4 Characterisation and Identification of Suspected *Lactobacillus* Isolates

The isolated colonies were carefully examined microscopically for characteristics such as shape, colour, size and consistency. The suspected *Lactobacillus* isolates were characterized based on colony morphology, Gram reactions and biochemical tests such as catalase, coagulase, citrate utilization, oxidase, indole, methyl red, Voges-Proskauer, starch hydrolysis, urease production and sugar fermentation (glucose, lactose, maltose and sucrose) (Muhammad *et al.*, 2020).

3.4.1 Microscopy

The suspected Lactic acid bacterial isolates were identified by picking a colony and placing it on a grease-free slide, Gram stained and viewed under a microscope.

3.4.1.1 *Gram staining*

Using a sterile loop, a light suspension of the test organism was streaked on a grease-free slide. The smear was airdried and heat-fixed by passing the slide twice through a flame. The slide was then allowed to cool. The slide was placed on a staining rack, flooded with crystal violet solution, and left for 60 seconds before washing off with running tap water. The slide was again flooded with Lugol's iodine solution and left for 30 seconds before washing off with running tap water. Acetone alcohol was added to the film and washed off immediately with running tap water. The film was flooded with safranin solution and left for 1 minute before washing off with running tap water. The film on the slide was allowed to air-dry. A drop of immersion oil was then placed on the film, and it was examined under the microscope using the ×100 oil immersion lens. The Gram-positive bacteria retained the colour of primary dye and showed purple colour. The shapes and arrangement of the cells were also recorded (Biswas *et al.*, 2020).

3.4.1.2 *Motility test*

A loopful of bacterial isolate was inoculated into already prepared motility agar that is semi-gelled by stabbing using a straight wire loop and incubated for 24 hours at 37°C. After 24 hours it was observed for the presence of motility. The presence of motility showed a cone-shaped growth by the organism (spread growth pattern). Non-motile organisms only grow along the line of stabbing (Maheswari and Vitendra, 2020).

3.4.2 Biochemical tests

3.4.2.1 *Catalase test*

A loopful of the bacterial isolate from the slant was transferred into a clean sterile glass slide containing 3% hydrogen peroxide and was mixed properly. Organisms were identified based on the ability to produce catalase that breaks down the hydrogen peroxide. The appearance of a gas bubble indicated a positive reaction (Begom *et al.*, 2020).

3.4.2.2 *Oxidase test*

Kovac's reagent (1% tetramethyl- p-phenylene diamine dihydrochloride) was dissolved in warm water and stored in a dark bottle. Using a wire loop, a colony of the test organism was transferred to the filter paper and rubbed on the moistened area. Purple colouration within 30 seconds indicated the production of cytochrome c oxidase (Begom *et al.*, 2020).

3.4.2.3 *Citrate utilization test*

Bacterial colonies from fresh (18 to 24 hours old) plates were inoculated onto a slope of Simmons citrate agar and incubated for 24 - 48 hours. at 37°C. A change in colour of medium from green to blue indicated a positive reaction, that is, the organism can utilize citrate as a sole source of carbon and energy (Begom *et al.*, 2020).

3.4.2.4 *Starch hydrolysis*

In the starch hydrolysis test, a starch-containing medium was employed. Iodine reagent was added after inoculation and incubation (16 hours) of the medium to detect the presence of starch. Iodine reacts with starch to form a blue-black colour in the culture medium. A clear zone of inhibition is an indication of the organism's ability to digest the starch in the medium (Begom *et al.*, 2020).

3.4.2.5 *Indole test*

A 24-hour test organism was inoculated in a Bijou bottle containing 3 ml of sterile tryptone water and incubated at 35–37°C for 48 hours. Indole was tested for by adding 0.5 ml (5 drops) of Kovac's reagent (isoamyl alcohol; para-dimethyl amino benzaldehyde; concentrated hydrochloric acid) and was agitated gently. A cherry colour in the surface layer within 10 minutes indicated a positive reaction while a yellow colour indicated a negative reaction (Maheswari and Vitendra, 2020).

3.4.2.6 *Urea test*

Urea agar slants were prepared, inoculated with test organism and incubated at 37˚C for 24-48 hours. A positive result showed a colour change from light red or peach to pink while a negative result showed no colour change (Biswas *et al.*, 2020).

3.4.2.7 *Methyl red (MR) and Voges Proskauer (VP)*

Five millilitres (5ml) of MR-VP broth were inoculated with the organism and incubated at 37˚C for 48-72 hours after which, 1ml of the broth was transferred into a test tube, and then, 2-3 drops of methyl red reagent were added. To the rest of the broth in the original tube, some drops of 4% potassium hydroxide (KOH) were added followed by the addition of some drops of 5% naphthalin ethanol. The tube (sealed with a cotton plug) was agitated and placed in a slanted position. The development of a red colour starting from the liquid-air interface within 1 hour indicated a VP positive result while the absence of colour change indicated a VP negative result (Biswas *et al.*, 2020).

3.4.2.8 *Sugar utilization test*

The sugar solutions used in this experiment were glucose, sucrose, maltose, lactose, galactose, fructose, sorbitol and mannose. The media prepared with each of the sugar were dispensed into different sets of test tubes containing inverted Durham tubes. The test organism was aseptically introduced into the sugar and medium mixed. Also, 0.1% phenol red indicator was added into test tubes. The indicator and inverted Durham tubes were used to detect respectively the production of acid and liberation of gas by the isolates. Un-inoculated tubes were used as control. Positive results were interpreted by the change in colour of phenol red from red to yellow/orange colour (Maheswari and Vitendra, 2020).

3.5 Molecular Identification of Isolate from Corn Steep Liquor

The method of Adeyemo and Onilude, (2014) was employed for the identification of isolate from Corn steep liquor. A single colony grown on a medium was transferred to 1.5 mL broth and placed in a shaker for 2 days (48 hours) at 28 °C. The cultures were centrifuged at 4600 rpm for five minutes. The resulting pellets were re-suspended in 520 µL of 10 mM Tris-HCL buffer (TE) and 1 mM Ethylenediaminetetraacetic acid (EDTA), pH 8.0. Fifteen microliters (15µl) of 20% Sodium Dodecyl Sulfate (SDS) and 3 µL of Proteinase K (20 mg/ml) were added. The mixture was incubated for 1 hour at 37°C, then 100 µL of 5M NaCl and 80 µL of 10 % Cetyl trimethylammonium bromide (CTAB) solution in 0.7 M NaCl were added and mixed. The suspension was incubated for ten minutes at 65°C and kept on ice for 15 minutes. An equal volume of chloroform: isoamyl alcohol (24:1) was added, and the mixture was incubated on ice for 5 minutes and centrifuged at 7200 rpm for 20 minutes. The aqueous phase was transferred into a new tube, isopropanol (1:06) was added and the DNA was precipitated at -20°C for 16 hours. The DNA was collected via centrifugation at 7200 rpm for 10 minutes, washed with 500 µL of 70 % ethanol, air dried at room temperature (28±2°C) for three hours and dissolved in 50 µL of TE buffer.

The polymerase chain reaction (PCR) cocktail consisted of 10 µL of 5x GoTaq colourless reaction, 3 µL of MgCl2, 1 µL of 10mM of dNTPs mix, 1 µL of 10 pmol each of 27F 5'-AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. The PCR was carried out in a GeneAmp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA) with a polymerase chain reaction profile consisting of an initial denaturation at 94°C for 5 min; followed by 30 cycles consisting of 94°C for 30s, 50°C for 60s and 72°C for 1 minute 30 seconds; and a

final termination at 72°C for 10 minutes. It was then allowed to chill at 4°C in a gel.

The integrity of the amplified (1.5Mb) gene fragment was checked on a 1% Agarose gel to confirm the amplification. This was carried out by mixing 8 µL of amplified product to 4 µL of loading dye and passed through solidified Agarose gel of 110 V for 60 minutes. A picture was taken under UV light and the amplified product was observed on a nanodrop of model 2000 from thermo scientific quality to determine the concentration of the amplified product.

After gel integrity, the amplified fragments were purified in ethanol to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each about 40µl PCR amplified products in a new sterile 1.5 µl tube eppendorf, mixed thoroughly by vortexing and kept at -20°C for at least 30 minutes. Centrifugation for 10 minutes at 13000 rpm and 4°C followed by removal of the supernatant by inverting the tube, after which the pellets were washed by adding 150 µl of 70% ethanol, the solution was mixed and then centrifuged for 15 minutes at 7500 g at 4°C. All supernatants were discarded on a paper tissue. This was allowed to dry in the fume hood at room temperature (28±2°C) for 10-15 minutes, then resuspended with 20 µl of sterile distilled water and was kept in -20°C before sequencing. The purified fragment was observed on a 1.5% Agarose gel ran on a voltage of 110V for about an hour to confirm the presence of the purified product.

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using the manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio-Edit software and MEGA 6 were used for all genetic analyses.

3.6 Determination of Microbial Load of *Kulikuli* Obtained from the Market

One gram (1g) of *Kulikuli* obtained from the market (Kmkt) was transferred aseptically into a sterile test tube containing 9 ml of sterile water and serially diluted up to dilution 10^{-4}. One millilitre of the diluent (10^{-4}) was inoculated in duplicates onto Nutrient Agar (NA), MacConkey Agar (MAC) and Sabouraud Dextrose Agar (SDA). The plates were incubated at 37°C for 24 hours for bacterial isolates and at 28°C for 3-5 days for fungal isolates (Wanjiku *et al.*, 2020).

The microbial load which is an estimate of total viable microorganisms in a sample was determined in colony forming unit per gram (CFU/g) of the sample. The Colony forming unit was calculated using Equation 3.1:

$$Colony\ forming\ unit\ (CFU) = \frac{\text{Number of colonies x Dilution factor}}{\text{Volume plated}} \quad (3.1)$$

3.6.1 Isolation and identification of microorganisms from *Kulikuli* obtained from the market

Discrete bacterial and fungal colonies were subcultured onto freshly prepared Nutrient Agar (NA) and Sabouraud dextrose agar (SDA) agar plates respectively to obtain pure cultures. Pure cultures obtained were stored on appropriate agar slants for further analysis.

The bacterial isolates were identified based on colony morphology (shape, texture and colour), Gram's reaction and various biochemical tests. Fungal isolates were identified macroscopically using their morphological characteristics (colony appearance, type of colonies, colony colour (surface and backside). Microscopy of the fungal isolates was carried out by placing a drop of lactophenol blue on a clean, grease-free glass slide. Using an inoculating needle, a minute piece of mycelium was picked and placed on the

slide and dispersed evenly, before covering it with a cover slip to avoid air bubbles and then viewed under a microscope using x10 and x40 objective lens (Wanjiku *et al.*, 2020).

3.7 Aseptic Preparation of *Kulikuli* in the Laboratory

One kilogram (1kg) of shelled groundnut was sorted, roasted and pulverized into a paste. The groundnut paste was transferred into a sterile mortar, 250 mL of sterile water, 100 g of sugar and 10 g of salt was added to the paste and stirred using a clean pestle. Oil was removed and 90 g of the resultant mixture was pressed, moulded and fried into *Kulikuli* flakes (labelled Unfermented *Kulikuli* (UK)), the flakes were allowed to cool and packaged in sterile polythene bags for microbiological analysis (Achimugu and Okolo, 2020). The remaining mixture was kept as stock for the formulation of fermented *Kulikuli.*

3.8 Preparation of Starter Culture

The starter culture of *Lactobacillus plantarum* (LP) isolated from corn steep liquor was prepared by transferring a loopful of an overnight culture of the organism from MRS agar into 10 mL MRS broth and incubated at 35°C for 24 hours. One hundred microliters (100 µL) of the 24 hours old culture were transferred into 10 mL MRS broth and incubated at 35°C for 16 hours. Subsequently, cells were harvested by centrifugation at 5000 g for 10 minutes (4°C) washed three times with 20 mL sterile diluent (Merck) pH 7.2 ± 0.2 and resuspended in 50 ml of sterile water to obtain an initial cell density of 7 log CFU/g which served as the inoculum (Akabanda *et al.*, 2014; Maidana *et al.*, 2020).

3.9 Formulation of Fermented *Kulikuli* Samples

Six (6) glass jars were sterilized and ninety grams (90 g) of the stock groundnut paste was placed in each. The jars were labelled and contained the following:

1. Jar containing only the groundnut paste (K)

2. Jar containing *Kulikuli* and *Lactobacillus plantarum* (5 mL) (KL)
3. Jar containing *Kulikuli* and all-purpose flour (9 g) (KF)
4. Jar containing *Kulikuli* and chia flour (9 g) (KC)
5. Jar containing *Kulikuli*, all-purpose flour (9 g) and *Lactobacillus plantarum* (5 mL) (KFL)
6. Jar containing *Kulikuli* and chia flour (9 g) and *Lactobacillus plantarum* (5 mL) (KCL)

The jars were tightly closed and kept in a room for three (3) days at room temperature to enable fermentation to take place. After fermentation, the content of each jar was moulded and fried separately. It was allowed to cool, packaged and labelled accordingly. Table 3.1 shows the constituents of the samples.

Table 3.1: Recipe for the Preparation of fermented *Kulikuli* Samples

Ingredients	**K**	**KL**	**KF**	**KC**	**KFL**	**KCL**
Groundnut paste (g)	90 g	90 g	90 g	90 g	90 g	90 g
Lactobacillus plantarum inoculum(mL)	-	5 mL	-	-	5 mL	5 mL
All-purpose flour (g)	-	-	9 g	-	9g	-
Chia powder (g)	-	-	-	9 g	-	9 g

K: fermented *Kulikuli;* KL: fermented *Kulikuli* and *Lactobacillus plantarum*; KF: fermented *Kulikuli and* all- purpose flour; KC: fermented *Kulikuli* and chia flour; KFL: fermented *Kulikuli*, all- purpose flour and *Lactobacillus plantarum*; KCL: fermented *Kulikuli*, chia flour and *Lactobacillus plantarum*

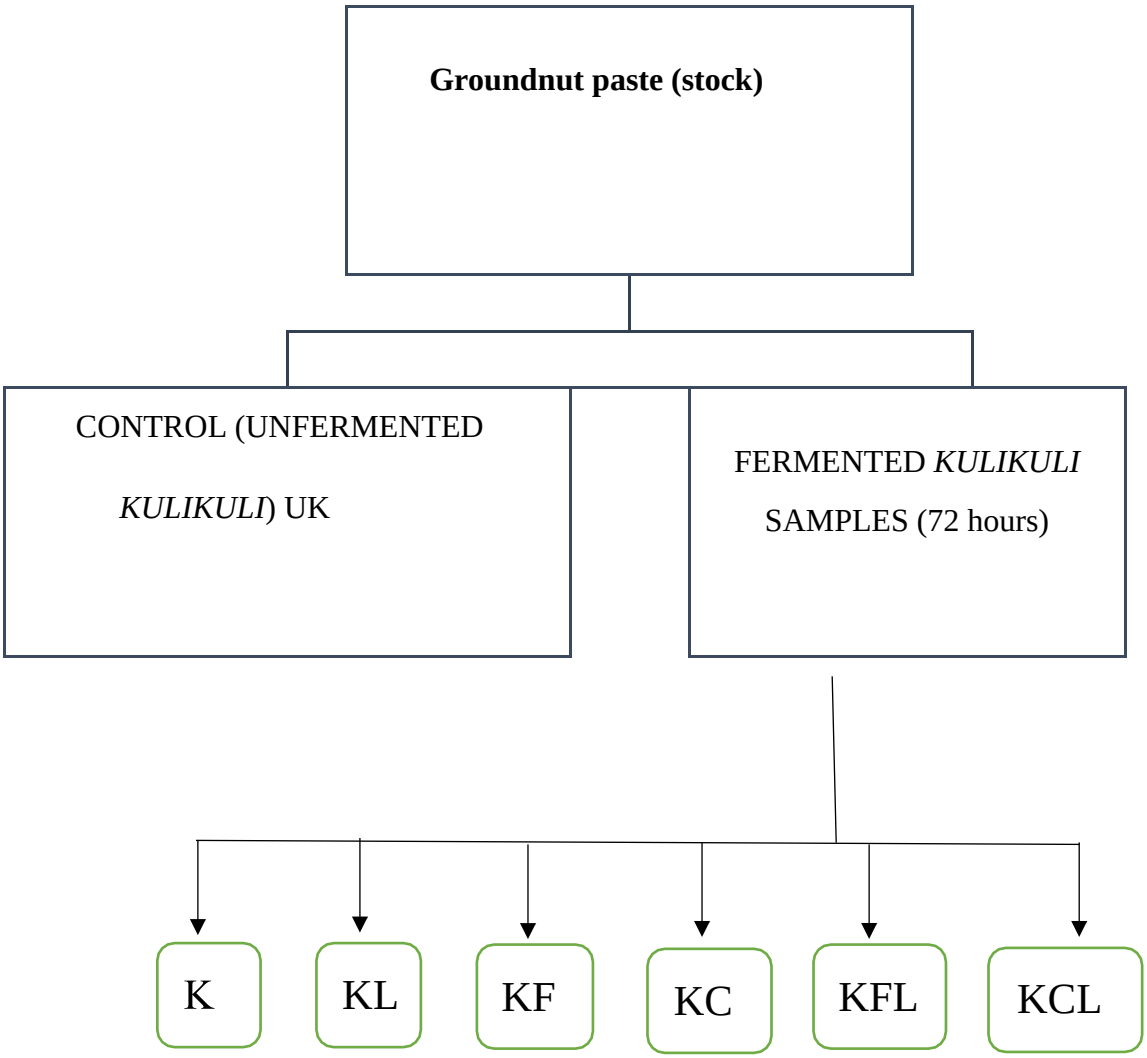


Figure 3.2: Laboratory preparation of the fermented *Kulikuli* samples.

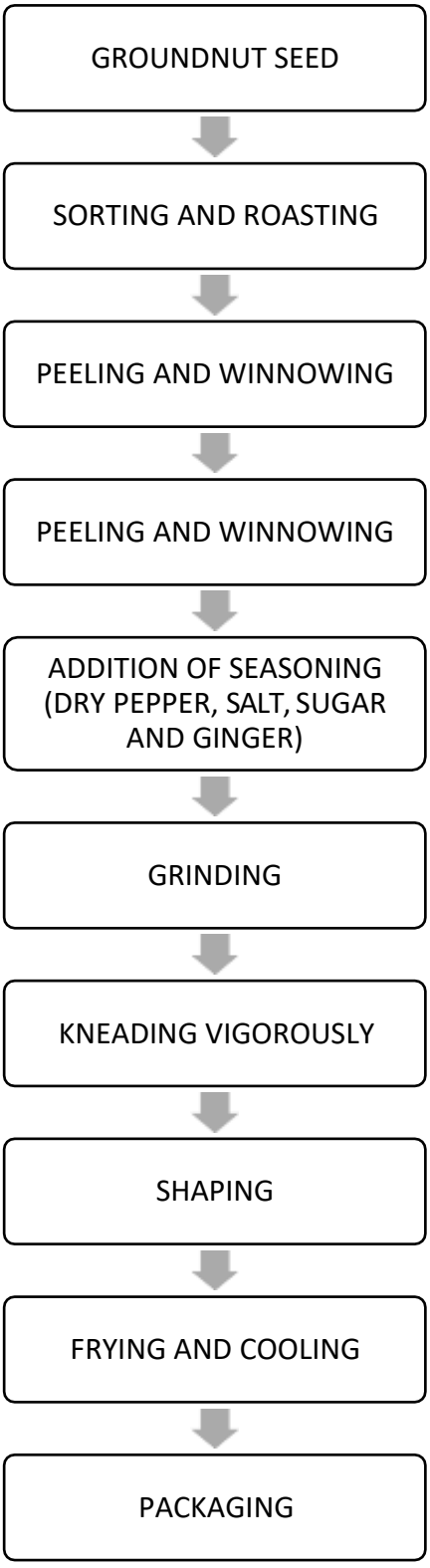


Figure 3.3: Preparation of *Kulikuli*
(Achimugu and Okolo, 2020)

Plate III: Fried Fermented *Kulikuli* Samples

3.10 Determination of Total Microbial Load of Laboratory-Prepared *Kulikuli* Samples

One gram (1 g) from each of the prepared *Kulikuli* samples; Unfermented *Kulikuli* (UK) served as one of the controls and the 6 fermented samples (K, KL, KF, KC, KFL, KCL) were transferred aseptically into a sterile test tube containing 9 ml of sterile water and serially diluted using the method of Wanjiku *et al.* (2020).

3.11 Characterisation and Identification of Microorganisms from Laboratory Prepared *Kulikuli* Samples

The method of Wanjiku *et al.* (2020) was employed for the characterization and identification of all the *Kulikuli* samples.

3.12 Determination of Frequency of Occurrence of Microbial Isolates from *Kulikuli*

The frequency of occurrence of the isolates was determined by counting the number of occurrences of a particular organism compared to the total organisms isolated from all the *Kulikuli* samples.

3.13 Determination of Nutritional Composition of the *Kulikuli* Samples

The methods of the Association of Official Analytical Chemists (AOAC, 2000) and Akalu and Geleta (2019) were used for the determination of moisture, crude fibre, protein, fat, ash and carbohydrate.

3.13.1 Determination of moisture content

About 1.5 grams of each of the sample was transferred into a dried weighed crucible (W1), mixed thoroughly and dried at 105 °C for 6 hours. The dried samples were dried in the desiccator for 30 minutes, allowed to cool and reweighed. The process was repeated until a constant weight was obtained (W2). The percentage moisture content

was calculated using the Equation 3.2:

$$\% \text{ Moisture } = \frac{W1-W2}{Wt} \; x \; 100 \qquad (3.2)$$

Where: W1 = initial weight of crucible + sample; W2 = final weight of crucible + sample; Wt = weight of the sample.

3.13.2 Determination of nitrogen and crude protein

One and a half (1.5) gram of the groundnut cake powder in an ashless filter paper was introduced into a 300 mL Kjeldahl flask. Twenty-five millilitres of concentrated H_2SO_4 and 3 g of digesting mixed catalyst (weighed separately into an ashless filter) was also dispersed into the Kjeldahl flask. The flask was then transferred to the Kjeldahl digestion apparatus. The sample was digested until a clear green colour was obtained. The digestion cooled and diluted to 100 mL with distilled water. Twenty millilitres (20 mL) of diluted digest were dispersed into 500 mL Kjeldahl flask containing anti-bumping chips and 40 mL of 40% NaOH was slowly added by the side of the flask. A 250 mL conical flask containing a mixture of 50 mL of 20% boric acid and 4 drops of the mixed indicator was used to trap the ammonia being liberated. The conical flask and the Kjeldahl flask were then placed on the kjeldahl distillation apparatus with the tubes inserted into the conical flask and the Kjeldahl flask. The flask was heated to distil out the NH_3 evolved. The distillate was collected into a boric acid solution. When the boric acid turned green, it was allowed for 10 minutes for complete distillation of the ammonia present in the digest. The distillate was then titrated with 0.1M HCl.

The crude protein content was calculated using Equation 3.3:

$$\% \text{ Crude protein} = \frac{14 \; x \; Mx \; Vt \; x \quad TV}{Weight \; of \; sample \; (mg) \; x \; V} \text{ x } 100 \qquad (3.3)$$

Where: % Crude protein = % nitrogen (N_2) x 6.25

M = actual molarity; TV= titre volume of HCl used

V_t = total volume of distilled digest V_a = aliquot volume distilled

3.13.3 Determination of crude fibre

Two grams (2.0 g) of the fine *Kulikuli* powder was transferred to a round bottom flask containing 100 mL of 0.25M sulphuric acid solution and the mixture was boiled under reflux for 30 minutes. The hot solution was quickly filtered under suction and the insoluble matter was washed several times with hot water until it was acid-free. It was quantitatively transferred into the flask and 100 mL of hot 0.31M sodium hydroxide solution was added and the mixture boiled again under the reflux for 30 minutes and quickly filtered under suction. The soluble residue was washed with boiling water until it was base free and was dried to constant weight in the oven at 100°C and recorded as C_1. The weighed sample (C_1) was then incinerated in a muffled furnace at 50°C for 2 hours, cooled in the desiccator and reweighed (C_2) The crude fibre content was calculated using Equation 3.4:

$$\%Crude\ fibre = \frac{C1-C2}{Weight\ of\ sample} \qquad (3.4)$$

Where; C_1 = Initial weight C_2 = Final weight

3.13.4 Determination of crude lipid

A clean, dried 500 mL round bottom flask containing few anti-bumping granules was weighed (W_1) and 300 mL of petroleum ether (40-60) was dispersed into the flask fitted with a soxhlet extraction unit. The extractor thimble containing 20 g of the sample was fixed into the Soxhlet extraction unit. The round bottom flask and a condenser were connected to the soxhlet extractors and cold-water circulation was introduced. The heating mantle was switched on and the heating rate was adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for 6 hours. The solvent was

recovered and the oil was dried in the oven at 70 °C for one hour. The round bottom flask and oil was cooled and then weighed (W2). The lipid content was calculated using Equation 3.5:

$$\%Crude\ lipid\ content = \frac{w2-w1}{Weight\ of\ sample}\ X\ 100 \quad (3.5)$$

Where; W1= Initial weight W2= Final weight

3.13.5 Determination of ash content

A porcelain crucible with a cover was dried for 2 hours at 100 °C, it was allowed to cool in a desiccator and weighed (W1). Two grams of the finely ground sample was placed into the previously weighed porcelain crucible and reweighed (W2). It was first ignited and then transferred into a furnace which was set at 550 °C. The sample was left in the furnace for eight hours to ensure proper ashing. The crucible containing the ash was removed and cooled in the desiccator after which it was weighed (W3). The percentage ash content was calculated using Equation 3.6:

$$\%\ \text{Ash content} = \frac{W3-W1}{W2-W1}\ X\ 100 \quad (3.6)$$

Where: W1 = initial weight of crucible;

W2 = initial weight of crucible + sample;

W3 = final weight of crucible + sample.

3.13.6 Determination of carbohydrates

The percentage carbohydrate content in both samples was determined by mathematical difference excluding crude fibre as follows (Equation 3.7):

Utilization carbohydrate (%) = 100 − ($moisture$ + $crude\ protein$ + $crude\ fat$ + ash + $fat\ content$) (3.7)

3.14 Determination of Antinutritional Composition of the *Kulikuli* Samples

3.14.5 Determination of tannins

One gram (1 g) of the test sample was weighed in a test tube. Then 10 mL of 1% HCl in methanol was prepared for extracting the tannin from the sample. The sample was extracted for a day at room temperature (28 ± 2°C) using a horizontal shaker. After extraction, the sample was centrifuged at 1000 rpm for 5 min. One millilitre of the extract was mixed with 5 mL of vanillin-HCl reagent (prepared by mixing 8% of concentrated HCl and 4% vanillin both prepared using methanol). D-catechin was used as a standard. The mixture was left to stand for 20 minutes and the absorbance was measured at 500 nm (Mesfin *et al*., 2021).

3.14.2 Determination of saponins

Saponin content was determined by extraction of samples in 20% aqueous ethanol in a water bath for 30 minutes. The extract was transferred into a separating funnel with dimethyl ether and homogenized. The diethyl ether layer was discarded and the aqueous phase was retained and the butanol layer was used for the extraction of saponin, the extract was dried in a hot air oven in a pre-evaporating dish. The difference in dish weight was considered the amount of saponin present in the sample (Manisha and Navjot, 2020).

3.14.3 Determination of phytates

Two grams (2 g) of the test sample was soaked in 100 mL of 2% HCl for 3 hours and filtered (Whatman No. 4). In a separate conical flask, a further 25 mL aliquot of the filtrate was added and 5 mL of 0.3 % NH_4SCN (Ammonium thiocyanate) solution was added. Precisely 53.5 mL of deionized distilled water was added and then titrated with the normal solution of iron (III) chloride for 5 minutes until a brownish-yellow colour appeared (Ramli *et al.*, 2021).

3.14.4 Determination of cyanides

Five grams (5 g) of the sample was introduced into a 300 mL volumetric flask, 160 mL of 0.1 M phosphoric acid was added and the mixture was homogenized for 15 minutes at low speed and made up to the mark. The solution was centrifuged at 10,000 rpm for 30 minutes and the supernatant was transferred into a screw cap bottle which was stored at 4°C. A 5 mL aliquot of the extract was transferred into a quick-fit stoppered test tube containing 0.4 mL of 0.2 M phosphate buffer pH (7.0) and 10 mL of diluted linamarase enzyme was added. The tube was incubated at 30°C for 15 min and the reaction was stopped by the addition of 0.2 M NaOH (0.6 mL). The absorbance of the solution was measured at 450 nm (Sobowale *et al.*, 2021). The cyanide content was determined using Equation 3.8:

Hydrogen cyanide content (mg⁄100g)

$$= \text{absorbance} \times \text{dilution factor extinction coefficient} \times 100 \quad (3.8)$$

3.14.5 Determination of oxalates

Soluble and insoluble oxalates were extracted with 15 mL of distilled water and 2N hydrochloric acid respectively. The sample suspensions were heated in a boiling water bath for 15-20 minutes and cooled down to room temperature. It was filtered through Whatman No. 1 filter paper, washed with distilled water and the volume was made up to 50 mL, 0.25 mL bromophenol blue, 0.4 mL potassium dichromate and 1 mL sulphuric acid were added to 1 mL sample and incubated in a boiling water bath for 10 minutes. The reaction was terminated by adding 1 mL sodium hydroxide and absorbance was read at 600 nm. The oxalate concentration was estimated using a standard curve prepared using oxalic acid (Sandya *et al.*, 2021).

3.15 Determination of Physicochemical Properties

3.15.1 Determination of free fatty acid value

The free fatty acid value was determined according to the method of Akter *et al.* (2021a) two grams (2 g) of each sample was weighed into a conical flask, afterwards 5 cm^3 of chloroform and a mixture of 25 cm^3 diethyl ether and ethanol 1:1 (v/v) was added. A few drops of phenolphthalein indicator were added and the mixture was titrated against 0.1M KOH. The endpoint was noted when a pink colour appeared and persisted for 30 seconds. The acid value was calculated using Equation 3.9:

$$AV = \frac{S-B \ X \ KOH \ X \ 5.61}{S} \qquad (3.9)$$

where

S = vol. in cm^3 of sample,

B = vol. in cm^3 of blank

and N = Normality of KOH Calculation

Acid value = Titre (ml) x 5.61/ weight of *Kulikuli* sample

The FFA is usually calculated as oleic acid (1mL 0.1M sodium hydroxide ≡ 0.00282g oleic acid), in which case the acid value = 2 x FFA. Determination of iodine value

3.15.2 Determination of iodine value

Zero point thirty grams (0.30g) of the oil sample was dissolved in 10 cm^3 of chloroform in a 100cm^3 stoppered flask, 25cm^3 of Wijj's solution was also added. The flask was allowed to stand in a dark place for 30 minutes. Twenty cubic centimetres (20cm^3) of 10% KI was then added and the mixture was titrated against 0.1M sodium thiosulphate with a few drops of starch as an indicator (Tsado *et al.*, 2018). A blank titration was also carried out. The iodine value was calculated using Equation 3.10:

$$IV = \frac{(b-a)\ X\ 1.269}{100} X\ 100 \qquad (3.10)$$

Where

A= Sample of the titre value B= Blank of the titre value

W= Weight of the sample used (g).

3.15.3 Determination of peroxide value

The method of Akter *et al*. (2021a) was used for the determination of the peroxide value. Three grams (3g) of the lipid sample were mixed with a concoction of chloroform and acetic acid (2:3) solution. Then, 1 mL of saturated potassium iodide was added and the solution was kept in the dark for 3 minutes. After stabilization, 100 mL distilled water and 1 mL starch solution (1 g 100 mL/1) was added into the solution and titrated with Sodium thiosulfate ($Na_2S_2O_3$) until reaching the end point (colorless). Peroxide values were calculated using Equation 3.11:

$$PV = \frac{S\ X\ N}{W}\ X\ 100 \qquad (3.11)$$

where, S = vol. in cm^3 of $Na_2S_2O_3$ N = normality of $Na_2S_2O_3$

W = weight of oil sample (g)

3.16 Safety Evaluation of *Kulikuli* Samples

3.16.1 Experimental animals

Thirty-six (36) healthy albino rats of average weight (80 g) were purchased from the Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria. The rats were kept in clean plastic cages and maintained under standard laboratory conditions. They were maintained at ambient temperature and observed under a 12-hour light/dark cycle of the prevailing time in a well-ventilated animal house for 2 weeks and fed with standard formulated feed and water *ad libitum* before the

commencement of the experiment. They were allowed unrestricted access to rat pellets and water. Soiled wood shavings we replaced often. The animal house was cleaned and disinfected regularly using jik and dettol. The feed, water containers and animal cages were washed regularly using water (tap). The animals were housed and cared for following the good laboratory practice (GLP) regulations of WHO (1998). The principle of laboratory animal care (National Institute of Environment and Health Services, NIEHS, 1985) was also followed throughout the study.

3.16.2 Experimental protocol

Toxicological testing of the *Kulikuli* samples was carried out using a completely randomised design (CRD) to examine the effects of *Kulikuli-formulated* feed on the body weight, biochemical parameters, haematological indices and histological evaluation. The feeding experiment was carried out according to Agbabiaka *et al.* (2013) procedure. The thirty-six (36) rats were distributed into nine groups with four rats each, and the groups were housed individually. For 28 days, the eight groups (A, B, C, D, E, F, G, H) were fed with feed containing (*Kulikuli* from the market (KMKT), (Unfermented *Kulikuli*) UK, Fermented *Kulikuli* (K), Fermented *Kulikuli* and *Lactobacillus plantarum* (KL), Fermented *Kulikuli* and all-purpose flour (KF), and Fermented *Kulikuli* and Chia flour (KC), Fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL), Fermented *Kulikuli*, Chia flour and *Lactobacillus plantarum* (KCL) while Group I served as a control (basal diet).

Feed as well as water was made available *ad libitum*, and other standard experimental animal management procedures were followed. Rats were weighed at the start of the experiment and thereafter weekly, and the amount of feed consumed was calculated by subtracting the amount of feed supplied from the amount left over the next morning.

3.17 Determination of Weekly Body-Weight and Relative Organ Weight

The body weights of the rats were measured weekly throughout the trial and afterwards, as stated by Bashir *et al.* (2015). The following formula was used to calculate the weight gain:

Weight gain of rat (g) = Final weight (g) − Initial weight (g)

The organs were weighed and relative organ weights (ROW) were computed and recorded using Equation 3.12 :

$$ROW = \frac{\text{Absolute Organ Weight (kg)}}{\text{Body Weight of Rat on Sacrifice Day (kg)}} X\ 100 \qquad (3.12)$$

Where ROW = Relative Organs Weight

3.18 Estimation of Haematological Parameters

Blood samples of the rats were screened for their haematological components which included haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), platelet count (PLT) and differential counts (basophils, lymphocytes, eosinophils, monocytes and neutrophils) were determined using the automated haematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan employing the methods described by Dacie and Lewis (1995).

3.19 Serum Biochemical Studies

3.19.1 Estimation of aspartate aminotransferase (AST) level

Aspartate aminotransaminase (AST) was assayed according to the method described by Ekanem and Yusuf (2005). Here, 0.25 cm^3 of reagent R1 (Phosphate buffer; L – aspartate, α –Oxoglutarate) was mixed with 0.05 cm^3 of sample and incubated for 30 minutes at 37°C. An Aliquot of 0.25 cm^3 of solution R2 (2, 4 – dinitrophenylhydrazine) was further added to the contents of the test tubes and 0.05 cm^3 of distilled water was added to the blank and allowed to stand for 20 minutes at 25°C. Two point five cubic centimetres (2.5 cm^3) of NaOH (0.4 M) was added, mixed and the absorbance was read at 546 nm after 5 minutes. The activity of AST in the sample was read off the standard curve.

3.19.2 Estimation of alanie aminotransaminase (ALT) level

Alanine aminotransaminase (ALT) was assayed according to the method described by Ekanem and Yusuf (2005). Here, 0.25 cm^3 of reagent R1 (Phosphate buffer; L – alanine, α –Oxoglutarate) was mixed with 0.05 cm^3 of sample and incubated for 30 minutes at 37°C. A measured quantity of 0.25 cm^3 of solution R2 (2, 4 – dinitrophenylhydrazine) was further added to the contents of the test tubes and 0.05 cm^3 of distilled water was added to the blank and allowed to stand for 20 minutes at 25°C, after which Two point five centimetre cube (2.5 cm^3) of NaOH (0.4 M) was added, mixed and the absorbance was read at 546 nm after 5 minutes. The activity of AST in the sample was read off the standard curve.

3.19.2 Estimation of bilirubin concentration

Total bilirubin was assayed using a test kit (Randox Laboratories Ltd., UK) as described by Tolman and Rej (1999). The contents of the kit were R1 (Sulphanilic acid and HCl), R2 (Sodium nitrite), R3 (Caffeine) and R4 (Tartrate and NaOH). Two hundred microliters (200 µl) of R1, 10.00 µl of R3 and 200 µl of serum sample were pipetted into each test tube. Fifty microliters (50 µl) of R2 was added to each sample tube while 50 µl of distilled H_2O was added to the corresponding blank tube. This was done for each sample. The contents in the test tubes were mixed and incubated for 10 minutes at 25°C and 10.00 µl of R4 was added, mixed and incubated for a further 30 minutes at 25°C. Absorbance of the sample (A sample) was read at 578 nm against sample blank. Total bilirubin was calculated using Equation 3.13:

$$\text{Total bilirubin concentration (mg/dl)} = 10.8 \times A\ sample \qquad (3.13)$$

Where: A= absorbance of the sample

10.8= Bilirubin standard concentration

3.19.4 Estimation of total protein concentration

A total protein test kit (Randox Laboratories Ltd, Crumlin, UK) was used for the estimation of plasma total protein as described by Ekanem and Yusuf (2005). The content of the test kit included R1a (10 cm^3 of bottled R1 (biuret reagent) in 40 cm^3 distilled water (dH2O), R21 (10 cm^3 of bottled R2 in 40 cm^3 dH2O) and protein standard. The reaction mixture contained 1.0 cm^3 of R1a and 0.02 m3 of plasma for the sample's test tube while blank and standard test tubes contained 1.0 cm^3 of R1a and 0.02 ml of dH2O or 0.02 cm^3 of standard respectively. Each test tube was mixed and incubated at 25°C for 30 minutes.

The absorbances of the standard (A standard) and sample (A sample) were measured against reagent blank at 546 nm and protein concentration was calculated using Equation 3.14:

$$\text{Total protein concentration mg/dl} = \frac{A\ sample}{A\ standard}\ X\ \text{Standard concentration} \qquad (3.14)$$

Where standard protein concentration = 5.85 g/dl

3.19.5 Estimation of albumin concentration

Albumin was assayed using a test kit (Randox Laboratories Ltd., UK) based on its reaction with bromocresol green as described by Doumas *et al.* (1997). Five millilitres (5mL) of bromocresol green buffered at pH 4.2 was added to 0.1 mL plasma, mixed and incubated at 37°C for 10 minutes. The absorption of the dye–albumin complex was measured spectrophotometrically at 630 nm against a reagent blank.

3.19.6 Estimation of urea concentration

A urea test kit (Randox Laboratories Ltd., Crumlin, UK) was used for this determination as described by Tietz (1995). The content of the kit was R1a (Sodium nitroprusside and urease solution), R2a (40 cm3 of R2 (phenol) in 280 cm^3 of dH2O), R3a (10 ml of R3 (Sodium hypochlorite) in 340.9 cm^3 of dH2O) and standard. Correspondingly, 10 µl of each serum sample was mixed with 100 µl of R1a, while 10 µl of standard and 10 µl of dH2O were mixed for standard and blank respectively. These were incubated for 10 minutes at 37°C. Thereafter, 2.50 cm^3 of R2a and 2.50 cm^3 of R3a were added and incubated at 37°C for additional 15 minutes. Absorbances of the sample (A sample) and standard (A standard) were read against the blank. Urea concentration was calculated using Equation 3.15:

$$\text{Urea concentration mg/dl} = \frac{A\ sample}{A\ standard} X\ \text{Standard concentration} \quad (3.15)$$

Where: Urea standard concentration = 80.5 mg/dl

3.19.7 Estimation of creatinine concentration

A creatinine test kit (Randox Laboratories Ltd., Crumlin, UK) was used for the creatinine assay according to the method of Perrone *et al.* (1992). The contents of the kit included R1a (picric acid), R1b (sodium hydroxide) and creatinine standard. The working reagent contained equal volumes of R1a and R1b. One hundred microlitres (100 µl) of plasma, 100 µl of creatinine standard and 100 µl of dH_2O were separately placed in different test tubes and 10.00 µl of working reagent was added to each tube. The contents of each tube were mixed gently and absorbances A1 sample and A1 standard were read at 492 nm against the blank after 30 seconds. Thereafter, after 2 minutes of initial reading, the Absorbances A2 sample and A2 standard were also determined. The concentration of creatinine was calculated using Equation 3.16:

$$\text{Creatinine concentration mg/dl} = \frac{\Delta\ A\ sample}{\Delta\ A\ standard} X\ \text{Standard concentration} \quad (3.16)$$

Where: Creatinine standard concentration = 1.97 mg/dl

3.19.8 Estimation of potassium concentration

Fifty microliters (50 µl) of serum sample was mixed with 500 µl (0.5 cm^3) of the precipitating reagent. The mixture was centrifuged at 4000 rpm for 10 minutes. The supernatant fluids were carefully decanted and kept. One hundred microlitres (100 µl) of each serum sample was transferred into a prelabelled corresponding test tube to which 1.0 cm^3 of the working reagent was already added. One cubic centimetre (1.0 cm^3) of the working reagent (equal quantities of sodium tetraphenyl boron and sodium hydroxide) was also added to each of the standard and blank tubes. One hundred microliters (100 µl) of potassium standard was added into the test tube labelled standard while a

corresponding volume of water was added to the 'blank'. The content of each tube was thoroughly mixed and kept standing for five minutes at ambient temperature (25±2°C). The absorbance was recorded at 600 nm wavelength against the reagent blank. Thus, the potassium concentration was calculated using Equation 3.17:

$$\text{Potassium concentration} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times 5.0\ \text{mmol/L} \quad (3.17)$$

3.19.9 Estimation of sodium concentration

Ten microliters (10 µl) of each serum sample were pipetted into the appropriate test tube. Correspondingly, 10µl of sodium standard (100 mmol) was added into the standard tube while the blank contained 10 µl of distilled water. Finally, 10.00 µl of the working reagent was added to each tube. The contents of each tube were well mixed and incubated for 5 minutes at 37°Cover a water bath. The absorbances of the sample and standard were measured against the reagent blank at 640 nm wavelength. The sodium concentration was calculated using Equation 3.18:

$$\text{Sodium concentration} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times \text{Concentration of standard (mmol/L)} \quad (3.18)$$

3.20 Lipid Profile

3.20.1 Estimation of plasma cholesterol concentration

A cholesterol test kit (Cypress Diagnostics, Vlaams-Brabant, Belgium) was used for this estimation. The content of the kit included R1 (Pipes PH .9 and phenol), R2 (CHE, CHOD, POD and 4- AP) and cholesterol standard solution. The working reagent was prepared by mixing 100 cm^3 of R1 with 10 cm^3 of R2. Each tube contained 1.00 cm^3 of working reagent and blank tubes. The contents of the tubes were mixed gently and incubated at 25°C for 10 minutes. Absorbance (A sample and A standard) were recorded

at 505 nm against the blank. The concentration of cholesterol in the serum sample was calculated using Equation 3.19:

$$\text{Cholesterol concentration (mg/dl)} = \frac{Asample}{Astandard} \text{ x 200 mg/dl} \quad (3.19)$$

Where: Cholesterol standard concentration is 200 mg/dl

3.20.2 Estimation of triglyceride concentration

A triglyceride test kit (Linear Chemicals, Barcelona, Spain) was used for this estimation as described by Fossati and Prencipe (1982). The contents of the kit included R1 (Monoreagent) and triglycerides standard. Each tube contained 1.00 ml of R1 and a blank tube. The contents of the tubes were mixed gently and incubated at room temperature (25±2°C) for 15 minutes and the absorbances (A sample and A standard) were determined at 500 nm against the reagent blank. The colour was protected from light. Triglyceride concentration was thus calculated using 3.20:

$$\text{Concentration of triglyceride (mg/dl)} = \frac{A\ sample}{A\ standard} \text{ x 200 mg/dl} \quad (3.20)$$

Where: Triglyceride standard concentration is 200 mg/dl

3.21 Histopathological Studies

The method of Babayi *et al.* (2019) was used for the study. After sacrificing the rats that had been fed with experimental diet, the organs (kidney, intestine, liver, spleen and stomach) were collected for histopathological studies. The organs were washed in normal saline and fixed immediately in 10% formol saline for 24 hours. Grossing was achieved by the selection of tissues to be processed. The organs were placed on tissue cassettes alongside their respective identification numbers. The selected tissues were processed using an automatic tissue processor (SLEE MTP Tissue processor), which involved four major stages (fixation, dehydration, clearing and impregnation). An embedding machine

was used to dispense wax into an embedding mould, unto which the processed tissues and tissue cassettes were placed and allowed to solidify. The solidified tissue in the cassette was placed in an MR3500 microtome and tiny sections were cut at 5 microns. The tissue sections were flooded with water on a heated water bath maintained at 3°C below the melting point of the wax. Tissue sections were picked using microscopic slides angled at 45°C for water to drain and dry. The slide was then placed on hot plate and allowed to fix at a maintained temperature of 3°C above the melting point of the wax. This was done to ensure a bond between the tissue and slides and allowed fixing for a minimum of 30 minutes. Thereafter, the slides were stained using Harri's haematoxylin and eosin method and allowed to air dry. The dried slides were mounted with Distyrene Plasticizer Xylene (DPX) and coverslips and examined under the microscope. Any alterations compared to the normal structures were recorded.

3.22 Statistical Analysis

Results are expressed as mean values. Within groups, comparisons were performed by the analysis of variance using the ANOVA test. Significant differences between control and experimental groups were assessed by Duncan's Multiple Range Test (Yalta, 2008).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Morphological characteristics of isolates from corn steep liquor

Table 4.1 shows the morphology of the colonies isolated from Corn steep liquor. The colonies were creamy white, circular and smooth when cultured on De Mann Rogosa Sharpe (MRS) agar.

4.1.2 Biochemical characteristics of isolates from corn steep liquor

The microscopic and biochemical characteristics of isolates from Corn steep liquor are shown in Table 4.2. The three isolates from corn steep liquor were Gram-positive rods, catalase, citrate, indole and Voges Proskauer negative. They utilized D-fructose, glucose, sorbitol and lactose.

4.1.3 Molecular identity of isolate from corn steep liquor

The result showed the sequential amplicons of LAB isolated from Corn steep liquor as the resulting alignment of the concentrated nucleotide (from $5^1 \rightarrow 3^1$ and $3^1 \leftarrow 5^1$ with other known sequence. The gel documented image of the isolated bacterial DNA after electrophoresis appeared at 1500 bp which is an indication of a pure isolate. Lane Mk represents the ladder (Molecular marker) and Lab1 represents DNA extracted from the isolate as shown in Plate III. The isolated organism had 99.87 percent similarity to *Lactobacillus plantarum* with the accession number AP019815.1 and a max score of 2806 (Table 4.3).

Table 4.1: Morphological Characteristics of Bacteria Isolated from Corn Steep Liquor

MRS Agar	Suspected Organism
Circular, creamy white smooth with opaque appearance	***Lactobacillus* sp.**
Large irregular dull white (cream) colonies with a dry surface	***Lactobacillus* sp.**
Round-shaped, creamy white, mucous, raised on the surface of the media	***Lactobacillus* sp**

MRS: De Mann Rogosa Sharpe Agar

Table 4.2: Biochemical Identities of Isolates from Corn Steep Liquor

Sample	Grams reaction	Shape	Cat	Cit	UR	VP	MR	Ind	SF	Oxi	Mot	Lac	Glu	Suc	Fru	Man	Sor	Suspected organisms
A	+	Rods	-	-	-	-	+	-	+	-	-	+	+	+	+	-	+	*Lactobacillus* sp.
B	+	Rods	-	-	-	-	+	-	+	+	-	+	+	+	+	-	+	*Lactobacillus* sp.
C	+	Rods	-	-	+	-	+	-	+	-	-	+	+	+	+	-	+	*Lactobacillus* sp.

Cat: Catalase; Cit: Citrate; UR: Urease; VP: Voges Proskeur; MR: Methyl Red; Ind: Indole; SF: Spore formation; Oxi: Oxidase;

Mot: Motility; Lac: Lactose; Glu: Glucose; Suc: Sucrose; Fru: Fructose; Man: Mannose; Sor: Sorbitol

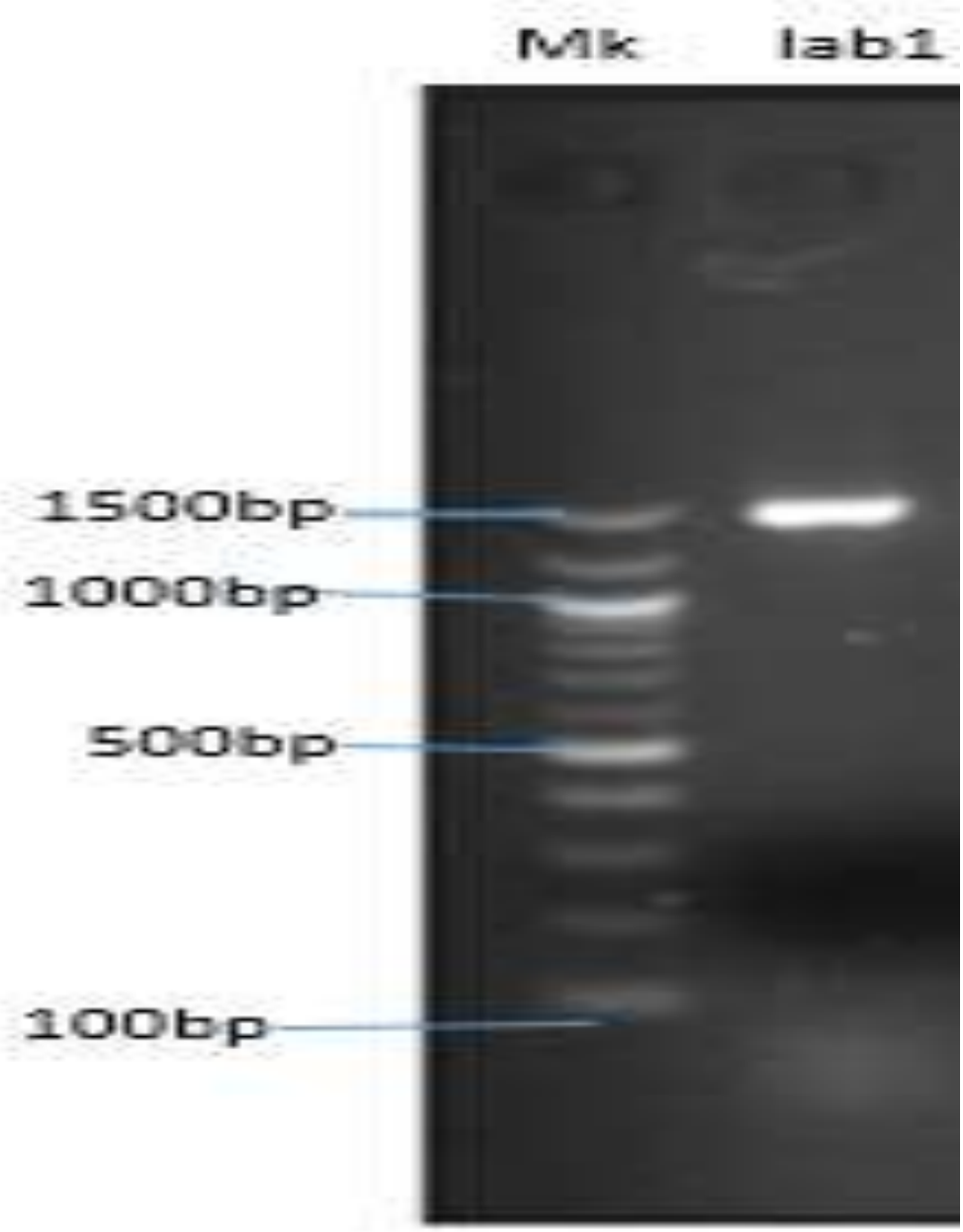


Plate IV: Agarose gel documented image of *Lactobacillus plantarum*

Table 4.3: Identity and Accession Number of Test Organism

sample ID	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
1	*Lactobacillus plantarum*	2806	14001	100%	0	99.87%	AP019815.1

4.1.4 Microbial count of *Kulikuli* obtained from the market

The total bacteria, coliform and fungal count obtained from the microbiological analysis of the *Kulikuli* from the market (Kmkt) were 3.0 $x10^4$, 0.0 and 1.0 $x10^4$ CFU/g respectively (Table 4.4).

Table 4.4: Microbial Count of *Kulikuli* Obtained from the Market (CFU/g)

Sample	TBC	TCC	TFC
KMKT	3.0 $x10^4$	0.0	1.0 x 10^4

KMKT – *Kulikuli* from market; TBC- Total bacterial count; TCC- Total coliform count; TFC: Total fungal count; CFU/g: Colony forming unit per gram

4.1.5 Characteristics of bacterial isolates from *Kulikuli* obtained from the market

The morphological, microscopic and biochemical characteristics of all the bacterial isolates obtained on nutrient and MacConkey agar are presented in Table 4.5a and 4.5b. The organisms isolated included species of *Klebsiella*, *Proteus* and *Staphylococcus.*

4.1.6 Morphological characteristics of fungal isolate from *Kulikuli* obtained from market

Aspergillus niger was the only fungal strain isolated from Kmkt on the basis of its colonial morphology and microscopic characteristics. The identity of the fungal isolate was cross-matched with those of standard taxa (Table 4.6).

4.1.7 Total microbial count for laboratory prepared *Kulikuli* samples

The total bacterial and fungal count ranged from 0.0 –2.00±0.00 x 10^4 CFU/g while the total coliform count was 0.0. The highest total bacterial count was recorded in fermented *Kulikuli*, all- purpose flour and *Lactobacillus plantarum* (KFL) (2.00±0.00 x 10^4 CFU/g) followed by fermented *Kulikuli* and all-purpose flour (KF) with 1.60±0.00 x 10^4 CFU/g, fermented *Kulikuli* and chia flour (KC) and fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) (1.00±0.00 x 10^4 CFU/g) while Unfermented *Kulikuli* (UK) (the control), fermented *Kulikuli* (K) and fermented *Kulikuli* and *Lactobacillus plantarum* (KL) had no bacterial growth. The highest total fungal counts observed was in fermented *Kulikuli* and *Lactobacillus plantarum* (KL) (2.00±0.00 x 10^4 CFU/g) followed by fermented *Kulikuli* and all-purpose flour (KF) and fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) with 2.00±0.00 x 10^4 CFU/g. No fungal growth was observed in fermented *Kulikuli* (K), fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL), fermented *Kulikuli* and chia flour (KC) and Unfermented *Kulikuli* (UK) as presented in Table 4.7

Table 4.5a: Morphological Characteristics of Bacterial Isolates from *Kulikuli* Obtained from market

Medium Nutrient agar	MacConkey Agar	Suspected organisms
Slightly yellow, smooth, large and circular colonies	NG	*Staphylococcus aureus*
Circular, entire convex, margins with Whitish mucoid colony	NG	*Klebsiella pneumoniae*
Small colonies, glistening and usually, the growth is irregular due to swarming.	NG	*Proteus* sp.

NG: No growth

Table 4.5b: Biochemical Characteristics of Bacteria Isolated from *Kulikuli* Obtained from Market

S/N	Grm	Sh	Cat	Coa	Cit	Oxi	Ure	Mot	Sth	MSA	Glu	Suc	Lac	H2S	MR	VP	Ind	Suspected organisms
1	+	Cocci	+	+	-	-	+	-	-	+	+	+	+	ND	-	+	-	*Staphylococcus aureus*
2	-	Rod	+	-	+	-	+	-	+	-	+	+	+	ND	-	+	-	*Klebsiella pneumoniae*
3	-	Rod	+	-		-	+	+	-	+	+	-	-	+	+	-	-	*Proteus* sp.

Sh:-Shape, Grm:-Gram reaction, Cat:-catalase, Coa:- coagulase, Cit:-citrate, Oxi:- oxidase, Ure:- urease, Suc:- sucrose, Lac:-lactose, Mot:-motility, Sth:-starch hydrolysis, MSA:- Mannitol Salt Agar Glu:- glucose, MR:- Methyl red, VP:- Voges Proskauer, Ind:- indole, H_2S:- Hydrogen sulfide production, ND:- Not determined.

Table 4.6: Morphological Characteristics of Fungal Species Isolated from *Kulikuli* Obtained from the Market

Cultural Characteristics	Microscopic Characteristics	Suspected Organisms
Black colony with white mat on SDA	Dark brown to black conidia heads splitting into several loose columns. conidiophores are smooth walled, turning dark towards the vesicle	*Aspergillus niger*

SDA:- Sabouraud Dextrose Agar

Table 4.7a: Total Microbial Count of Laboratory Prepared *Kulikuli* Samples

	CFU/g		
Sample	TBC ($\times 10^4$)	TCC ($\times 10^4$)	TFC ($\times 10^4$)
UK	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
K	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
KL	0.00 ± 0.00^a	0.00 ± 0.00^a	2.00 ± 0.00^c
KF	1.60 ± 0.00^c	0.00 ± 0.00^a	1.30 ± 0.00^b
KC	1.00 ± 0.00^b	0.00 ± 0.00^a	0.00 ± 0.00^a
KFL	2.00 ± 0.00^d	0.00 ± 0.00^a	0.00 ± 0.00^a
KCL	1.00 ± 0.00^b	0.00 ± 0.00^a	1.30 ± 0.00^b

L: *Lactobacillus plantarum;* UK: Unfermented *Kulikuli* K: fermented *Kulikuli;* KL: fermented *Kulikuli* and L; KF: fermented *Kulikuli and* all- purpose flour; KC: fermented *Kulikuli* and chia flour; KFL: fermented *Kulikuli*, all- purpose flour and L; KCL: fermented *Kulikuli*, chia flour and L; TBC: Total bacterial count; TCC: Total coliform count; TFC: Total fungal count; CFU/g: Colony forming unit per gram.

Values are presented as mean± standard deviation of the three replicates. Values with different superscript in a column are significantly different at $p \leq 0.05$.

Table 4.7b: Microorganisms Isolated from Laboratory Prepared *Kulikuli* Samples

Samples	Bacteria isolated from *Kulikuli samples*	Fungi isolated from *Kulikuli samples*
UK	NIL	NIL
K	NIL	NIL
KL	NIL	*Aspergillus niger, A. flavus*
KF	*Micrococcus, proteus*	*A.fumigatus,A. terreus*
KC	*Bacillus*	NIL
KFL	*Proteus, Enterobacter*	NIL
KCL	*Proteus, Staphylococcus aureus*	*Tricophyton* sp.

L: *Lactobacillus plantarum;* UK: Unfermented *Kulikuli* K: fermented *Kulikuli;* KL: fermented *Kulikuli* and L; KF: fermented *Kulikuli and* all- purpose flour; KC: fermented *Kulikuli* and chia flour; KFL: fermented *Kulikuli*, all- purpose flour and L; KCL: fermented *Kulikuli*, chia flour and L

4.1.8 Characteristics of bacterial isolates obtained from laboratory prepared *Kulikuli* samples

The morphological, microscopic and biochemical characteristics of isolated bacterial species are shown in Tables 4.8 and 4.9. The organisms isolated include *Bacillus* sp., *Enterobacter* sp., *Klebsiella pneumoniae, Micrococcus* sp., *Proteus* sp. and *Staphylococcus aureus Bacillus* sp., *S. aureus* and *Micrococcus* sp. were Gram-positive while *Klebsiella pneumoniae and Enterobacter* sp. were Gram negative. All the isolates were catalase positive and indole negative, all the isolates except *Micrococcus* sp. were oxidase negative. All the isolates were unable to hydrolyze starch except for *Bacillus sp.* For sugar fermentation tests, *Enterobacter* sp. and *Micrococcus* sp. were the only isolates that did not utilize glucose and sucrose. *K. pneumoniae, S. aureus* and *Micrococcus* sp. utilized lactose while *Bacillus* sp., *Proteus* sp. and *Enterobacter* sp. were unable to ferment lactose.

4.1.9 Cultural and Morphological characteristics of fungal isolates from laboratory prepared *Kulikuli*

Based on the colonial morphology, and microscopic characteristics, the fungal isolates were identified as *Aspergillus niger, A. flavus, A. fumigatus, A. terreus* and *Trichophyton* sp. Table 4.10 shows the characteristics of fungal isolates from *Kulikuli* samples

4.1.10 Frequency of occurrence of isolates from *Kulikuli* samples

The frequency of occurrence of bacterial isolates are presented in Table 4.11a. *Proteus* sp. was the most frequently isolated bacterial species while *Enterobacter* sp. was the least.

Aspergillus niger was the frequently isolated fungal species while *Aspergillus fumigatus and A. terreus* were the least isolated species from the test *Kulikuli* samples. The frequency of occurrence of fungal isolates is presented in Table 4.11b.

Table 4.8: Cultural Characteristics of the Bacterial Isolates Obtained from Laboratory prepared *Kulikuli* Samples

Medium **Nutrient Agar**	**MacConkey Agar**	**Suspected organisms**
Large, slimy colony no pigmentation and very mucoid	NG	*Klebsiella pneumoniae*
Circular small colonies with yellow pigment	NG	*Staphylococcus aureus*
Large, irregular spreading colony	NG	*Bacillus* sp.
Circular, entire, convex with yellow or red pigmentation	NG	*Micrococcus* sp.
Greyish to white-colored large, circular, and convex colonies	NG	*Enterobacter* sp.
Small colonies, glistening and usually, the growth is irregular due to swarming.	NG	*Proteus* sp.

NG-No Growth

Table 4.9: Biochemical Characteristics of the Bacterial Isolates

GR	Sh	Cat	Coa	Cit	Oxi	Ure	Mot	Sth	Glu	Suc	Lac	Hss	MR	VP	Ind	Suspected organisms
+	Rod	+	-	+	-	+	+	+	+	+	-	-	-	+	-	*Bacillus* sp.
-	Rod	+	-	-	-	+	-	-	+	+	+	ND	-	+	-	*Klebsiella pneumoniae*
+	Cocci	+	+	-	-	+	-	-	+	+	+	ND	-	+	-	*Staphylococcus aureus*
-	Rod	+	-	+	-	+	-	-	+	+	-	-	-	+	-	*Enterobacter* sp.
+	Cocci	+	-	+	+	+	-	-	-	-	+	ND	-	-	-	*Micrococcus* sp.
-	Rod	+	-	-	-	+	+	-	+	+	-	+	+	-	+	*Proteus* sp.

Sh:-Shape, Gr:-Gram reaction, Cat:-catalase, Coa:- coagulase, Cit:-citrate, Oxi:- oxidase, Ure:- urease, Suc:- sucrose, Lac:-lactose, Mot:- motility, Sth:-starch hydrolysis, Hem:-hemolysis, Glu:- glucose, MR:- Methyl red, VP:- Voges Proskauer, Ind:- indole, H_2S:- Hydrogen sulfide production, ND:- Not determined.

Table 4.10: Cultural and Morphological Characteristics of Fungal Isolates from Laboratory prepared *Kulikuli*

Cultural characteristics	Microscopic characteristics	Organisms suspected
Black colony on SDA	Septate hyphae, dark brown to black and rough walled spores	*Aspergillus niger*
Grey-green filaments with smooth colony	Branched septate conidiophore	*Aspergillus fumigatus*
Olive- green and wrinkled colony with whitish edges	Thick- walled and colourless globose conidiophore	*Aspergillus flavus*
Velvety, cinnamon brown on SDA	Conidial heads that are compact, biseriate, and densely columnar	*Aspergillus terreus*
White and cottony surface	Macroconida are small, (cylindrical), with thin cell walls, and they occur in clusters	*Trichophyton* sp.

SDA: Sabouraud Dextrose Agar

Table 4.11a: The Frequency of Occurrence of Bacterial Isolates from the *Kulikuli* Samples

Bacterial Isolates	Number	Frequency of Occurrence (%)
Proteus sp.	5.0	35.71
Staphylococcus aureus	3.0	21.43
Bacillus sp.	2.0	14.29
Micrococcus sp.	2.0	14.29
Klebsiella sp.	1.0	7.14
Enterobacter sp.	1.0	7.14
Total	**14.0**	**100**

4.11b: The Frequency of Occurrence of Fungal Isolates from *Kulikuli* Samples

Fungal Isolates	Number	Frequency of Occurrence (%)
Aspergillus niger	3.0	33.33
Aspergillus flavus	2.0	22.22
Aspergillus fumigatus	1.0	11.11
Aspergillus terreus	1.0	11.11
Tricophyton sp.	2.0	22.22
Total	**9**	**100**

4.1.11 Nutritional composition of the *Kulikuli* samples

Table 4.12 displays the nutritional composition of the *Kulikuli* samples. fermented *Kulikuli* (K),fermented *Kulikuli* and *Lactobacillus plantarum* (KL) , fermented *Kulikuli* and all-purpose flour (KF), fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) and fermented *Kulikuli* and chia flour (KC) had significantly higher moisture contents of 4.60±0.14, 4.55±0.07, 4.55±0.11, 4.40±0.20 and 4.03±0.05 respectively at ($p \geq 0.05$) followed by fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) (3.27±0.02) and *Kulikuli* from the market (KMKT) (3.40±0.02) while the lowest moisture content (2.71±0.08) was observed in Unfermented *Kulikuli* (UK).Significantly higher protein content at ($p \geq 0.05$) was recorded in fermented *Kulikuli* and all-purpose flour (KF) (30.07±0.08) and *Kulikuli* from the market (KMKT) (31.21±0.49) followed by Unfermented *Kulikuli* (UK) (27.09±0.40), fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) (26.99±0.08) and fermented *Kulikuli* and *Lactobacillus plantarum* (KL) (25.00±0.05) while lowest values were observed in fermented *Kulikuli* (K) (23.35±0.03) and fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) (22.53±0.25). However, the protein values obtained from this study were lower than the Standards Organisation of Nigeria (SON) (2015) permissible limit. In the case of Crude fibre content, fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) (0.86±0.05), *Kulikuli* from the market (KMKT) (0.82±0.04) and Unfermented *Kulikuli* (UK) (0.68±0.13) were significantly higher than the other samples. Similar crude fibre contents were recorded for fermented *Kulikuli* and chia flour (KC) (0.60±0.10), fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) (0.45±0.05) and fermented *Kulikuli* and all-purpose flour (KF) (0.43±0.08) while lower crude fibre contents were recorded for fermented *Kulikuli* (K) (0.40±0.00) and fermented *Kulikuli* and *Lactobacillus plantarum* (KL) (0.25±0.05).

The highest lipid value was observed in fermented *Kulikuli* and all-purpose flour (KF) (35.33±0.45) followed by fermented *Kulikuli* (K) (34.48±0.10), fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) (33.38±0.25), fermented *Kulikuli* and *Lactobacillus plantarum* (KL) (33.34±0.01) and fermented *Kulikuli* and chia flour (KC) (33.33±0.32) while the lowest values were observed in *Kulikuli* from the market (KMKT) (31.41±0.02), Unfermented *Kulikuli* (UK) (30.86±0.15) and fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) (30.64±0.40). Unfermented *Kulikuli* (UK) (4.53±0.05) and fermented *Kulikuli* (K) (4.52±0.00) had significantly higher ($p \geq 0.05$) ash content value compared to fermented *Kulikuli* and chia flour (KC) (3.52±0.15), fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) (3.59±0.25), *Kulikuli* from the market (KMKT) (3.69±0.04), fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) (3.81±0.16), fermented *Kulikuli* and *Lactobacillus plantarum* (KL) (3.47±0.09) and KF (3.70±0.20). All the obtained values were within the SON (2015) specified limit. Fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) (39.52±0.15) and fermented *Kulikuli* and chia flour (KC) (37.46±0.25) had the highest ($p \geq 0.05$) carbohydrate content when compared to the controls (Unfermented *Kulikuli* (UK) and *Kulikuli* from the market (KMKT)) while fermented *Kulikuli* and *Lactobacillus plantarum* (KL) (27.56±0.64) had the lowest carbohydrate content. Unfermented *Kulikuli* (UK), fermented *Kulikuli* and all-purpose flour (KF) and fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) had similar carbohydrate contents of (36.33±0.03) (30.97±0.1) (30.97±0.05), followed by fermented *Kulikuli* (K) (31.44±0.11) and *Kulikuli* from the market (KMKT) (29.77±0.28) which had significantly lower carbohydrate contents.

Table 4.12: Nutritional Composition of *Kulikuli* Samples

	Sample	Moisture (%)	Protein (%)	fibre (%)	Lipid (%)
Unfermented	Ctrl (UK)	$2.71^{a}\pm0.08$	$27.09^{c}\pm0.40$	$0.68^{cd}\pm0.13$	$30.86^{a}\pm0.15$
	KMKT	$3.40^{b}\pm0.02$	$31.21^{d}\pm0.49$	$0.82^{d}\pm0.04$	$31.41^{a}\pm0.02$
	Fermented *Kulikuli* (Incubation period of 72 hours)				
	K	$4.60^{c}\pm0.14$	$23.35^{a}\pm0.03$	$0.40^{ab}\pm0.00$	$34.48^{bc}\pm0.10$
	KL	$4.55^{c}\pm0.07$	$25.00^{c}\pm0.05$	$0.25^{a}\pm0.05$	$33.34^{b}\pm0.01$
	KF	$4.55^{c}\pm0.11$	$30.07^{d}\pm0.08$	$0.43^{abc}\pm0.08$	$35.33^{c}\pm0.45$
	KC	$4.03^{c}\pm0.05$	$24.87^{b}\pm0.85$	$0.60^{abc}\pm0.10$	$33.33^{b}\pm0.32$
	KFL	$3.27^{b}\pm0.02$	$22.53^{a}\pm0.25$	$0.86^{d}\pm0.05$	$33.38^{b}\pm0.25$
	KCL	$4.40^{c}\pm0.20$	$26.99^{c}\pm0.05$	$0.45^{abc}\pm0.05$	$30.64^{a}\pm0.40$
	SON	6.0	35.0	2-4	20.0

L : *Lactobacillus plantarum* ; UK: Unfermented *Kulikuli*; KMKT: *Kulikuli* from the market K: fermented *Kulikuli;* KL: fermented *Kulikuli* and L; KF: fermented *Kulikul and* Flour; KC: fermented *Kulikuli* and Chia flour; KFL: fermented *Kulikuli*, Flour and L; KCL: fermented *Kulikuli*, Chia and L, SON-Standards Organisation of Nigeria.

Values are means± standard error of mean of duplicate determinations. Means with dissimilar letter (s) differ significantly according to the Least Significant Different.

4.1.12 Antinutritional composition of *Kulikuli* samples

The antinutritional composition of the *Kulikuli* samples is presented in Table 4.13. In the current investigation, a significant difference existed for the tannin content among all the samples except for Unfermented *Kulikuli* (UK) (17.54 mg/100g) and fermented *Kulikuli* (K) (17.74mg/100g) at ($p \geq 0.05$). Fermented *Kulikuli* and *Lactobacillus plantarum* (KL) showed the highest tannin content of 18.58 mg/100g whereas *Kulikuli* from the market (KMKT) showed the lowest tannin content of 16.65 mg/100g. Lower tannin contents of the *Kulikuli* samples were observed in fermented *Kulikuli,* all-purpose flour and *Lactobacillus plantarum* (KFL) (18.13mg/100g), fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) (18.46 mg/100g) and fermented *Kulikuli* and all-purpose flour (KF)(18.49mg/100g).

The lowest saponin content was recorded in fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) (19.84 mg/100g) while fermented *Kulikuli* and chia flour (KC) had the highest 58.0mg/100g. fermented *Kulikuli* and all-purpose flour (KF) (28.20 mg/100g), fermented *Kulikuli* (K) (35.05 mg/100g), *Kulikuli* from the market (KMKT) (37.12 mg/100g) and 39.02mg/100g fermented *Kulikuli* and *Lactobacillus plantarum* (KL) recorded higher saponin contents. Unfermented *Kulikuli* (UK) and fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) showed no significant difference from one another, recording tannin values of 26.27 mg/100g and 24.99mg/100g.

All the phytate contents differed significantly from each other at ($p \geq 0.05$). The phytate content of the test *Kulikuli* samples ranged from 12.60 mg/100g in fermented *Kulikuli* and all-purpose flour (KF) to 94.86mg/100g in fermented *Kulikuli* and chia flour (KC). Fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL), fermented

Kulikuli, chia flour and *Lactobacillus plantarum* (KCL), fermented *Kulikuli* (K), fermented *Kulikuli* and *Lactobacillus plantarum* (KL) and *Kulikuli* from the market (KMKT) exhibited increasing phytate values of 27.75mg/100g, 35.06 mg/100g, 40.03 mg/100g, 56.53 mg/100g and 84.25 mg/100g respectively. Fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) had the least cyanide content of3.60 mg/100g when compared to the other test *Kulikuli* samples while fermented *Kulikuli* and *Lactobacillus plantarum* (KL) exhibited the highest content of 513.57mg/100g. Different cyanide contents were observed in Unfermented *Kulikuli* (UK) (15.45 mg/100g), *Kulikuli* from the market (KMKT) (22.40 mg/100g), fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) (39.40mg/100g), fermented *Kulikuli* and all-purpose flour (KF) (62.60 mg/100g), fermented *Kulikuli* (K) (72.60 mg/100g) and fermented *Kulikuli* and chia flour (KC) (110mg/100g).

The highest oxalate content of 0.15mg/100g was recorded in fermented *Kulikuli* and *Lactobacillus plantarum* (KL). Similar lower oxalate was revealed in *Kulikuli* from the market (KMKT) (0.07 mg/100g), Unfermented *Kulikuli* (UK) (0.09 mg/100g) and fermented *Kulikuli* and chia flour (KC) (0.1 mg/100g), however the lowest oxalate contents were observed in fermented *Kulikuli* and all- purpose flour (KF) (0.03 mg/100g), fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) (0.03mg/100g) *and* 0.04 mg/100g for fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL). The above-mentioned values did not differ significantly from one another at ($p \geq 0.05$).

Table 4.13: Antinutritional Composition of *Kulikuli* Sample

Sample	Tannins (mg/100g)	Saponins (mg/100g)	Phytates (mg/100g)	Cyanides (mg/100g)	Oxalates (mg/100g)
KMKT	16.65^{a}	37.12 ^{e}	84.25 ^{g}	22.40 ^{f}	0.07^{b}
UK	17.54^{bc}	26.27^{b}	14.33^{b}	15.45^{b}	0.08^{b}
K	17.74^{bc}	35.05^{d}	40.03^{e}	72.60^{f}	0.10^{b}
KL	18.58^{e}	39.02^{f}	56.53^{f}	513.57^{h}	0.15^{c}
KF	18.49^{e}	28.20^{c}	12.60^{a}	62.60^{e}	0.03^{a}
KC	17.21^{ab}	58.04^{g}	94.86^{h}	110.10^{g}	0.09^{b}
KFL	18.13^{cd}	19.84^{a}	27.75^{c}	39.40^{d}	0.03^{a}
KCL	18.46^{de}	24.99^{b}	35.06^{d}	3.60^{a}	0.04^{a}

L: *Lactobacillus plantarum;* KMKT: *Kulikuli* from the market; UK: Unfermented *Kulikuli* K: fermented *Kulikuli;* KL: fermented *Kulikuli* and L; KF: fermented *kulikul and* all-purpose flour; KC: fermented *Kulikuli* and chia flour; KFL: fermented *Kulikuli*, all-purpose flour and L; KCL: fermented *Kulikuli*, chia flour and L

Means with dissimilar letter (s) differ significantly according to the DMRT. ($P<0.05$).

4.1.13 Physicochemical properties of *Kulikuli* samples

The stability of the *Kulikuli* samples towards oxidative deterioration was analyzed by monitoring the oxidation parameters such as free fatty acid value (FFA), iodine value (IV) and peroxide value (PV) for a duration of 0 week to 3 weeks of storage period after a 3-day fermentation period. The physicochemical properties of the *Kulikuli* samples are shown in Table 4.14.

For the free fatty acid value, a significant difference existed ($p \geq 0.05$) between the two controls (Unfermented *Kulikuli* (UK) and KMKT (*Kulikuli* from the Market)) at week zero (0), however, control Unfermented *Kulikuli* (UK) did not differ from the other laboratory prepared *Kulikuli* samples (fermented *Kulikuli* and *Lactobacillus plantarum* (KL), fermented *Kulikuli and* all- purpose flour (KF), fermented *Kulikuli* and chia flour (KC) , fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) and fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL). At week 1, the controls (Unfermented *Kulikuli* (UK) and KMKT (*Kulikuli* from the Market)) were the same and they bore no significant difference ($p \geq 0.05$) from fermented *Kulikuli* (K), fermented *Kulikuli* and chia flour (KC) and fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL).

At week 21, fermented *Kulikuli* and *Lactobacillus plantarum* (KL), fermented *Kulikuli* and chia flour (KC) and fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) were significantly different from the controls (Unfermented *Kulikuli* (UK) and KMKT (*Kulikuli* from the Market)) and the other laboratory prepared *Kulikuli* samples (fermented *Kulikuli* (K), fermented *Kulikuli and* all- purpose flour (KF) and fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL)).

The free fatty acid value of the controls (Unfermented *Kulikuli* (UK) and KMKT (*Kulikuli* from Market)) samples at week zero were 7.64mg KOH/g and 6.75mg KOH/g after three weeks of storage the values changed to 9.36 and 8.47 mg KOH/g respectively. Fermented *Kulikuli* (K) and fermented *Kulikuli* and *Lactobacillus plantarum* (KL) at week zero were 7.50 mg KOH/g and 7.61 mg KOH/g, after 3 days of fermentation changed to 8.32 mg KOH/g and 8.99 mg KOH/g respectively after three weeks of storage.

Fermented *Kulikuli*and all-purpose flour (KF) was 9.41 mg KOH/g, changed to 9.47 mg KOH/g after a three-week interval. Fermented *Kulikuli* and chia flour (KC) at a storage duration of 0 week to 3 weeks after a 3-day fermentation was 8.42mg KOH/g - 10.32 mg KOH/g. 8.39 mg KOH/g – 9.17 mg KOH/g were the free fatty acid contents recorded for fermented *Kulikuli* and all-purpose flour and *Lactobacillus plantarum* (KFL) after a 3-day fermentation after a 0 week to 3 weeks storage time. Fermented *Kulikuli* and chia flour and *Lactobacillus plantarum* (KCL) recorded a free fatty acid content of 9.41 mg KOH/g – 9.47mg KOH/g after a 3-day fermentation period after a 0 week to a 3 weeks storage interval. The acceptable level of free fatty acids in a frying medium cannot be higher than 2.5 mg KOH/g (Theah and Akanbi, 2023).

There was a sequential increase in the peroxide value as the storage duration increased (0 to 3 weeks).The peroxide value at week zero showed that Unfermented *Kulikuli* (UK) and *Kulikuli* from the Market (KMKT)) had no significant difference ($p \geq 0.05$) from one another, significantly higher peroxide values were observed between fermented *Kulikuli* and *Lactobacillus plantarum* (KL), fermented *Kulikuli*and all-purpose flour (KF), fermented *Kulikuli* and chia flour (KC) and fermented *Kulikuli* and all-purpose flour and *Lactobacillus plantarum* (KFL). The controls (Unfermented *Kulikuli* (UK) and *Kulikuli* from the Market (KMKT)) differed from each other at ($p \geq$

0.05) at the end of week 1, however fermented *Kulikuli* (K), fermented *Kulikuli* and chia flour (KC), fermented *Kulikuli* and all-purpose flour and *Lactobacillus plantarum* (KFL) and fermented *Kulikuli* and chia flour and *Lactobacillus plantarum* (KCL) were significantly the same as *Kulikuli* from the Market (KMKT) whereas fermented *Kulikuli* and *Lactobacillus plantarum* (KL) and fermented *Kulikuli*and all-purpose Flour (KF) showed no difference from Unfermented *Kulikuli* (UK). At week 3, no significant difference at ($p \geq 0.05$) existed between Unfermented *Kulikuli* (UK) and *Kulikuli* from the Market (KMKT). All the other laboratory- prepared *Kulikuli* samples except for fermented *Kulikuli* and *Lactobacillus plantarum* (KL) showed significantly equal values. Unfermented *Kulikuli* (UK) and *Kulikuli* from the market (KMKT) which served as the controls and samples after a 3 day of fermentation at week zero were 2.67meq/kg and 2.19 meq/kg after three weeks of storage, the values changed to 8.44 meq/kg and 7.96 meq/kg for the peroxide value content.

Fermented *Kulikuli* (K) and fermented *Kulikuli* and *Lactobacillus plantarum* (KL) recorded a peroxide value of 2.80meq/kg and 3.05 meq/Kg after a 3-day fermentation period and after a zero- week to a 3 weeks storage interval changed to 6.81meq/kg and 7.11 meq/kg. Fermented *Kulikuli*and all-purpose flour (KF) had a peroxide value of 3.61meq/kg and 8.75 meq/kg after a three-week storage time. 2.88 meq/kg to 8.55 meq/kg were recorded for fermented *Kulikuli* and chia flour (KC) after a 3-day fermentation after a week zero to 3 weeks storage duration. Fermented *Kulikuli* and all-purpose flour and *Lactobacillus plantarum* (KFL) at a storage duration of 0 day to 3 weeks after a 3-day fermentation was 3.50 meq/kg - 7.67 meq/kg and lastly fermented *Kulikuli* and chia flour and *Lactobacillus plantarum* (KCL) at zero time was 3.61 meq/kg, after 3 days of fermentation, changed to 8.75 meq/kg after three weeks of storage.

The iodine value at week zero of all the laboratory prepared samples with exemption of fermented *Kulikuli* and *Lactobacillus plantarum* (KL) were significantly ($p \geq 0.05$) the same with control *Kulikuli* from the Market (KMKT), UK (the second control) and fermented *Kulikuli* and *Lactobacillus plantarum* (KL) were not significantly different from one another. Controls (Unfermented *Kulikuli* (UK) and *Kulikuli* from the Market (KMKT)) and all the laboratory prepared *Kulikuli* samples showed no significant difference ($p \geq 0.05$) except for KL in terms of the iodine content. The iodine values at week 3 showed no difference at ($p \geq 0.05$) between controls (Unfermented *Kulikuli* (UK) and *Kulikuli* from the Market (KMKT)) and fermented *Kulikuli* and all- purpose flour (KF), fermented *Kulikuli* and chia flour (KC) and fermented *Kulikuli* and chia flour and *Lactobacillus plantarum* (KCL). Initially the iodine value of the Unfermented *Kulikuli* (UK) and *Kulikuli* from the Market(KMKT) were 72.92 g/100g and 66.55 g/100g, after 3 weeks of storage, recorded values of 73.52 g/100g and 72.28 g/100g. Fermented *Kulikuli* (K) had iodine values of 74.51 g/100g – 75.08 g/100g. Fermented *Kulikuli* and *Lactobacillus plantarum* (KL) at week zero were 74.01 g/100g after 3 days of fermentation changed to 74.88 g/100g after three weeks of storage. Fermented *Kulikuli* and all-purpose flour (KF) after a 3-day fermentation at week zero were 74.47 g/100g to 75.48 g/100g after a three-week interval. Fermented *Kulikuli* and chia flour (KC) at a storage duration of 0 week to 3 weeks after a 3-day fermentation was 74.27 g/100g - 75.23 g/100g.Fermented *Kulikuli* and all- purpose flour and *Lactobacillus plantarum* (KFL) recorded a free iodine value of 73.81 g/100g – 73.95 g/100g after 3 weeks of storage, 74.47 g/100g – 75.48 g/100g were the iodine value recorded for fermented *Kulikuli* and chia flour and *Lactobacillus plantarum* (KCL) after a 3-day fermentation after a zero-day to 3 weeks storage time. It was observed that there was an increment in the iodine value as the storage days increased.

Table 4.14: Physicochemical Properties of *Kulikuli* Samples

Time (Weeks)	SAMPLE	Free Fatty Acid (mgKOH/g)	Peroxide Value (meq/kg)	IodineValue (g/100g)
0	**KMKT**	6.75^{a}±0.23	2.19^{a}±0.02	66.55^{a}±0.05
1		7.66^{b} ±0.02	3.88^{b} ±0.11	71.95^{b} ±0.01
3		8.47^{b} ±0.19	7.96^{c} ±0.07	72.28^{b} ±0.10
Laboratory Prepared *Kulikuli*				
0	UK	7.64^{b} ±0.22	2.67^{a}±0.25	72.92^{b} ±0.54
	K	7.50^{b} ±0.10	2.80^{a} ±0.20	74.51^{b} ±0.21
	KL	7.61^{b}±0.28	3.05^{b}±0.07	74.01^{a}±0.05
	KF	9.41^{b} ±0.25	3.61^{b} ±0.25	74.47^{b} ±0.35
	KC	8.42^{b} ±0.50	2.88^{b} ±0.00	74.27^{b} ±0.00
	KFL	8.39^{b}±0.27	3.50^{b}±0.35	73.81^{b}±0.71
	KCL	$9.41^{a\,b}$ ±0.21	3.61^{c} ±0.15	74.47^{b} ±0.10
1	UK	8.55^{b}±0.00	4.36^{c} ±0.00	73.96^{b} ±0.16
	K	8.75^{bc}±0.30	3.44^{b}±0.08	76.77^{b} ±0.07
	KL	8.62^{c} ±0.00	4.33^{c} ±0.01	75.14^{c} ±0.20
	KF	9.63^{c} ±0.63	4.28^{c} ±0.14	76.00^{b}±0.20
	KC	9.56^{bc}±0.01	3.90^{b}±0.15	75.35^{b} ±0.40
	KFL	8.54^{a}±0.32	4.04^{b}±0.14	75.05^{b}±0.14
	KCL	9.63^{ab}±0.11	4.28^{b}±0.11	76.00^{b}±0.05
3	UK	9.36^{b}±0.04	8.44^{c}±0.24	73.52^{b}±0.30
	K	8.32^{b}±0.30	6.8^{b}±0.55	75.08^{c} ±0.14
	KL	8.99^{c} ±0.12	7.11^{d}±0.12	74.88^{bc} ±0.30
	KF	9.47^{b} ±0.25	8.75^{b}±0.37	75.48^{b}±0.73
	KC	10.32^{c}±0.12	8.55^{b} ±0.22	75.23^{b} ±0.14
	KFL	9.17^{b}±0.25	7.67^{b}±0.49	73.95^{b}±0.21
	KCL	9.47^{c} ±0.19	8.75^{b} ±0.21	75.48^{bc} ±0.30

L: *Lactobacillus plantarum;* KMKT: *Kulikuli* from the market; UK: Unfermented *Kulikuli*; K: fermented *Kulikuli;* KL: fermented *Kulikuli* and L; KF: fermented *Kulikuli and* all – purpose flour; KC: fermented *Kulikuli* and chia flour; KFL: fermented *Kulikuli*, all- purpose flour and L; KCL: fermented *Kulikuli*, chia flour and L

Values are means± standard error of mean of duplicate determinations. Values with the same superscript do not differ significantly at ($P \leq 0.05$).

4.1.14 Effect of *Kulikuli* supplemented diet on the feed intake of rats

The effect of *Kulikuli* consumption by the albino rats was shown in Table 4.15. A significant difference existed between the rats fed with *Kulikuli* from the market (KMKT) when compared with the basal diet. There was a significant change in the treatment groups in all 4 weeks. The animals fed meals containing basal diet had the highest feed intake, followed by fermented *Kulikuli* and all-purpose flour (KF) while the animals fed Unfermented *Kulikuli* (UK) had the lowest. Animals fed with basal diet consumed the most, while those fed fermented *Kulikuli* and chia flour (KC) consumed the least.

Table 4.15: Weekly Feed Intake of Rats Fed with *Kulikuli* Supplemented Diet

Treatment	Week 1	Week 2	Week 3	Week 4
KMKT	38.88±0.57 ^{c}	45.54±0.98 ^{b}	41.22±0.01 ^{d}	48.67±0.24 ^{d}
UK	42.15±0.01 ^{e}	33.17±0.05 ^{c}	43.33±0.12 ^{e}	45.32±0.11 ^{c}
BASAL DIET	44.00±0.22 ^{f}	39.80±1.08 ^{ef}	48.12±0.01 ^{g}	54.22±0.01 ^{g}
K	44.92±0.70 ^{f}	44.27±0.01 ^{e}	46.64±0.09 ^{f}	49.84±0.02 ^{e}
KL	35.70±0.49 ^{a}	35.42±0.21 ^{b}	36.72±0.01 ^{b}	41.88±0.44
KF	47.24±0.42 ^{g}	40.47±0.65 ^{f}	46.53±0.32 ^{f}	51.23±0.02 ^{f}
KC	37.48±0.02 ^{b}	35.28±0.37 ^{a}	34.27±0.15 ^{a}	36.53±0.21^{a}
KFL	37.49±0.08 ^{b}	45.82±0.61 ^{d}	41.61±0.01 ^{d}	49.32±0.01 ^{e}
KCL	40.12±0.01 ^{d}	38.37±0.06 ^{d}	38.93±0.51 ^{c}	41.53±0.01 ^{b}

L: *Lactobacillus plantarum;* KMKT: *Kulikuli* from the market; UK:Unfermented *Kulikuli*; K:fermented *Kulikuli;* KL: fermented *Kulikuli* and L; KF: fermented *Kulikul and* all – purpose flour; KC: fermented *Kulikuli* and chia flour; KFL: fermented *Kulikuli*, Flour and L; KCL: fermented *Kulikuli*, chia and L; mg/kgbw: milligramme per kilogramme body weight

Values are means ± standard error of mean. Values with the same superscript do not differ significantly at ($P > 0.05$).

4.1.15 Effect of *Kulikuli* supplemented diet on the body weight of rats

Table 4.16 reveals the effect of *Kulikuli* on the body weight of Wister albino rats. The body weight was not significantly different ($P > 0.05$) from the control (basal diet). The weight gain for rats fed the basal diet (45.0%) was higher when compared with the weight gain of the other rats fed the other feeds. Weight gain by rats fed the control Unfermented *Kulikuli* (UK) (41.6%), fermented *Kulikuli* (K) (42.5%), Fermented *Kulikuli* and *Lactobacillus plantarum* (KL) (37.6%), fermented *Kulikuli* and all-purpose flour (KF) (44.1%), Fermented *Kulikuli*and chia flour (KC) (28.7%), fermented *Kulikuli* and all -purpose flour and *Lactobacillus plantarum* (KFL)(40.0%) and fermented *Kulikuli* and chia flour and *Lactobacillus plantarum* (KCL)(35.5%). However, rats fed fermented *Kulikuli* and chia flour (KC) supplemented diet (28.75%) had the least weight gain when compared with the other groups. The body weights of all the treatment groups were not significantly different ($P > 0.05$) from the control.

4.1.16 Effect of *Kulikuli* supplemented diet on the relative organ weight of rats

Table 4.17 shows the effect of *Kulikuli* from the market on the relative organ weight of Wister albino rats. There was no significant difference ($P > 0.05$) from the control group (basal diet). The computed relative weights of the stomach, kidney, liver, spleen, and intestine of the treated groups were not significantly different ($P > 0.05$) from the control groups.

Table 4.16: Effect of *Kulikuli* Supplemented Diet on Body Weight of Rats

Treatments	Day O	Day 7	Day 14	Day 21	Day 28	% Weight Gain
UK	97.96±19.7[a]	133.7±23.5[a]	151.95±29.4[a]	162.2±29.8[a]	167.8±25.0[a]	41.6%
KMKT	107.2±6.97[a]	138.4±12.0[a]	138.7±13.1[a]	165.2±7.49[a]	178.5±4.04[a]	39.9%
BASAL DIET	89.39±8.17[a]	103.5±8.39[a]	107.8±11.7[a]	141.3±9.89[a]	162.5±10.2[a]	45.0%
K	87.39±3.78	123.0±5.26[a]	130.5±2.73[a]	138.3±4.03[a]	152.1±3.45 [a]	42.5%
KL	89.03±20.7[a]	117.3±29.5[a]	124.3±33.5[a]	134.43±28.6[a]	142.6±28.3[a]	37.6%
KF	83.08±4.36[a]	109.3±8.23[a]	130.9±9.88[a]	141.7±12.5[a]	148.5±14.6[a]	44.1%
KC	101.3±0.13[a]	125.4±9.07[a]	135.3±2.77[a]	137.±3.68[a]	142.4±0.02[a]	28.7%
KFL	96.14±4.5[a]	129.7±4.16[a]	147.2±3.23[a]	149.9±5.48[a]	157.6±5.11[a]	40.0%
KCL	104.1±29.2[a]	137.3±41.6[a]	151.5±41.2[a]	154.7±41.9[a]	161.4±47.7[a]	35.5%

L: *Lactobacillus plantarum;* KMKT: *Kulikuli* from the market; UK: Unfermented *Kulikuli*; K: fermented *Kulikuli;* KL: fermented *Kulikuli* and L; KF: fermented *Kulikul and* all – purpose flour; KC: fermented *Kulikuli* and chia flour; KFL: fermented *Kulikuli*, Flour and L; KCL: fermented *Kulikuli*, chia and L. mg/kgbw: milligramme per kilogramme body weight; % : percentage

Values are in ± mean S.E. (S.E = Standard error of Mean)

Values between experimental treatments within groups bearing the same superscript are not significantly different at the 5% level ($P<0.05$).

Table 4.17: Relative Organ Weight of Rats Fed with *Kulikuli* Samples Supplemented Diet

Treatment	Stomach	Spleen	Intestine	Kidney	Liver
UK	3.32±19.7[a]	0.39±23.5 [a]	0.73±29.4 [ab]	5.70±29.8 [a]	4.27±25.0 [a]
KMKT	3.36±6.97 [a]	0.47±12.0[ab]	0.71±13.1 [ab]	6.77±7.49 [a]	3.89±4.04 [a]
BASAL DIET	3.34±8.17 [a]	0.79±8.39 [ab]	0.91±11.7 [ab]	6.86±9.89 [a]	4.26±10.2 [a]
K	3.35±3.78 [a]	0.63±5.26 [ab]	0.88±2.73 [ab]	5.74±4.03 [a]	4.05±3.45 [a]
KL	3.34±20.7 [a]	0.44±29.5 [a]	0.99±33.5 [ab]	6.76±28.6 [a]	3.65±28.3 [a]
KF	3.33±4.36 [a]	0.42±8.23 [a]	0.94±9.88 [ab]	6.74±12.5 [a]	4.13±14.6 [a]
KC	3.37±0.13 [a]	0.50±9.07 [a]	0.94±2.77 [ab]	6.81±3.68 [a]	3.74±0.02 [a]
KFL	3.33±4.52 [a]	0.44±4.16 [a]	1.12±3.23 [b]	5.72±5.48 [a]	4.07±5.11 [a]
KCL	3.27±29.2 [a]	0.43±41.6 [a]	0.07±41.2 [a]	5.68±41.9 [a]	3.99±47.7 [a]

L: *Lactobacillus plantarum;* KMKT: *Kulikuli* from the market; UK: Unfermented *Kulikuli*; K: fermented *Kulikuli;* KL: fermented *Kulikuli* and L; KF: fermented *Kulikul and* all – purpose flour; KC: fermented *Kulikuli* and chia flour; KFL: fermented *Kulikuli*, Flour and L; KCL: fermented *Kulikuli*, chia and L. mg/kgbw: milligramme per kilogramme body weight

Values are in ± mean S.E. (S.E = Standard error of Mean). Values between experimental treatments within groups bearing the same superscript are not significantly different at the 5% level ($P<0.05$).

4.1.17 Effect of *Kulikuli* supplemented diet on the haematological parameters of ` rats

The effect of the *Kulikuli* supplemented diet on the haematological parameters of rats is shown in Table 4.18, no significant difference occurred between this treatment and the control (basal diet). All the haematological parameters of rats fed with laboratory-prepared supplemented diet were not significantly ($P > 0.05$) different from the control group. However, platelets were significantly ($p<0.05$) higher in rats fed fermented *Kulikuli* (K).

Table 4.18: Effect of *Kulikuli* Supplemented Diet on the Haematological Parameters of Rats

Treatment	PCV (%)	Hb (g/dl)	RBC ($x10^9$/L)	WBC ($x10^9$/L)	PLATELETS ($x10^6$/L)	MCV (fl)	MCH (g/dl)	MCHC (g/dl)
UK	$41.50^{a}\pm1.50$	$13.80^{ab}\pm0.50$	$5.45^{ab}\pm0.35$	$5.80^{a}\pm1.50$	$195.00^{ab}\pm0.00$	$90.00^{ab}\pm7.00$	$31.50^{a}\pm1.50$	$33.20^{a}\pm0.00$
KMT	$43.00^{a}\pm2.00$	$13.80^{ab}\pm0.20$	$4.80^{ab}\pm0.60$	$6.55^{a}\pm1.45$	$121.50^{a}\pm83.50$	$84.00^{ab}\pm9.00$	$29.05^{a}\pm3.95$	$33.20^{a}\pm0.10$
Basal diet	$43.00^{a}\pm2.00$	$14.30^{ab}\pm0.70$	$4.60^{ab}\pm0.40$	$7.00^{a}\pm0.10$	$210.00^{ab}\pm30.00$	$78.00^{a}\pm4.00$	$33.35^{a}\pm2.15$	$33.20^{a}\pm0.10$
K	$44.50^{a}\pm0.50$	$14.80^{ab}\pm0.20$	$5.10^{ab}\pm0.10$	$5.10^{a}\pm0.00$	$375.00c\pm35.00$	$103.00^{b}\pm1.00$	$32.35^{a}\pm2.35$	$33.20^{a}\pm0.10$
KL	$43.00^{a}\pm3.00$	$14.30^{ab}\pm1.00$	$4.90^{ab}\pm0.20$	$6.20^{a}\pm0.10$	$295.00^{b}c\pm55.00$	$87.50^{ab}\pm2.50$	$28.40^{a}\pm1.60$	$33.20^{a}\pm0.00$
KF	$44.50^{a}\pm0.50$	$14.60^{ab}\pm0.00$	$5.05^{ab}\pm0.05$	$5.70^{a}\pm0.30$	$305.00^{b}c\pm15.00$	$87.00^{ab}\pm1.00$	$28.90^{a}\pm0.30$	$33.10^{a}\pm0.00$
KC	$42.50^{a}\pm2.50$	$14.15^{ab}\pm0.85$	$4.15^{a}\pm0.05$	$4.55^{a}\pm0.45$	$265.00^{b}c\pm25.00$	$101.00^{b}\pm4.00$	$28.75^{a}\pm1.25$	$33.20^{a}\pm0.00$
KFL	$38.50^{a}\pm0.50$	$12.80^{ab}\pm0.20$	$4.45^{ab}\pm0.25$	$4.50^{a}\pm0.50$	295.50 ± 44.50	$86.00^{ab}\pm6.00$	$28.85^{a}\pm2.05$	$33.20^{a}\pm0.10$
KCL	$40.00^{a}\pm0.00$	$13.30^{ab}\pm0.00$	$4.70^{ab}\pm0.40$	$5.15^{a}\pm0.15$	$237.50^{ab}\pm12.50$	$85.50^{ab}\pm7.50$	$28.30^{a}\pm1.70$	$33.20^{a}\pm0.00$

L: *Lactobacillus plantarum;* KMKT: *Kulikuli* from the market; UK: Unfermented *Kulikuli*; K: fermented *Kulikuli;* KL: fermented *Kulikuli* and L; KF: fermented *Kulikul and* all – purpose flour; KC: fermented *Kulikuli* and chia flour; KFL: fermented *Kulikuli*, Flour and L; KCL: fermented *Kulikuli*, chia and L; fl: flemolitre; g/dL : gramme per decilitre; mg/kgbw : milligramme per kilogramme body weight; % : percentage

Values are means± standard error of mean of duplicate determinations. Mean values with the same superscript on the same column are not significantly different from each other using DMRT ($P<0.05$).
PCV= packed cell volume; Hb=Haemoglobin; WBC=White blood cells; RBC=Red blood cells; PLT= platelets count; MCV=Mean corpuscular volume; MCH= mean corpuscular haemoglobin; MCHC=Mean corpuscular haemoglobin concentration.

4.1.18 Effect of *Kulikuli* supplemented diet on the differential blood count of rats

The effect of the *Kulikuli* supplemented diet on the differential blood count of rats is presented in Table 4.19, there was no significant difference between the treatment and control. All the treatment groups were not statistically different from the control groups ($P > 0.05$) (rats fed with Unfermented *Kulikuli* (UK)) for the neutrophil, monocytes, eosinophil and basophils. However, lymphocyte counts significantly ($P < 0.05$) increased when compared with the control.

4.1.19 Effect of *Kulikuli* supplemented diet on liver and kidney function

The effect of *the Kulikuli* supplemented diet on the liver and kidney function is shown in Table 4.20. A significant difference existed betweenthe Total protein, albumin, alanine aminotransaminase, creatinine, urea levels, aspartate aminotransferase and bilirubin for the fermented *Kulikuli* samples from the control ($P > 0.05$).

4.1.20 Effect of *Kulikuli* supplemented diet on the lipid profile of rats

The effects of *Kulikuli* supplemented diet on the lipid profile in rats are shown in Table 4.21. The total cholesterol, triglycerides, low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C) concentrations in rats of laboratory-prepared *Kulikuli* samples supplemented diet were significantly different ($P > 0.05$) from the control groups.

Table 4.19: Effect of *Kulikuli* Supplemented Diet on Differential Blood Count of Rats

Treatment	Neutrophils (%)	Lymphophytes (%)	Monocytes (%)	Eosinophils (%)	Basophil (%)
UK	$46.50^{ab}\pm4.50$	$44.00^{a}\pm5.00$	$5.50^{a}\pm1.50$	$3.50^{a}\pm0.50$	$0.00^{a}\pm0.00$
KMT	$48.00^{ab}\pm2.00$	$39.50^{a}\pm0.50$	$8.50^{a}\pm1.50$	$4.00^{a}\pm0.00$	$0.00^{a}\pm0.00$
BASAL DIET	$47.00^{ab}\pm3.00$	$41.50^{a}\pm1.50$	$7.00^{a}\pm1.00$	$4.50^{a}\pm0.50$	$0.00^{a}\pm0.00$
K	$41.50^{ab}\pm1.50$	$50.00^{ab}\pm1.00$	$7.00^{a}\pm2.00$	$2.50^{a}\pm2.50$	$1.50^{a}\pm1.50$
KL	$47.00^{ab}\pm8.00$	$45.00^{a}\pm5.00$	$4.50^{a}\pm1.50$	$3.50^{a}\pm1.50$	$0.00^{a}\pm0.00$
KF	$34.50^{a}\pm3.50$	$56.00^{b}\pm4.00$	$6.50^{a}\pm0.50$	$3.00^{a}\pm0.00$	$0.00^{a}\pm0.00$
KC	$49.00^{b}\pm4.00$	$45.50^{a}\pm0.50$	$3.50^{a}\pm1.5$	$2.00^{a}\pm2.00$	$0.00^{a}\pm0.00$
KFL	$47.00^{ab}\pm3.00$	$42.50^{a}\pm3.50$	$8.00^{a}\pm5.00$	$2.50^{a}\pm1.50$	$0.00^{a}\pm0.00$
KCL	$49.50^{b}\pm0.50$	$43.00^{a}\pm2.00$	$5.00^{a}\pm1.00$	$2.50^{a}\pm0.50$	$0.00^{a}\pm0.00$

L: *Lactobacillus plantarum;* KMKT: *Kulikuli* from the market; UK: Unfermented *Kulikuli*; K: fermented *Kulikuli;* KL: fermented *Kulikuli* and L; KF: fermented *Kulikuli and* all – purpose flour; KC: fermented *Kulikuli* and chia flour; KFL: fermented *Kulikuli*, all- purpose flour and L; KCL: fermented *Kulikuli*, chia flour and L; %: percentage; mg/kgbw: milligramme per kilogramme body weight.

Values are means ± standard error of mean. Values with the same superscript do not differ significantly at ($P > 0.05$).

Table 4.20: Effect of *Kulikuli* Supplemented Diet on the Liver and Kidney Function

Treatment	Total protein (g/dL)	Albumin (g/mL)	AST (µ/L)	ALT (µ/L)	Creatinine (µmol/L)	Bilirubin (mg/dL)	Urea
UK	$3.86^{a}\pm0.74$	$2.39^{a}\pm0.30$	$55.10^{e}\pm1.89$	$71.82^{c}\pm2.28$	$89.40^{cd}\pm2.06$	$0.43^{a}\pm0.11$	$58.80^{ab}\pm1.66$
KMKT	$4.90^{ab}\pm0.23$	$2.94^{a}\pm0.18$	$51.21^{de}\pm2.28$	$66.65^{bc}\pm1.44$	$94.18^{cd}\pm2.06$	$0.93^{d}\pm0.04$	$76.23^{e}\pm1.89$
Basal diet	$7.91^{c}\pm1.02$	$5.01^{bcd}\pm0.66$	$44.13^{bc}\pm1.19$	$66.49^{bc}\pm1.74$	$92.39^{cd}\pm2.94$	$0.62^{ab}\pm0.06$	$67.18^{bc}\pm1.73$
K	$7.43^{c}\pm0.46$	$3.56^{ab}\pm0.42$	$40.72^{ab}\pm2.49$	$59.05^{a}\pm1.07$	$89.47^{cd}\pm2.13$	$0.78^{cd}\pm0.10$	$58.68^{ab}\pm1.56$
KL	$6.43^{bc}\pm0.45$	$3.95^{ab}\pm0.17$	$47.46^{cd}\pm1.46$	$63.34^{ab}\pm2.89$	98.61 ± 1.83	$0.85^{cd}\pm0.09$	$72.23^{de}\pm2.01$
KF	$8.15^{c}\pm0.68$	$5.73^{cd}\pm1.17$	$38.49^{a}\pm1.63$	$59.01^{a}\pm1.12$	$78.03^{a}\pm2.09$	$0.70^{bc}\pm0.08$	$70.39^{cde}\pm1.95$
KC	$7.73^{c}\pm0.61$	$5.60^{cd}\pm0.61$	$47.56^{cd}\pm0.66$	$69.31^{bc}\pm1.90$	$96.93^{de}\pm2.47$	$0.41^{a}\pm0.02$	$55.01^{a}\pm2.89$
KFL	$8.73^{c}\pm0.61$	$6.38^{d}\pm0.37$	$46.51^{cd}\pm0.61$	$73.26^{c}\pm3.14$	$81.27^{ab}\pm1.95$	$0.53^{ab}\pm0.07$	$54.72^{a}\pm3.27$
KCL	$8.73^{c}\pm0.84$	$6.11^{d}\pm033.$	$37.13^{a}\pm1.81$	$71.82^{c}\pm2.50$	$86.98^{bc}\pm2.35$	$0.63^{ab}\pm0.04$	$63.50^{bc}\pm1.38$

L: *Lactobacillus plantarum;* KMKT: *Kulikuli* from the market; UK: Unfermented *Kulikuli*; K: fermented *Kulikuli;* KL: fermented *Kulikuli* and L; KF: fermented *Kulikuli and* all – purpose flour; KC: fermented *Kulikuli* and chia flour; KFL: fermented *Kulikuli*, all-purpose flour and L; KCL: fermented *Kulikuli*, chia flour and L.; ALT : Alaninetransaminase; AST : Aspartatetransaminase; g/dL: gramme per decilitre; g/mL: grams per millilitre; mg/dL : milligramme per decilitre ; µmol/L: micromole per litre; µ/L: microlitre

Values are means ± standard error of mean and values with the same superscript do not differ significantly at ($P > 0.05$). mg/dL: milligram per decilitre.

Table 4.21: Effect of *Kulikuli* Supplemented Diet on the Lipid Profile of Rats

Treatment	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
UK	175.61^{ab}±2.74	259.38^{a}±2.44	119.18^{d}±2.40	162.35^{b}±1.68
KMKT	218.61^{e}±2.83	299.52^{d}±1.97	88.28^{a}±1.94	181.52^{cd}±2.98
BASAL DIET	196.34^{d}±2.11	286.33^{c}±1.12	99.51^{b}±1.95	174.66^{c}±2.28
K	184.01^{c}±3.56	265.17^{a}±2.40	108.63^{c}±1.91	$151.9^{a}5$±2.09
KL	229.91f±2.43	298.65^{d}±2.22	93.17^{ab}±1.95	195.77^{e}±2.56
KF	211.90^{e}±1.34	297.98^{d}±1.23	88.92^{a}±1.48	165.68^{b}±1.45
KC	201.92^{d}±2.58	295.78^{d}±2.57	92.63^{ab}±1.59	182.72^{c}±2.27
KFL	171.90^{a}±2.33	273.36^{b}±2.77	108.65^{c}±1.90	152.45^{a}±1.87
KCL	180.89^{bc}±1.44	276.89^{b}±1.56	96.28^{b}±1.85	163.67^{b}±1.78

L: *Lactobacillus plantarum;* KMKT: *Kulikuli* from the market; UK: Unfermented *Kulikuli*; K: fermented *Kulikuli;* KL: fermented *Kulikuli* and L; KF: fermented *Kulikuli and* all – purpose flour; KC: fermented *Kulikuli* and chia flour; KFL: fermented *Kulikuli, and* all – purpose flour and L; KCL: fermented *Kulikuli*, chia flour and L; HDL-C: High density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol.

Mean values with the same superscript on the same column are not significantly different from each other using DMRT (P>0.05). mg/dL: milligram per decilitre.

4.1.21 Effect of *Kulikuli* supplemented diet on the organs of rats

The microscopic examination of the liver sections of the control group (basal diet) showed normal architecture of structural units of the liver. There were no significant ($P>0.05$) histopathological presentations observed in the rats placed on the *Kulikuli* supplemented diets and the control group. The liver appeared normal with preserved hepatic architecture, hepatocytes arranged as radial plates, and eosinophilic cytoplasm and basophilic central nuclei. No cytoplasmic inclusions were seen and no portal inflammation.

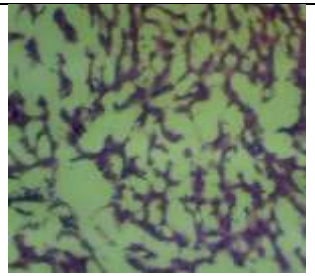
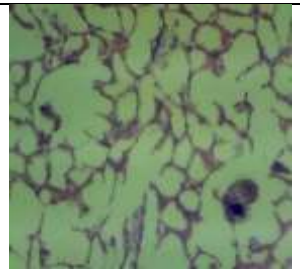
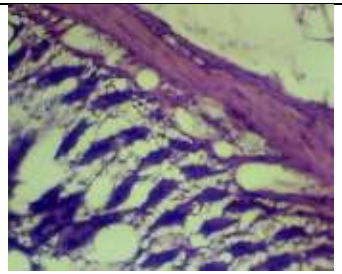
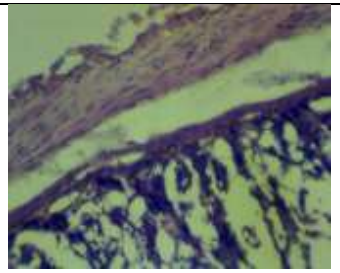
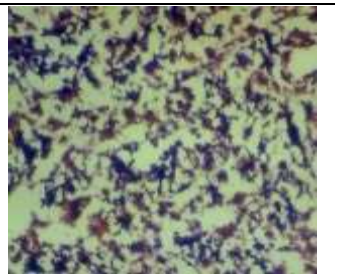
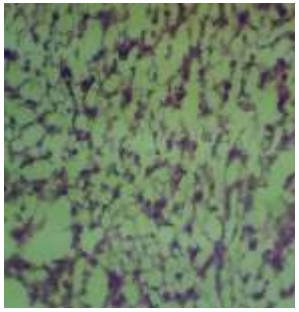
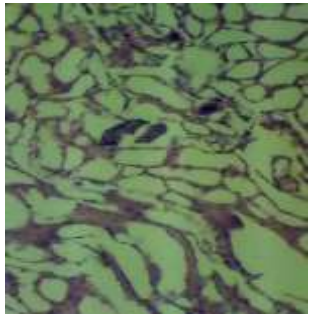
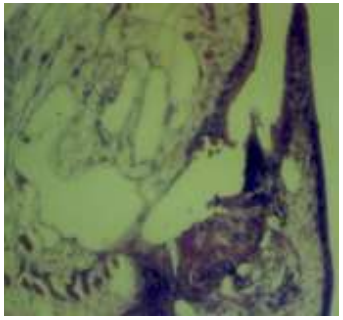
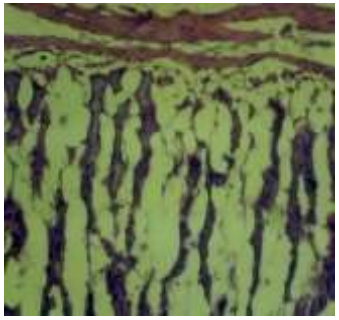
Examination of kidney sections of rats placed on the laboratory-prepared *Kulikuli* supplemented diet indicated no structural difference compared to the control. The microscopic architecture of sections of the kidney had a similar appearance to that of the control in which renal corpuscles maintained their normal size of urinary space and normal tubular structures was observed. No necrosis was observed.

The microscopic structures of the spleen, intestine and stomach showed unnoticeable differences between the rats placed on laboratory-prepared *Kulikuli* supplemented diets and the control group. There was no alteration in cell structure or any unfavourable effects when viewed under the light microscope (Table 4.22, Plate V).

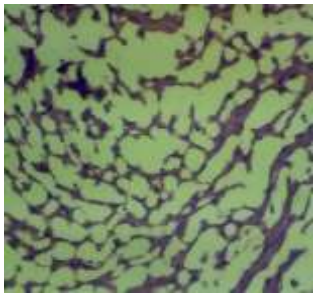
Table 4.22: Effect of *Kulikuli* Supplemented Diet on the Organs of Rats

Organs	**Groups**								
	UK	**KMKT**	**BD**	**K**	**KL**	**KF**	**KC**	**KFL**	**KCL**
Liver	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Stomach	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Spleen	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
Kidney	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
intestine	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

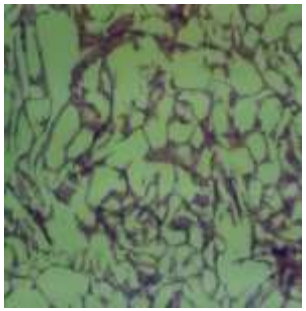
NAD: No adverse effect; BD: Basal Diet

	Liver	Kidney	Intestine	Stomach	Spleen
BASAL DIET	 Photomicrograph of the Liver tissue showing normal histological architecture with no necrosis	 Photomicrographof the Kidney tissue showing normal histological architecture.	 Photomicrograph of the intestinal tissue showing normal histological architecture with intact Laminapropria and epithelial cells	 Photomicrograph of the Gastric mucosa tissue showing normal histological architecture with intact mucosal layers	 Photomicrograph of the spleen tissue with intact histological architecture
UK	 Hepatic tissue with Unaltered hepatic cells	 Photomicrograph of the Kidney tissue showing normal sinusoids	 No visible alteration in lamina propria and epithelialcells	 Gastric mucosa tissue showing normal histological architecture with intact mucosal layers	 Photomicrograph of the spleen tissue showing intact histological architecture

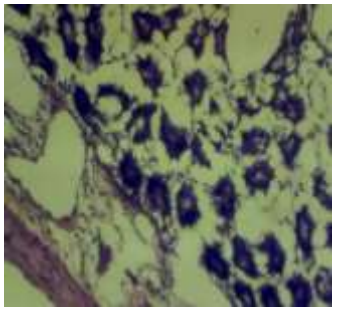
K



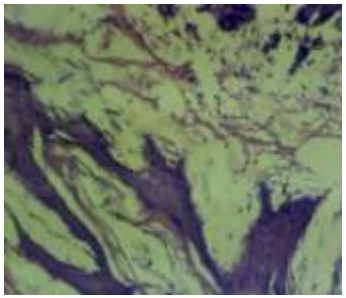
Photomicrograph of the Liver tissue showing no hepatic damage



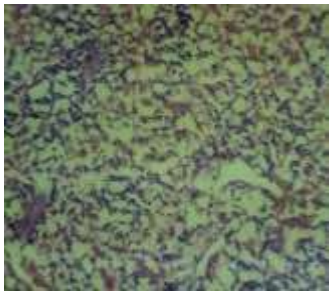
Photomicrograph of the kidney tissue showing intact histological architecture



No visible alteration in lamina propria and epithelial cells

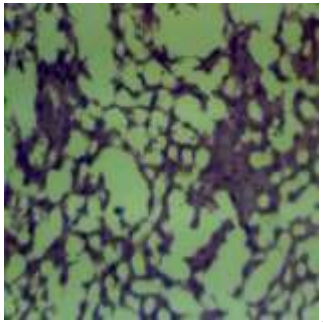


Photomicrograph of the Gastric mucosa tissue showing normal histological architecture with intact mucosal layers

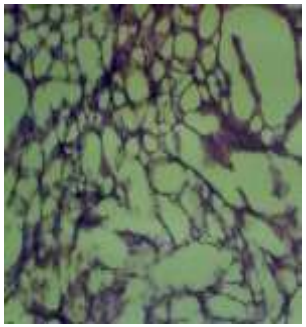


Photomicrograph of the spleen tissue showing normal trabecular artery muscle cells and infiltration of inflammatory cells

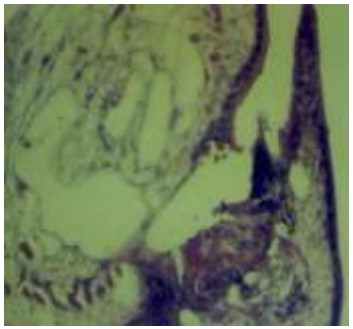
KF



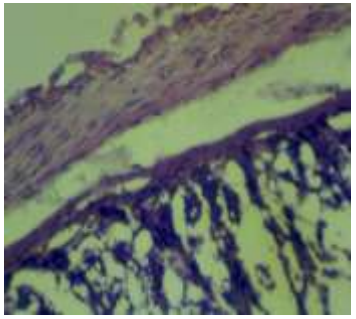
Unaltered hepatic cells



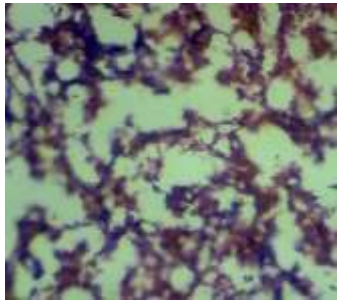
No Capsular and glomeruli degeneration



No visible alteration in lamina propria and epithelial cells

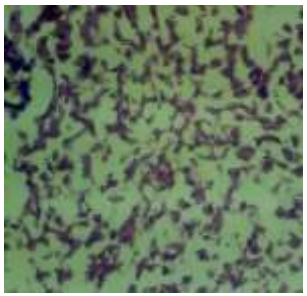


Photomicrograph of the Gastric mucosa tissue showing normal histological architecture with intact mucosal layers

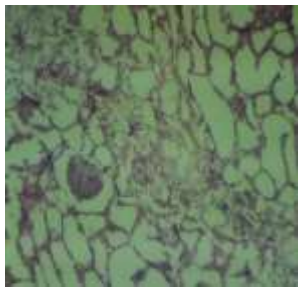


Spleen tissue showing intact histological architecture

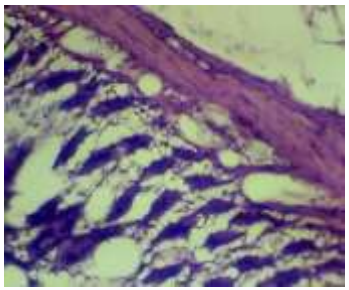
KC



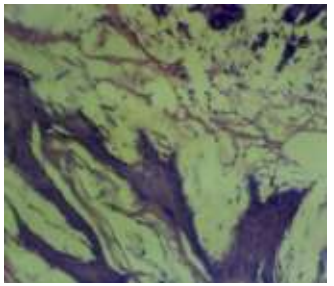
No significant hepatocellular damage



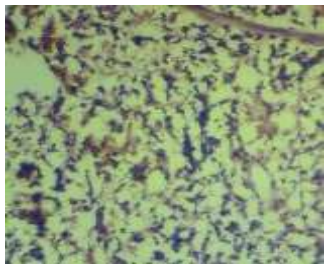
No significant cellular damage



Photomicrograph of the intestinal tissue showing normal histological architecture with intact Laminapropria and epithelial cells

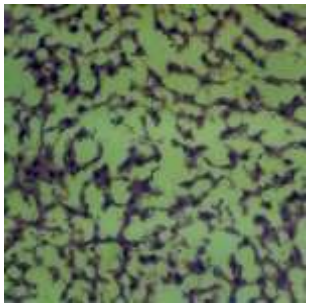


Photomicrograph of the Gastric mucosa tissue showing normal histological architecture with intact mucosal layers

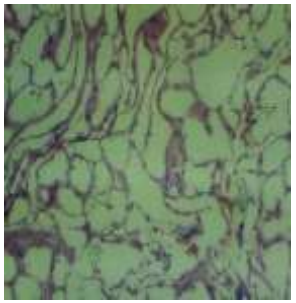


No visible alteration in histological architecture

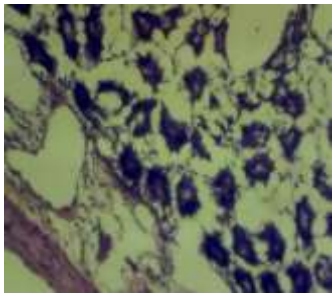
KL



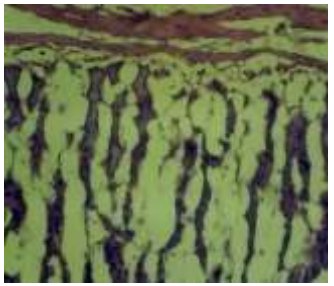
No cirrhosis or inflammation



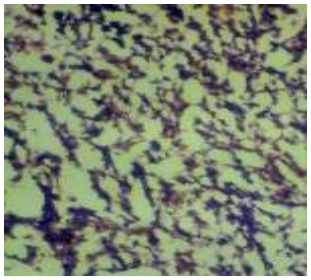
No significant cellular damage



No visible alteration in lamina propria and epithelial cells

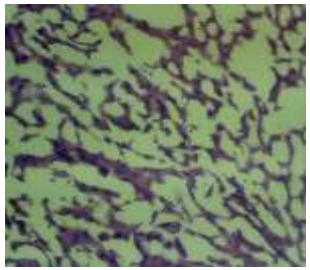


Gastric mucosa tissue showing normal histological architecture with intact mucosal layers

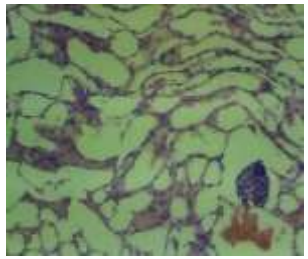


Spleen tissue Showing intact histological architecture

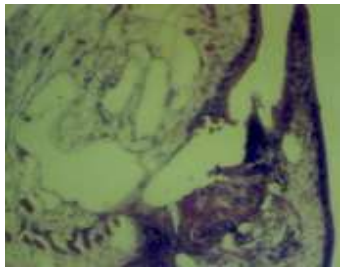
KFL



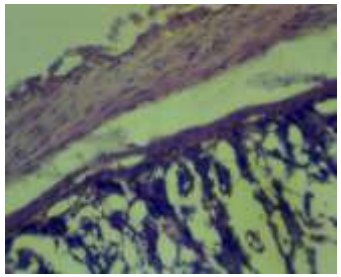
No significant hepatocellular damage



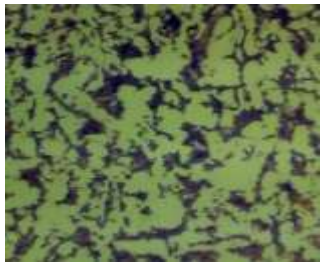
Intact nephrocytes and glomerulus no damage shown



No visible alteration in lamina propria and epithelialcells

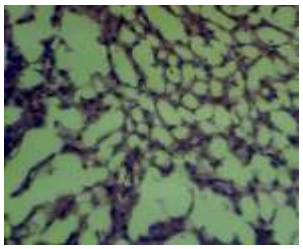


Photomicrograph of the Gastric mucosa tissue showing normal histological architecture with intact mucosal layers

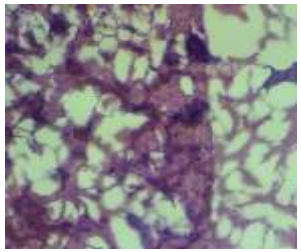


Spleen tissue showing unaltered histological architecture

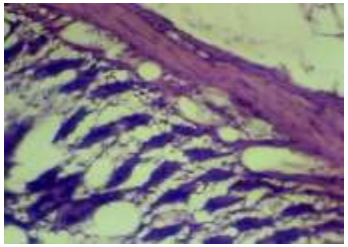
KCL



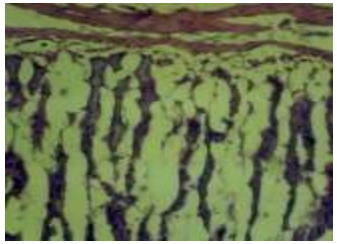
No significant hepatocellular damage



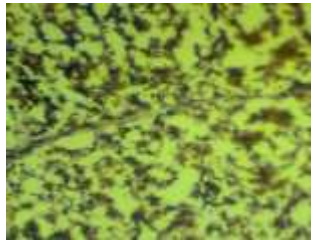
No significant alterations



Photomicrograph of the intestinal tissue showing normal histological architecture with intact Laminapropria and epithelial cells

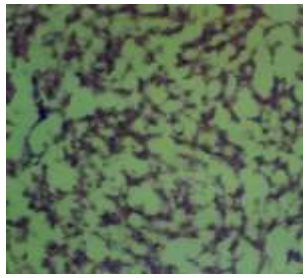


Gastric mucosa tissue showing normal histological architecture with intact mucosal layers

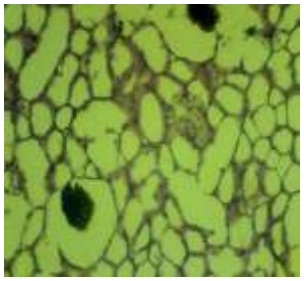


Spleen tissue showing unaltered histological architecture

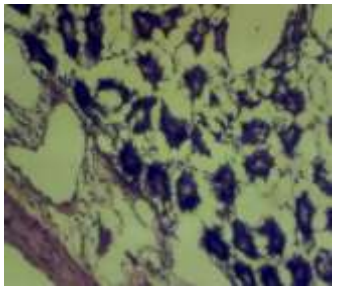
KMKT



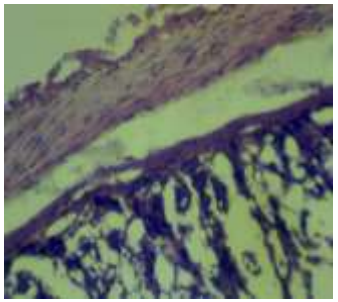
Photomicrograph of the liver showing intact hepatocytes



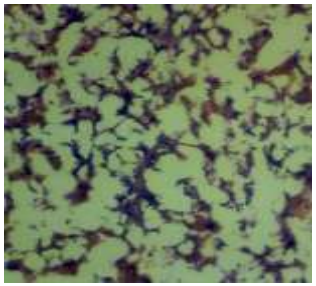
Intact glomerulus no significant damage shown



No visible alteration in lamina propria and epithelial cells



Photomicrograph of the Gastric mucosa tissue showing normal histological architecture with intact mucosal layers



No visible damage is shown in the histological architecture

Plate V: Photomicrographs of histological sections of rats fed with Rat feed, UK (Unfermented *Kulikuli*), K (fermented *Kulikuli);* KL (fermented *Kulikuli* and *Lactobacillus plantarum*); KF (fermented *Kulikuli and* all – purpose flour); KC (fermented *Kulikuli* and chia flour); KFL (fermented *Kulikuli*,all- purpose flour and *Lactobacillus plantarum*); KCL (fermented *Kulikuli*, chia flour and *Lactobacillus plantarum*) and KMKT (*Kulikuli* from the market).

4.2 Discussion

4.2.1 Biochemical characteristics of isolates from corn steep liquor

The investigation revealed that the isolates were catalase, citrate, urease, Voges Proskauer, oxidase negative. They utilized lactose, glucose, sucrose, fructose and sorbitol. Tawfik *et al.* (2022) identified similar organisms using these biochemical tests. The isolates were non- motile, Gram-positive, rod-shaped cells occurring in pairs or short chains. Fallo and Sine (2022) made similar observations using Gram stain and motility test to identify the organisms from Corn steep liquor.

4.2.2 Molecular identity of isolates from corn steep liquor

The isolate was identified as *Lactobacillus plantarum* now known as *Lactiplantibacillus plantarum* (Table 3) Adeyemo and Onilude (2014) and Ohaegbu *et al.* (2022) identified the same organism using16s rRNA.

4.2.3 Total microbial count of *Kulikuli* samples (*Kulikuli* from market and laboratory- prepared *Kulikuli)*

The total microbial count is one of the most important indices that reflect the quality of food products. Results from the present study showed that some of the *Kulikuli* samples analyzed were contaminated with microorganisms. The total plate counts for bacteria ranged from 0.0 x 10^4 - 3.00±0.00 x 10^4 CFU/g and fungal counts ranged from 0.0 x 10^4 – 2.00±0.00 x 10^4 CFU/g (Tables 4 and 7). The microbial counts of the *Kulikuli* from the market (KMKT) and Laboratory prepared *Kulikuli* samples were within the specified range of 10^4 – 10^6 CFU/g microbiological limits recommended for Ready- To-Eat foods such as *Kulikuli* by the International Commission on Microbiological specifications for foods, ICMSF (2018), Food and Agriculture Organization/ World Health Organization (FAO/WHO) (2005) and Centre for Food Safety Food and Environmental Hygiene Department, Microbiological guidelines for Ready-To-Eat

foods (2014). According to the International Commission for Microbiological Specification for ready-to-eat-food, a total viable microbial count between 0 to 10^3 CFU/g is regarded as acceptable, 10^4 to 10^5 CFU/g is tolerable and $\geq 10^7$ CFU/g is unacceptable (Omorodion and Odu, 2022).

Ajala *et al.* (2020) observed a total bacterial count ranging from 1.3± 0.10 x 10^3 to 4.10± 0.10 x 10^3 CFU/ml from *Kulikuli* which was similar to the findings in this study. Muhammad *et al.* (2020) reported a total bacterial count of 4.0 x 10^6 to 2.2 x 10^7 CFU/g for groundnut cake (*Kulikuli*), which was higher than the values obtained from this investigation. The total bacterial counts obtained by Ezekiel *et al.* (2011) and Oko *et al.* (2015) for *Kulikuli* samples were within the range,1.01 x 10^5 to 2.37 x 10^5 and 4.2 x 10^6 to 1.0 x 10^7 CFU/g respectively. The variations in the findings from this study with observations made by other researchers could be as a result of the differences in seed type, environmental factors (climate, soil type, storage conditions and method of processing) and level of hygiene practiced during the production and packaging of the samples (Musa, 2020; Idrissi *et al.*, 2022; Esan *et al.*, 2023).

The total fungal count of the *Kulikuli* in the present study was also within the recommended limit of 10^4 – 10^6 CFU/g set by the International Commission on Microbiological Specifications for foods (2018) and FAO/WHO (2005). Lower fungal counts of 1.5 x 10^3 –3.4 x 10^3 CFU/g were obtained by Odeniyi *et al.* (2019) and 1.00± 0.300 x 10^3 - 3.87± 0.10 x 10^3 CFU/g by Ajala *et al.* (2020) for *Kulikuli* samples respectively. Salau *et al.* (2017a) who studied the mycological quality assessment of groundnut products in Sokoto State reported 4.7 x 10^4 CFU/g, 1.5 x 10^4 CFU/g, 3.8 x 10^4 CFU/g and 3.2 x 10^4 CFU/g for groundnut cake (*Kulikuli*). Their findings were similar to some of the results of the fungal counts obtained from this study.

Fungi in this food product might have originated from the raw groundnut used in the individual groundnut cake processing as well as the postproduction exposure of these snacks to fungal spores resident in the air (Salau *et al.*, 2017a). The fermentation processes might have also increased the acidity of the sample which is known to favour fungal growth in food samples. Furthermore, some of the materials (chia seed and flour) added to the *Kulikuli* might have been infested with fungi during the processing stage. Some fungal species can survive under harsh conditions making them widely distributed and they produce spores that are heat resistant and allow for them to proliferate easily. In storage, some fungi cause physical and chemical changes in the tissues of the seeds, causing loss of lipids, carbohydrates, proteins, and increased fatty acid, besides influencing the seed's germination (Aswini *et al.*, 2022).

Fidelis *et al.* (2020) who studied the detection, transmission and pathogenic fungi in chia seeds isolated *Aspergillus* sp., attributed their presence to inadequate drying conditions and storage of seeds causing the reduction of grain. Similarly, species of *Aspergillus* and *Penicillium* were the most common genera of fungi isolated from some flour production sites, hence the isolation of some species of *Aspergillus* in this investigation (Bernardi *et al.*, 2019).

The results from this study indicate that not all the contamination of the finished product is ascribable to the unhygienic processing of *Kulikuli.* Only a marginal reduction in the level of contamination by microorganisms existed in the laboratory-prepared *Kulikuli* when compared with the samples obtained from the market. This could be because a major portion of the contamination could have already occurred during the preharvest, postharvest and pre- processing stages of the groundnut (Ayoade and Adegbite, 2016).

Unfermented *Kulikuli* (UK) samples and fermented *Kulikuli* (K) were free of microbial contamination. This may be due to the fact that the samples were prepared under

controlled environmental conditions, fermented *Kulikuli* and *Lactobacillus plantarum* (KL) recorded the highest fungal count. This may be due to the optimum pH (acidic) attained during the processes of fermentation which maybe favourable for the growth of fungi (Cui *et al.*, 2023).

The highest bacterial count was recorded in fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) with a bacterial load of 2 x10^4 CFU/g followed by 1.6 x 10^4 CFU/g recorded from fermented *Kulikuli* and all-purpose flour (KF). This combination increased the nutritional content of the samples, thereby creating an enabling environmental condition for the growth of bacteria (Ahmed *et al.*, 2022). Ajobiewe *et al.* (2022) also reported that microbial contamination in flour could result from the crop production chain (stages of pre- harvest, harvest, transportation, storage and processing). Furthermore, different weather conditions such as precipitation and relative humidity level, as well as specific field microflora can influence the type and amount of microbial load. *Escherichia coli*, *Bacillus cereus, Aspergillus flavus and A. niger* were the microorganisms isolated from the microbiological examination of wheat flour (Ajobiewe *et al.*, 2022), which shares some similarity with some of the microbes obtained from this investigation.

4.2.4 Bacterial isolates from *Kulikuli* samples

The organisms isolated from this study include *Proteus* sp., *Staphylococcus aureus, Bacillus* sp., *Micrococcus* sp., *Klebsiella pneumoniae* and *Enterobacter* sp. *Proteus* species has been frequently isolated from food and can be conveyed into food, drink or water by vectors or faecally-contaminated handlers who maintain a low level of hygiene (Miller *et al.*, 2021). *Proteus* sp. was isolated from *Kulikuli* in the previous studies undertaken by Ezekiel *et al.* (2011) and Ayoade and Adegbite (2016). The microbe infects the human lower respiratory tract and also cause nosocomial urinary and septic

infections (Wasfi *et al.*, 2020; Hasan *et al.*, 2021). They are widely distributed in nature as saprophytes, being found in decomposing animal matter, sewage, manure soil, the mammalian intestine and human and animal faeces (Nwachukwu *et al.,* 2022). Severe cases can also lead to sepsis (blood poisoning). In recent years, this pathogen has increasingly developed resistance to common antibiotics (including beta-lactams), which makes it difficult to combat the infections it causes (Mokrani *et al.*, 2023).

Staphylococcus aureus was the second most prevalent bacterium isolated from the *Kulikuli* samples screened in this study. The detection or presence of *S. aureus* in ready-to- eat foods is strong evidence of poor handling during food processing and packaging. *Staphylococcus aureus* are pathogenic microorganisms and have been isolated from *Kulikuli* (Ajala *et al.*, 2020; Muhammad *et al.*, 2020*;* Galadima, 2021). *Staphylococcus aureus* can survive in the potentially dry and stressful habitat. They are also part of the normal flora of the human skin (Saunte *et al.*, 2020), the throat and nose of the producer or seller of the *Kulikuli. Staphylococcus aureus* infections range from mild skin infections to life- threatening ones; such as bacteremia, endocarditis, necrotizing pneumonia, toxic shock syndrome and food poisoning (Roetzer *et al.*, 2022). *Staphylococcus aureus* gets access to foods through food handlers and processing equipment. Although bacteria can be found on the skin of animals, water, soil, and other surfaces, bacteria from food handlers and other human sources are thought to be the major contributors to food poisoning (Pal *et al.,* 2022). Staphylococcal food poisoning is a major public health concern. Although staphylococcal food poisoning is generally self-limiting and resolves within 24-48 hours of onset, it can cause life-threatening infections in children, the elderly, and immunocompromised people. *Staphylococcus aureus* infections cause significant morbidity and mortality, it accounts for up to one-third of foodborne gastrointestinal illnesses (Pal *et al.*, 2022).

Bacillus sp. was another microbial contaminant of the *Kulikuli* samples in this study. A high incidence of *Bacillus* sp. was reported in the microbiological assessment of selected groundnut- based snacks '*Kulikuli'* and 'donkwa' in Ogbomosho and Ilorin (Ajala *et al.*,2020). Oko *et al.* (2015) reported the presence of *Bacillus* sp. in groundnut cake sold in an open market in Samaru, Zaria-Kaduna State. The presence of this organism in food products is likely evidence of poor handling, poor quality of raw materials or poor temperature control. Diseases like septicemia/ bacteremia, endocarditis and respiratory tract infection are caused by *Bacillus* sp., they are abundant spore formers in air, water, dust, soil, bodies of insects, animals and humans who handle the groundnut during planting, processing, production and packaging (Muhammad *et al.*, 2020). This particular microbe is not completely inactivated by heat, thus causing spoilage or health risks if the conditions are favourable for their germination. They can also serve as reservoirs for the transmission of antibiotic-resistant genes hence increasing their pathogenicity (Muhammad *et al,* 2020; Badul *et al,* 2021; Bowley *et al.*, 2021).

This study also revealed the presence of *Micrococcus* sp. in the *Kulikuli* samples. Similarly, Ajala *et al.* (2020) isolated *Micrococcus* sp. in selected groundnut-based snacks in Ogbomosho and Ilorin. This group of microorganisms are harmless saprophytes present on animal and human skin (Bello and Echevarría, 2023). They are also found in many other places in the environment, including water, dust, and soil. *Micrococcus* is generally thought to be a commensal organism, though it can be an opportunistic pathogen, particularly in hosts with compromised immune systems, such as HIV patients. It can be difficult to identify *Micrococcus* as the cause of an infection, since the organism is normally present in skin microflora, and the genus is seldom linked to disease. In rare cases, death of immunocompromised patients has occurred from pulmonary infections caused by *Micrococcus* sp. Micrococci

may be involved in other infections, including recurrent bacteremia, septic shock, septic arthritis, endocarditis, meningitis, and cavitating pneumonia in immunosuppressed patients (Garcia-Gonzalez and Hernandez, 2022).

Though not a spore former, *Micrococcus* cells can survive for an extended period both at refrigeration temperatures and in nutrient-poor conditions. Micrococci can grow well in environments with little water or high salt concentrations which might account for its presence in the *Kulikuli* samples.

Enterobacter sp. was isolated from some of the *Kulikuli* samples, it is an indication of faecal contamination which might have resulted from the water used during sample preparation (Manzanas *et al.*, 2023). *Enterobacter* sp. was reportedly isolated from peanut cake (*Kulikuli)* sold in Nigerian Markets (Ezekiel *et al.*, 2011). The genus *Enterobacter* is a member of the coliform group of bacteria. It does not belong to the fecal coliforms group of bacteria, unlike *Escherichia coli*, because it is incapable of growth at 44.5 °C in the presence of bile salts.

Some strains of these bacteria are pathogenic and cause opportunistic infections in immunocompromised (usually hospitalized) hosts and in those who are on mechanical ventilation. The urinary and respiratory tracts are the most common sites of infection. *Enterobacter* are ubiquitous; their presence in the intestinal tracts of animals results in their wide distribution in soil, water, and sewage (Moxley, 2022). They can also be found in plants. In humans, multiple *Enterobacter* species are known to act as opportunistic pathogens including *E. cloacae*, *E. aerogenes*, *E. gergoviae*, and *E. agglomerans*. Pathogenic *Enterobacter* can cause any of a variety of conditions, including eye and skin infections, meningitis, bacteremia (bacterial blood infection), pneumonia, and urinary tract infections.

Klebsiella, although found in the normal flora of the mouth, skin and intestines, they can

also be found in the soil, water, plants and other vegetation. Ezekiel *et al.* (2011) and Oko *et al.* (2015) also reported the isolation of *Klebsiella* sp. from peanut cake in Nigerian Markets and groundnut cake sold in an open market in Samaru, Zaria-Kaduna State respectively. It can cause destructive changes to human and animal lungs if aspirated, specifically to the alveoli resulting in bloody, brownish or yellow coloured jelly sputum (Perelomov *et al.,* 2022). In recent years, *Klebsiella* species have become important pathogens in nosocomial infections. The capsule on the organism increases its virulence, acting as a physical barrier to easily evade the hosts' immune system and the capsule protects the cell from desiccation. *Klebsiella* is one of the leading pathogens of septicemia, bacteremia, urinary tract infection, diarrhoea and pneumonia (Anastasia *et al.*,2023). Infections caused by this microbe are spread from person to person through contact by the contaminated hands of people in hospitals. It is an agent of hospital-acquired infections and community-acquired bacterial pneumonia. The presence of *Klebsiella* sp. in food serves as a potential food hazard because the gastrointestinal route is considered one of the main routes of infection of the pathogen.

4.2.5 Fungal isolates from *Kulikuli* samples

The fungal isolates identified were *Aspergillus niger, A. flavus, A. fumigatus, A. terreus* and *Trichophyton* sp. According to Adebesin *et al.* (2001) and Boli *et al.* (2013), the mycoflora commonly associated with groundnut products belongs to the genera *Aspergillus*, *Penicillium*, *Rhizopus* and *Fusarium*. These fungal species are reported to be commonly associated with raw materials such as groundnut, chia seeds and spices amongst others in their product preparation, packaging or storage of finished products (Nguyen *et al.,* 2023). Fungi are ubiquitous, they can be found in the air, and soil and a few of them live only in aquatic habitats.

They interact with other organisms by either forming beneficial or mutualistic

associations (mycorrhizae and lichens) or by causing serious infections (Vitte *et al.*,2022).

The presence of *Aspergillus* species in the analyzed *Kulikuli* samples pose a toxicological threat to the consumers since the majority of the strains of these fungal species are toxigenic. The species of these fungi are abundantly distributed in the soil, water and air, and they can easily contaminate exposed or improperly handled food products. Some of these species release toxic metabolites known as mycotoxins under certain environmental conditions. Mycotoxins are secondary metabolites secreted by fungi which produce toxic responses when ingested by animals or humans (Alsalabi *et al.*, 2023).

Mycotoxicosis is a term used to denote the intoxication that results from the ingestion of mycotoxin by animals and humans. Different types of mycotoxins have been reported, but the agro-medically important types include aflatoxins, citrinin, fumonisins, ochratoxin A, trichothecenes, tremorgenic toxins, ergot alkaloids, zeareleone and patulin. Aflatoxins are mycotoxins produced by *Aspergillus* sp. (Badmos *et al.*, 2021; Popoola *et al.*, 2021). that grow in oil seeds and cereals which could be carcinogenic, hepatoxic and teratogenic. Vomiting, abdominal pain, coma, convulsion and even death are associated with acute aflatoxicosis in humans (Aljamali, 2021; Sitena, 2021).

The incidence of the aflatoxigenic fungi, *Aspergillus* in food products can result from the raw material (groundnut, flour and chia seed), in the spore forms, and materials used in processing, packaging or storage methods (Abdul-Lateef *et al.*,2020). Odeniyi *et al.* (2019) also stated that this may also lead to the deterioration of the food material and could produce spores which aids in their survival. By the virtue of their immediate handling, packaging and storage conditions, groundnut products are usually exposed to the environment and these may contribute to their fungal contamination (Odeniyi *et al.*,

2019).

Trichophyton species cause various forms of fungal infection of the skin known as dermatophytosis (Nikkholgh *et al.,* 2022). These species are usually found on the skin, hair and nails of infected persons while some of these species exist in the soil developing on soil- decomposing keratinous materials. They may cause both human and animal infections. They might have contaminated the samples from the body of the handlers or the environment and the spores produced by this group of fungi are usually extremely difficult to eliminate (Tokarski *et al.*, 2022).

Fungal infestation of food commodities results in spoilage and is associated with the production of off-colours and flavours, rancidity and discolouration, which can lead to the deterioration of these food commodities as manifested in loss of weight, nutritional value and toxicity due to the production of mycotoxins (Fidan *et al., 2022*). The infestation and the intensity of damage are found to be closely linked to agroclimatic conditions including the storage methods and the ambient temperature and humidity (Neya *et al.*, 2023).

4.2.6 Frequency of occurrence of isolates

Six (6) bacterial species were identified in this study amongst which *Proteus* sp. was highest with 35.71%, *Staphylococcus aureus* 21.43%, *Bacillus* sp. (14.29 %), *Micrococcus* sp. (14.29 %). The least frequently isolated organisms were *Klebsiella pneumoniae* (7.14 %) and *Enterobacter* sp. (7.14%) (Table 8a). The findings are consistent with that of Muhammed *et al.* (2020) who isolated similar organisms from *Kulikuli*. Some of the identified microorganisms are of public health interest due to their ability to cause harm to human life (Delorme *et al.*, 2020). Due to its high nutritive content, peanut cake in Nigeria is prone to contamination by a wide variety of microorganisms including many bacterial species ranging from simple commensals to

pathogenic types and fungal organisms Ayoade and Adegbite, (2016). Among the bacterial contaminants are *enterobacteriaceae*, a group of Gram-negative intestinal bacteria that are extremely pathogenic to man and animals (Heroes *et al.*, 2023). *Enterobacteriaceae* are used to access the general hygienic status of food products. All *Enterobacteriaceae* are known to be killed by heat used in food production. Their presence in heat-treated food such as *Kulikuli*, therefore, signifies inadequate cooking temperature (*Proteus* and *klebsiella* have been known to withstand temperatures as high as 45 °C) or post-processing contamination (Zafar *et al.*, 2022).

The percentage occurrence of *Aspergillus niger* was 33.33%, followed by *A. flavus* (22.22%), *Trichophyton* sp. (22.22%), *A. fumigatus* (11.11%) and the least was *A. terreus* (11.11%). Salau *et al.* (2017b) and Ajala *et al.* (2020) isolated *Aspergillus flavus*, *A. fumigatus* and *A. niger*. The majority of the fungal isolates identified in this study produce mycotoxins. The presence of *A. niger*, *A. flavus* and *A. fumigatus* in the investigated *Kulikuli* samples is of great health concern because, these microorganisms can thrive in environments with low moisture activity, making them important in post-harvest contamination.

4.2.7 Nutritional composition of the *Kulikuli* samples

From the current study, the moisture content of the *Kulikuli* obtained from the market and laboratory-prepared *Kulikuli* samples differ significantly ($p \geq 0.05$). The Unfermented *Kulikuli* (UK) had the lowest moisture content (2.71±0.08) while fermented *Kulikuli* (K) (4.60±0.14) had the highest. The higher moisture content observed in the fermented *Kulikuli* sample could be as a result of soaking during fermentation and the temperature of the fermenting medium (Ojokoh *et al.*, 2020).

Moisture content recorded in this study were lower than those reported by Abdul-Lateef *et al.* (2020) (6.64±0.3- 11.91±0.78) and Musa, (2020) (6.36±0.17 and 6.90±0.1) in *Kulikuli* samples. The observed difference in values could be attributed to the initial

dryness of the groundnut seeds and the season the study was conducted, as food items tend to contain more moisture during the rainy season due to increased atmospheric humidity (Achimugu and Okolo, 2020). The low moisture content ascertained from the resultant data could be due to the deep frying of snacks, which can enhance the keeping quality of the products and prevent rancidity (Adeyeye *et al.*, 2020).

The protein content of the controls, unfermented *Kulikuli* (UK) (27.09±0.40) and *Kulikuli* from the market (KMKT) (31.21±0.49) were significantly different ($p \geq 0.05$) from one another and were higher than the protein contents observed from the other fermented *Kulikuli* samples. The protein contents obtained from this study were lower (31.21± 0.49, 27.09±0.40, 23.35±0.30, 25.00±0.05, 30.07± 0.08, 24.87±0.85, 22.53±0.25 and 26.99±0.05) than those observed by Odeniyi *et al.* (2019) (34.95± 0.01, 40.45±0.03, 40.43±0.20 and 43.93±0.05) and Abdul-Lateef *et al.* (2020) (43.18±9.44 - 59.23±1.43) for *Kulikuli* samples. This variation in values may be as a result of the different seed cultivars, storage conditions, processing methods and environmental factors including climate, season and soil (Musa, 2020). Fermentation is one of the best food processing techniques that can improve the protein levels of legumes, however previous literature has reported a decrease in protein levels which could be attributed to the different study durations, raw materials and experimental designs (Abdallah *et al.*, 2023).

After fermentation, the test *Kulikuli* samples had protein values ranging from 23.35±0.03 to 26.99±0.05. The reduction in the protein contents of the fermented *Kulikuli* samples could be attributed to the fermenting microorganisms utilizing the amino acids which lowered the protein content and quality of some fermented foods. Moreso, the effect of fermentation on proteins has yielded inconsistent reports due to variations in the initial protein or amino acid profile of foods, study duration and varying experimental designs (Nkhata *et al.* 2018; Karimi *et al.*, 2023).

The unfermented *Kulikuli* (UK) and *Kulikuli* from the market (KMkt) were low in fibre content (0.68±0.82) and there was no significant difference between them at ($p \geq 0.05$), this data is comparable to Musa (2020) who reported 0.565±0.1 for the *Kulikuli* made from Espanola (Yar madali) specie of groundnut consequently higher than the values of 1.0±0.1 for Kresting (Kampala) and 1.06±0.14 for Malagache (Bahusa) species of groundnut for the same study. However, the values obtained from this study were lower than the Standards Organisation of Nigeria (SON, 2015) limit of 2.0%-4.0%.

The fibre content of the laboratory-prepared *Kulikuli* samples were relatively lower than the *Kulikuli* from the market (KMKT) except for fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) with a fibre content of 0.86±0.05. The decrease in fibre could be attributed to the release of extracellular enzymes by microbes that hydrolyze and metabolize insoluble polysaccharides. Also, the decrease in fibre content after fermentation is a sign of the softening of the fibrous tissues and increases the digestibility due to the activities of the microorganisms which is known for the bioconversion of carbohydrate and lignocellulose in protein (Lavanya *et al.*, 2023). Diets low in fibre are undesirable as it could lead to constipation and have been associated with diseases of the colon like piles, appendicitis and cancers (Adeyeye *et al.*, 2020; Musa, 2020).

Lipid content is of immense importance in diets as it is known to promote fat-soluble vitamin absorption. The lipid content observed in unfermented *Kulikuli* (30.86±0.15) and *Kulikuli* from the market (KMkt) (31.41±0.02) were high and did not differ significantly from one another at ($p \geq 0.05$). An increase in lipid value was observed in all the fermented *Kulikuli* samples except for fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL), this sample showed no significant difference from both controls.

Odeniyi *et al.* (2019) reported a similar value range of (28.41±1.20 - 33.51±0.03) for vended groundnut. The high lipid content could be attributed to the high oil content of groundnut (Emelike and Akusu, 2018). Lower lipid contents were observed in the findings of Abdul- Lateef *et al.* (2020) (3.26±0.37 - 4.83±0.19) and Musa (2020) (5.5 ±0.1 -7.46±0.1).

The increase in lipid content may be attributed to the heat treatment of legumes which disrupted the lipid bodies of the peanut expelling more oil (Rapando *et al.* 2020). One of the binding agents employed in this study, chia seed powder contains mainly polyunsaturated fatty acid (Linoleic and Linolenic acid) which increases the saturated fat at high temperatures (Nyingi and Mburu, 2021; Rashid *et al.*, 2021). Nonetheless, lipid plays a significant role in the shell life of food as it promotes rancidity (Bhunia *et al*., 2023). Nonetheless, the lipid content of the *Kulikuli* samples were higher than the SON specified limit of (20.0).

The ash content of the unfermented *Kulikuli* (UK) (4.53±0.05) and *Kulikuli* from the market (KMkt) (3.69±0.04) were significantly different ($p \geq 0.05$). These values are in line with the reports of Emelike and Akusu (2018) which ranged from (3.19±0.29 - 4.63±0.07). Odeniyi *et al.* (2019) also reported a value of (3.46±0.03 - 6.17±1.20). The high ash content is a reflection of the mineral content preserved in the groundnut (Okolo and Makanjuola, 2021; Oyeyinka *et al.* 2021) and the obtained values are in line with the SON (2015) specification. However, the ash content in this study decreased significantly at ($p \geq 0.05$) after fermentation of the test *Kulikuli* samples with a value of (3.47±0.09 - 4.52±0.00). The decrease in the ash content of the laboratory-prepared *Kulikuli* samples (fermented) could be due to the general activities of the fermenting microorganisms (breaking down of substrate into absorbable forms) (Ahnan-Winarno *et al.*, 2021; Ibrahim *et al.*, 2021). Low ash contents serve as an indication of the low level

of organic impurities and this qualifies the test sample as a good mineral source (Santana *et al.*, 2020).

The changes in the carbohydrate content of the test *Kulikuli* differed significantly at ($p \geq 0.05$) from each other. A significant difference existed at ($p \geq 0.05$) between controls unfermented *Kulikuli* (UK) and *Kulikuli* from the market (KMkt) with recorded values of 36.33±0.03 and 29.77±0.28 which were comparable to Musa (2020) who recorded 30.54±00 and 31.07±0.00 while Odeniyi *et al.* (2019) had a lower value of 19.41±0.04 - 24.35±0.03. The carbohydrate content of groundnut indicates that it could supplement for the energy required for some daily activities (Muhammad *et al.*, 2020). The fermentation process led to an increase in the carbohydrate content of two (2) fermented *Kulikuli* samples fermented *Kulikuli* and chia flour (KC) (37.46±0.25), fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) (39.52±0.15) which were higher than the 36.33±0.03 for unfermented *Kulikuli* (UK) (one of the controls) while the other 4 samples were lower in carbohydrate value (27.56±0.64 - 31.44±0.11).

The increased carbohydrate content is in contrast with several works in the literature that reported decreased levels of carbohydrates with fermentation. This could be due to the reduced moisture content of the fermented test *Kulikuli* samples (Loo *et al.*, 2023) and the addition of the chia flour which contains 39-41% of carbohydrates with a substantial number of polyphenols. Owing to this composition, they tend to formulate mucilage (Din *et al.*, 2021). This mucilage serves as a source of polysaccharides and can also be used industrially to produce polymer blends for consumable coating and films (Nyingi and Mburu, 2021) while the diminution in carbohydrates is attributed to the starch hydrolyzing enzymes such as α-amylase and maltose which have starch degrading abilities in maltodextrins and simple sugars. The glucose released during fermentation is the preferred substrate for microorganisms and further explains the decrease in

carbohydrate (Ayodele *et al.*, 2023).

4.2.8 Antinutritional composition of *Kulikuli* Samples

For the tannin content, an increase in value was observed in fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL), fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL), fermented *Kulikuli* and all-purpose flour (KF) and fermented *Kulikuli* and *Lactobacillus plantarum* (KL) exhibited increased tannin content. This is similar to the observation of Olapade *et al.* (2021) who reported an increase in tannin content in fermented tiger nut flour. The increase in tannin during fermentation may be attributed to the hydrolysis of condensed tannins (proanthocyanidins). Tannins bind minerals and reduce their bioavailability depending on the duration of fermentation. This result agrees with the findings of Omeje *et al.* (2021) who reported higher values of tannins (25.18-52.28mg/100g) in fermented soybean and roasted Moringa and Duhan *et al.* (2021) who observed an increase in tannin content from 21.33mg/g to 245.33 mg/g in fermented peanut press cake. Prolonged fermentation decreases the tannin contents as a result of the enzyme phenyl oxidase activity. The transformation of tannins to phenols which takes place during fermentation increases the phenol content that interacts with minerals leading to the hindrance of mineral bioavailability (Olapade *et al.* (2021). Also, the hydrothermal treatment of seeds before fermentation breaks protein–polyphenol complex, resulting in higher leaching of water-soluble compounds in steep water such as tannins (Villacres *et al.*, 2020). The fermented *Kulikuli* and chia flour (KC) showed a slightly lower tannin value than Unfermented *Kulikuli* (UK) (17.54 mg/100g) whereas *Kulikuli* from the market (KMKT) (16.65 mg/100g) was the lowest. This reduction of tannins after heat application could be because these compounds in addition to their predominance in seed coats are water soluble and consequently leached into the processing medium**.** It is

however imperative to note that the toxicity effects of tannins depend upon their chemical structure and dosage since the total acceptable tannic acid daily intake for a man is 560 mg/100g as reported by Makinde and Abolarin, (2020).

The fermented *Kulikuli* and chia flour (KC) had the highest phytate content of 94.86mg/100g which is lower than the phytate content of 828.5 mg/100g in fermented maize observed by Anaemene and Fadupin (2020). All the test *Kulikuli* samples showed an increased phytate content except for fermented *Kulikuli* and all-purpose flour (KF) (12.60 mg/100g). This could be as a result of the microbial activities during fermentation, a wide range of microflora have been known to possess phytate activity which could be responsible for the reduction in the phytate content of the fermented samples (Olapade *et al*., 2021). A similar observation was reported by Yakubu *et al*, (2022) on fermented locust beans from 12.48 mg/g- 4.95 mg/g after 72 hours of fermentation at 38 °C.

The fermented *Kulikuli* and all-purpose flour (KF) had the least phytate content of 12.60mg/100g which was higher than the values of 5.98 mg/100g, 4.45mg/100g and 4.90mg/100g for Bambara groundnut reported by Adebiyi *et al.* (2019). The differences may be due to the different geographical locations and the soil on which they were cultivated or as a result of the method of processing as well as the difference in cultivar (Kakagida *et al.*, 2021). The observed reduction in the phytic acid content of legume seeds during heat treatment may be partly due to the heat liable nature of phytic acid. Heat processing inactivates heat-sensitive phytic acid, however, phytic acid is more tolerant of heat cooking than other antinutritional factors. On a positive note, there is evidence that dietary phytate at low levels may have a beneficial role as an antioxidant, and anti-carcinogen and may likely play an important role in controlling hypercholesterolemia and atherosclerosis (Makinde and Abolarin *et al.*, 2020).

The Unfermented *Kulikuli* (UK) had the highest oxalate content of 0.08/100g than the other *Kulikuli* samples evaluated. This value is similar to the oxalate content of 0.08 mg/100g and 0.05mg/100g for 14 and 21 days of *Jatropha curcas* kernel fermentation recorded by Okomoda *et al.* (2020). Samples of fermented *Kulikuli* and all-purpose flour (KF), fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) and fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) showed significantly lower values of 0.03, 0.03 and 0.04. The depletion in the level of oxalate as a result of processing is comparable with the findings of Omeje *et al.* (2021) for fermented soybean and roasted moringa seed flour blends (3.71 mg/100g- 1.84mg/100g) and Sandya *et al.* (2021) for *Lactobacillus plantarum* fermentation to reduce anti-nutritional contents in peanut, mustard and sesame (700 mg/g – 300mg/g). The *L. plantarum* has been proven to produce oxalate decarboxylase, an oxalate degrading enzyme. Oxalates can have a harmful effect on human nutrition and health by binding with calcium and magnesium, interfering with their metabolism and subsequently causing muscular weakness and paralysis.

The total intake of oxalate in the human diet should not exceed 50–60 mg per day as indicated by Makinde and Abolarin (2020). The antinutritional content of *Kulikuli* samples varied significantly from each other after 72-hour fermentation with a value range of 19.84% - 58.04%. fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) (19.8mg/100g) had a lower saponin content than Unfermented *Kulikuli* (UK) (26.27%) which could be due to the processing of fermentation which helps to reduce the anti-nutritional factors. This in turn helps to enhance digestibility and nutritional value. The antinutrients have been shown to have both adverse and beneficial effects on humans. Saponin has been shown to have both beneficial and deleterious properties and to exhibit structure-dependent biological activities (Omeje *et al.*, 2021).

However, an increase in saponin content was observed in the other *Kulikuli* samples fermented *Kulikuli*(K), fermented *Kulikuli* and *Lactobacillus plantarum* (KL), fermented *Kulikuli* and all-purpose flour (KF), fermented *Kulikuli* and chia flour (KC) and fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL).

A high concentration of saponins can lead to a decrease in mineral and vitamin bioavailability and an astringent or bitter taste of the products (Pontonio *et al.*, 2020). Omeje *et al.* (2021) who worked on fermented soybean and roasted moringa seed flour blends reported lower saponins values than the present study with a value range of (1.74-2.69%) as well as 1.14% and 1.50% by Olapade *et al.* (2021) for freshly fermented tiger nut and dry fermented tiger nut respectively. Pontonio *et al.* (2020) also reported a value of 32.9% for yoghurt which could be compared to 35.05% of fermented *Kulikuli* (K) in this study. An upsurge of the saponin content in bambara groundnut (*Vigna Subterranean* L) enriched bio-fortified gari by Odunsi (2021) increased from 1.72 mg/g to 3.39 mg/g, this could be due to an increase in the temperature of the fermenting jar (Yakubu *et al.*, 2022).

The cyanide content in the *Kulikuli* samples evaluated differed significantly from each other at ($p \leq 0.05$). The fermented *Kulikuli* and *Lactobacillus plantarum* (KL) had the highest cyanide content of 513.57 mg/100g while fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) (3.60 mg/100g) had the lowest.

Damayanti *et al.* (2021) who studied fermentation of cyanide reported cyanide contents of 75.57 mg/100g with inoculum and 78.09 mg/100g without inoculum which is comparable to fermented *Kulikuli* (K) with 72.60 mg/100g. The low cyanide content could be attributed to lactic acid fermentation which could aid in the increment of the nutritional profile and enhance the bioavailability of vitamins, minerals, and other nutrients (Rodríguez-España *et al.*,2022). This is achieved by hydrolyzing coatings and

cell walls which are indigestible both chemically as well as physically, resulting in the release of nutrients trapped within the plant's complex structure (Sharma *et al.*, 2020).

Adeoye *et al.* (2020) reported a cyanide content of 2.93mg/100g - 3.71mg/100g for enriched protein meals. These values are comparable to the cyanide content of 3.60mg/100g obtained from fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL). The cyanide content is due to the presence of cyanogenic glucosides, linamarin contained in cassava tuber which is the source of the cassava flour present in the composite flours (Adeoye *et al.* 2020). Lower cyanide contents ranging from 0.13mg/100g - 1.62 mg/100g were revealed from the findings of Sobowale *et al.* (2021) and Okomoda *et al.* (2020) who reported a reduced cyanide content of 0.23mg/100g – 0.07mg/100g over a 3 weeks fermentation period of *Jatropha curcas*. Agbara and Chikaodili (2020) also reported a cyanide content of 0.11 mg/100g - 0.34 mg/100g for cassava gari supplemented with melon and furthermore, a cyanide value range of 1.63-2.32 mg/100g was reported by Igbua *et al.* (2020) for the antinutrients in maize, cassava and soybeans.

Microbial fermentation can enhance the nutritional value as well as the digestibility of plants with low nutrient bioavailability, however, microbial enzymes display maximum activity under environmental conditions that are favourable for their growth and metabolic function such as temperature ranges varying between 22°C and 25°C under acidic conditions, duration of the study and varying experimental designs. Hence the observed inconsistent reports from several works of literature (Manzoor *et al.*, 2021).

4.2.9 Physicochemical properties of *Kulikuli* samples

Free fatty acids (FFA) are produced as a result of the hydrolysis process that occurs due to heat and exposure to water (Salih *et al.* 2021). The FFA of the controls (Unfermented *Kulikuli* (UK) and *Kulikuli* from the market (KMKT) were 7.64 mg KOH/g and 6.75mg

KOH/g which is comparable to the value of 6.46 mg KOH/g obtained by Balogun *et al.* (2020) but was higher than oil extracted from bread and oil extracted from chia seeds with values of 3.41mg and 0.85mg KOH/g reported by Kowalski *et al.* (2020).

An increase in FFA was observed in the laboratory-prepared *Kulikuli* samples. The presence of a high amount of FFA can facilitate the lipid oxidation rate and the difference in values may be due to the source of groundnut and the method of processing (Balogun *et al.*, 2020). Data illustrated by Zahran and Tawfiek (2019) indicated the acceptable level of FFA in a frying medium cannot be higher than 2.5mg KOH/g of fat. The values obtained from this research were lower than the acceptable value and ranged from 7.50-9.41mg KOH/g for the initial value of day 0 and to (8.32-10.32) mg KOH/g by week 3.

Peroxide value (PV) is an indicator of peroxidation and a high peroxide value of a sample is a sign of weak oil resistance and can be a sign of rapid deterioration (Zahran and Tawfiek, 2019). Initially, the PV of the control was 2.67 meq/kg which was higher than bleached palm oil (1.10 meq/kg), Margarine (2.60 meq/kg) and Lard (1.25 meq/kg) presented by Makanjuola and Adepegba, (2020) for the evaluation of the quality of bread produced from bleached palm oil, margarine and lard. However, Balogun *et al.* (2020) reported a higher value of 5.66 meq/kg for the effect of potash on *Kulikuli*.

After 3 weeks, the PV for all the laboratory-prepared *Kulikuli* samples increased, these findings correlate with the research of Mansour *et al.* (2020) who reported an increment of (1.17-1.51) meq/kg after a 6 weeks storage period. In another study, fungi and Lactic acid bacteria were employed on flaxseed oil cake by Lopusiewicz *et al.* (2020) who reported a PV of (4.59 –12) meq/kg and (6.89 - 23.34) meq/kg after 3 weeks of storage. The higher PV in the flaxseed cake could be due to the high proportion of unsaturated fatty acid compared to peanuts (Zahran and Tawfiek, 2019). The FAO/WHO (2005) recommended limit for PV is ≤ 10 meq/kg, so the values obtained from this storage are

within the recommended limit. Oils with high PV are unstable and become easily rancid (Makanjuola and Adepegba, 2020). The result from this study indicates that the fermented *Kulikuli* is rancidity free and suitable for consumption, although, with the progressive increase, it would become rancid with a longer storage time which in turn decreases the quality of the *Kulikuli* sample. Afolabi *et al.* (2018) stated that the increase in PV with increased storage is due to an interaction with oxygen, high heat or sunlight.

The high iodine value in the *Kulikuli* in this research showed a high content of unsaturated fatty acids which could be confirmed by the fatty acid profile and this could be attributed to the fatty acid content including the unsaturated ones due to the chia seed content are susceptible to rancidity and could lead to lowered consumer acceptability (Kowalski *et al.*, 2020).

According to literature, iodine value (IV) is an indication of the quality of unsaturated fatty acids present in the fatty sample and has been used to predict shelf life. A high IV shows that the samples are a rich source of polyunsaturated fatty acids which are beneficial to health and helps to regulate and lower blood cholesterol levels as well as high blood pressure. It also shows that the sample has good qualities of edible oil (Makanjuola and Adepegba, 2020). Lopusiewicz *et al.* (2020) reported an increased IV of flaxseed oil cake (156.95 - 204.08 g/100g) after 3 weeks of storage. Lower IV of oil samples would contribute to its greater oxidative storage stability, the FAO/WHO (2005) recommended standard for oil-containing samples is 112-129 g/100g.

4.2.10 Effect of *Kulikuli* supplemented diet on feed intake

Accumulative feed intake is an indicator of the palatability and the acceptability of diets by the animals (Olarotimi and Adu, 2022). The feed intake values varied among the experimental diets. This could be attributed to the adaptation which in turn works to maintain body weight gain (Ludwig *et al.*, 2022). Feed intake was different among the

groups of experimental animals which is in line with the results obtained by Hamad *et al.* (2015) who noted a significant difference in the feed intake of rats fed with fermented wheat bran. Mbaeyi-Nwaoha *et al.* (2021), reported that in rats, feed intake may be influenced by factors such as the taste, smell, and texture of the test diets among other things. The low consumption of fermented *Kulikuli* and chia flour (KC) and fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) could probably be attributed to the colour, taste and smell of the blends. This could be a result of the strong smell and colour of the chia seed, which was contained in fermented *Kulikuli* and chia flour (KC) and fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) samples. Low feed intake could also be attributed to poor palatability and aroma (Qui *et al.* 2023).

4.2.11 Effect of *Kulikuli* supplemented diet on body weight

Body weight is a method of monitoring the level of nutrition and growth of animals. In this research, there was no significant difference ($P > 0.05$) in the body weight of the experimental animals in all the groups when compared to the control groups (Unfermented *Kulikuli* (UK), *Kulikuli* from the market (KMKT) and the Basal diet). This showed that the laboratory- prepared *Kulikuli* samples had no significant effect on the body weight of experimental animals. Hassaan *et al.* (2018), Oladejo *et al.* (2020) and Musliu *et al.* (2021) made similar observations in the studies conducted on fermented sunflower, fermented African yam beans and fermented soya beans. High body weight in albino rats fed with laboratory-prepared *Kulikuli* could be ascribed to optimal bioavailability of nutrients, ease of absorption and robust utilization due to pre-processing of the peanuts to assimilable diet by enzymatic activities (Picó *et al.*, 2019). Protein hydrolysis is known to yield amino acids that are readily digestible and absorbed compared with intact protein. Probiotics are also known to produce many important

enzymes and increase the bioavailability of vitamins and calcium as well as the enhancement of mineral availability such as iron (Samtiya *et al.,* 2021).

4.2.12 Effect of *Kulikuli* supplemented diet on relative organ weight

Organ weight is an integral part of the physiological and pathological state in animals (Bozhkov *et al*.,2022). The relative organ weight is important to determine whether the organs were exposed to any form of harm during the experiment. The findings from this study indicated that there was no significant difference in the weight of the kidney, liver, spleen, stomach and intestine of the experimental animals fed with laboratory-prepared *Kulikuli* samples when compared to the control groups (Unfermented *Kulikuli* (UK), *Kulikuli* from the market (KMKT) and basal diet). This observation implies that the formulated feed did not cause harm to the organs of the experimental rats. This is in contrast with the findings of Fabersani *et al.* (2018) who reported an increase in the weight of the small intestine and a decrease in the spleen of animals fed with goat milk yoghurt supplemented with yacon flour.

4.2.13 Effects of *Kulikuli* supplemented diet on haematological parameters

Haematological parameters are important indices of the physiological and pathological status of both animals and humans (Sindete *et al.*, 2021). It can be used to determine the deleterious effect of foreign compounds, including plant extracts on the blood of the experimental rats (Enenebeaku *et al*., 2021). The present study showed that there was no significant differences ($P > 0.05$) in hemoglobin (Hb), red blood cells (RBC) and white blood cells (WBC) of rats fed with laboratory-prepared *Kulikuli* samples. This corroborates with the findings of Hassaan *et al.* (2018) who reported no significant differences ($P > 0.05$) in haematological parameters of rats fed with fermented sunflower while Oladejo *et al.* (2020) reported improved pack cell volume, Hb, and RBC for albino rats fed with hydrolyzed African Yam Bean. The improved PCV, Hb and RBC might be

due to the presence of mineral and vitamin contents that helps in the formation and maintenance of the blood (Kolawole *et al.*, 2021).

This also suggests that the treated diets improved the RBC and Hb which are the oxygen-carrying compounds present in the RBC. The total Hb concentration depends primarily on the number of RBC in the blood sample while PCV and Hb are used for monitoring quantitative changes in RBC. The packed cell volume (PCV), haemoglobin concentration (HbC), white blood cells (WBC) and red blood cells (RBC) had no significant difference when compared to the control. These findings differ from Oladejo *et al.* (2020) who observed an increased PCV, HbC, RBC when the experimental animals were fed with fermented soybean. Kumar *et al.* (2023b) established that diets containing quality protein and iron can enhance the production of haemoglobin and immunity in animals.

The values of the mean cell haemoglobin concentration (MCHC), and mean cell haemoglobin (MCH) and mean cell volume (MCV) of the experimental rats fed on the formulated diets were not significantly different when compared to the control. The MCHC, MCH, and MCV are useful indices of the average Hb concentration of red blood cells, and a low concentration of this haematological parameter in animals is indicative of hemolytic anaemia while an increase in these parameters is suggestive of massive intravascular hemolysis (Yadav *et al.*, 2022). The values derived from the haematological parameters of the rats fed with laboratory-prepared *Kulikuli* in this study were within the standard reference range in humans for WBC (4 to 10 x 10^9/L), RBC (4.5 to 6.5 x 10^{12}/L), and platelets (150 to 400 x 10^9/L), PCV (40 to 52 %), Hb (13 to 17 g/dL), MCV (80 to 100 fL) and MCHC (30 to 35 g/dL) (Farinde, 2019).

4.2.14 Effect of *Kulikuli* supplemented diet on differential blood count

The differential blood count parameters neutrophil, lymphocytes, monocytes, eosinophils and basophils are used to diagnose an infection, inflammation, leukaemia, or an immune system disorder (Obiorah *et al.*, 2021). The findings from this study indicate that there is no significant difference ($p > 0.05$) in the differential blood counts of the experimental animals compared with the controls (Unfermented *Kulikuli* (UK), *Kulikuli* from the market (KMKT) and basal diet) except for the lymphocytes of animals who consumed fermented *Kulikuli* and all-purpose flour fermented *Kulikuli* and all-purpose flour (KF). This shows that the formulated feed administered to the animals had no toxic effect that could trigger an immune response in the rats (Muhammad, 2021). This result is not in agreement with the result obtained by Adejuwon *et al.* (2021) who reported significant differences between all the differential blood counts of the experimental rats.

4.2.15 Effect of *Kulikuli* supplemented diet on liver and kidney function

The safety or dangers associated with the use of plant materials at the cellular level have always been monitored through enzyme activities. Cellular necrosis is usually monitored using ALT (alanine aminotransaminase) and AST (aspartate aminotransferase), and high levels in serum may signal malfunctioning of the liver. Both enzymes occupy a central point in amino acid metabolism and are usually found in the liver, heart, kidney, skeletal muscle and other tissues. They are indicators of liver damage, majorly resulting from exposure to chemicals, however, alanine aminotransferase is more liver-specific (Ogunbode, 2021).

There was an overall significant ($P > 0.05$) increase in serum of aspartate aminotransferase (AST) and alanine aminotransaminase (ALT) activity in this study. Hassaan *et al.* (2018) reported an increase of ALT and AST in Nile tilapia fed high

inclusion level of fermented soybean meal diet. However, Novriadi *et al.* (2018) findings showed there were no significant differences in total protein, albumin, cholesterol, alanine aminotransaminase and aspartate aminotransferase activities in all treatments. These contradictory results might be due to the fermentation methods, species of microorganisms and growth conditions as these may influence the levels of the active components present in the final products incorporated into diets (Amin and Mao, 2021). Thus, based on these findings, the collection of blood samples at different time after the last feeding is needed to clarify whether the inclusion of fermented food samples might influence the haematological conditions and other enzyme activities (Novriadi *et al.*, 2018). The total blood protein and albumin concentrations ranged from 3.86 to 8.73 g/dl and 2.39–6.38 g/dl respectively. The total blood protein was highest in rats fed fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL) and fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) whereas the serum albumin concentration was highest in rats fed fermented *Kulikuli*, all-purpose flour and *Lactobacillus plantarum* (KFL), both parameters are not comparable to the control. The serum protein and albumin concentrations obtained in this study were higher than the normal ranges (5.6–7.6g/dl; 3.4–5.8 g/dl, respectively) reported by Adejuwon *et al.* (2021).

The urea nitrogen and the creatinine of rats fed with the laboratory-prepared *Kulikuli* samples and the controls ranged from 54.72 to 98.61 mg/dl. The values were way higher than the normal range reported by Adejuwon *et al.* (2021). Healthy kidneys are designed to remove creatinine and urea nitrogen from the blood, the higher the creatinine and urea value, the less effective the kidney function (Chen *et al.*, 2021). The levels of AST and ALT in serum are used in the diagnosis of the health status of the liver (Akter *et al.*, 2021b) whereas the AST and ALT levels in the blood are directly related to the level of

tissue damage (Samadi-Noshahr *et al.*, 2021). The AST values of the experimental rats ranged from 37.13 to 55.10 u/L and were highest in the control group, while the fermented *Kulikuli*, chia flour and *Lactobacillus plantarum* (KCL) fed group was lowest. However, fermentation of the diet substrate significantly affected the AST response in test animals. These AST concentrations obtained were within the normal range (45.70–80.50 u/L) previously reported for healthy animals (Adejuwon *et al.*, 2021). The ALT values of the experimental rats ranged from 59.01 to 73.26 u/L. The ALT values were above the normal range (17.50 – 30.20 u/L) reported for healthy animals. A high concentration of AST or ALT in the blood is indicative of potential liver damage (Otuechere *et al.*, 2023).

4.2.16 Effect of *Kulikuli* supplemented diet on lipid profile of rats

Lipids are a group of fats and fat-like constituents that are important components of cells as well as being expensive roots of energy. A lipid panel estimates the level of definite lipids in the blood (Ogunbode, 2021). Tissue triglyceride profiles are usually used as an indicator reflecting lipid metabolism (Desoye and Herrera, 2021) in animal studies. Lipoprotein content can be a measure of the degree of promotion of reversed cholesterol and metabolism where high- density lipoprotein (HDL-C) aids in the removal of cholesterol from the periphery for delivery to the liver and excretion into the bile (Endo *et al.*, 2023) and LDL-C is the major cholesterol carrier in circulation and its physiological function is to convey cholesterol to the cells (Zanoni *et al.*, 2021). Thus, HDL-C/LDL-C is usually related to reflect the transport of cholesterol, and the higher ratio indicates higher lipid content (Patel and Kashfi, 2021).

In this research, the total cholesterol, triglycerides, low density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C) concentrations in rats fed laboratory- prepared *Kulikuli* samples supplemented diet were significantly different (P

> 0.05) from each other. There was an increment in the cholesterol, triglycerides and low-density lipoprotein- cholesterol (LDL-C) levels in rats fed with the laboratory-prepared *Kulikuli* samples. This observation indicates that the test samples have hyperlipidemic tendencies and can cause coronary heart disease (Martin-Campos *et al.*, 2021). This finding is not in line the observation of Fabersani *et al.* (2018) who reported no significant differences in total cholesterol and LDL-C in all experimental groups fed with goat milk yoghurt and a decrease in the triglycerides and high-density lipoprotein-cholesterol (HDL-C) concentrations. However, Ogunbode (2021) reported a significant difference for all the lipid parameters, with the control diets having the highest lipid values.

4.2.17 Histopathological studies on the organs of rats fed with *Kuilkuli* supplemented diet

Histopathology, which is the study of tissue disorders and entails looking at microscopic slides with tissues, cells, and other components, has been widely utilized to diagnose many diseases in animals and human beings (Salman *et al.*, 2022). When a metabolic reaction is caused by food, the kidney, liver, spleen, stomach, and intestine are the main organs impacted (Rehman *et al.,* 2021). No obvious liver damage was observed during this investigation, the histological sectioning is consistent with the biochemical data. The histopathological findings from this study is in agreement with the work of Adeoti *et al.* (2018) who researched the histopathological study on rats fed with a maize-based complementary diet enriched with fermented and germinated *Moringa oleifera* seed flour. Salman *et al.* (2022) reported variable hepatic injury in the form of thickening in the Glisson capsule, as well as dissociation and disorganization of hepatic cords in the liver after rats were fed with cultured white soft cheese. The general liver histological architecture and its functions were not affected in any way by the supplemented *Kulikuli*

samples as compared to the control. Other organs, the stomach, small intestine and the spleen showed normal morphological characteristics when compared with the control. Therefore, it is safe to assume that during the experiment, the supplemented *Kulikuli* did not have any toxic effect on the organs of the rats.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Lactobacillus species was isolated from Corn steep liquor and were further identified using 16s rRNA as *Lactobacillus plantarum.*

The total bacterial count of *Kulikuli ranged* from 0.0 to 0.0 –3.00±0.00 x 10^4 CFU/g while the total fungal counts ranged from 0.0 to 0.0 –2.00±0.00 x 10^4 CFU/g. *Aspergillus niger, A. fumigatus, A. flavus, A. terreus, Klebsiella pneumoniae, Staphylococcus aureus* and species of *Bacillus*, *Enterobacter*, *Micrococcus*, *Proteus* and *Tricophyton* were isolated from the *Kulikuli* samples.

The moisture content in the *Kulikuli* samples ranged from 2.71±0.08 to 4.60±0.14%, ash (3.47±0.09 to 4.53±0.05%), protein (22.53±0.25 to 31.21±0.49%), fibre (0.25±0.05 to 0.86±0.05%), lipids (30.64±0.40 to 35.33±0.45%) and carbohydrate (27.56±0.64 to 36.33±0.03%).

The *Kulikuli* samples contained antinutrients such as tannins (16.65 to 18.58 mg/100g), saponins (19.84 to 37.12 mg/100g) phytates (12.60 to 84.25 mg/100g), cyanides (3.60 to 513.57 mg/100g) and oxalates (0.03 to 0.15mg/100g).

The Free fatty acid (6.75 – 10.32) mg KOH/g, peroxide (2.17 meq/kg – 8.75 meq/kg) and iodine values ranged from (66.55g/100g – 75.23g/100g).

Kulikuli supplemented diet did not alter the red blood cells, white blood cells, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and mean corpuscular volume. However, basophils and monocyte counts were decreased while lymphocytes and platelets counts were increased. Consumption of fermented *Kulikuli* led to an increase in aspartate aminotransferase (AST), alanine aminotransaminase (ALT), albumin and protein concentration. Higher triglycerides, total cholesterol,

low-density lipoproteins-cholesterol (LDL-C) were observed in rats fed fermented *Kulikuli* (KF).

The histopathological studies carried out on the rat organs (liver, kidney, spleen, stomach and intestine) revealed that the fermented *Kulikuli* -supplemented diet did not affect any of the organs.

5.2 Recommendations

It is recommended that:

i. fermentation of legumes can increase its protein, lipids and carbohydrates contents, therefore making it a good food product for consumption.

ii. small to medium-scale production of *Kulikuli* be carried out under standard hygienic operation procedures. This will drastically reduce contamination that may result in the consumption of improperly produced food and food products as well as popularize the nutritional benefits of the product.

iii. this snack may serve as a supplement to low-nitrogen foods such as cereal-based snacks and foods, therefore it is advised that *Kulikuli* be eaten within 20 days of production to avoid the consumption of nutritionally deficient food.

iv. promotion of these products through enlightenment for the local producers and vendors of the snack is necessary to enhance improved nutrient intake and optimal nutrition for the low- and middle-income earners who are the major consumers of *Kulikuli*.

5.3 Contribution to Knowledge

The study established that the microbial load of the fermented *kulikuli* samples (0.0 - 2.0±0.00 x 10^4 CFU/g) was within the acceptable range in food products. The fermentation of *Kulikuli* with *Lactobacillus plantarum* showed enhancement in protein (27.09±0.4% - 30.07±0.08%), carbohydrate (36.33±0.03 - 39.52±0.15) and lipids

(30.86±0.15- 35.33±0.45) content of the food product. The inherent anti-nutrients phytate and oxalate were reduced from (14.33%-12.60%) and (0.08%-0.01%) respectively. The physicochemical properties examined such as free fatty acids, peroxide and the iodine values increased from (6.75 – 10.32) mgKOH/g, (2.17 – 8.75) meq/kg and 66.55g/100g – 75.23g/100g. Histopathology of the analyzed organs of the rats (liver, kidney, spleen, small intestine and stomach) exhibited normal architecture. Fermented *Kulikuli* with *Lactobacillus plantarum* was enriched by reducing the antinutritional composition, improving the iodine value, polyunsaturated fatty acids as well as increasing the protein and carbohydrate contents. The study therefore suggests that fermented *Kulikuli* with *Lactobacillus plantarum* is relatively safe for consumption.

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APPENDICES

Appendix A

Composition of Experimental Diets per Gram Utilised in the Feed Trial (g)

Ingredients (g)	Groups				
	1	2	3	4	5
Maize	450	450	450	450	450
Maize offal	350	350	350	350	350
Soya beans	100	75	50	25	00
Kulikuli	00	25	50	75	100
Palm kernel cake	40	40	40	40	40
Bone meal	20	20	20	20	20
Minerals	7.5	7.5	7.5	7.5	7.5
Methionine	2.5	2.5	2.5	2.5	2.5
Lysine	2.5	2.5	2.5	2.5	2.5
Vitamins	25	25	25	25	25
Salt	2.5	2.5	2.5	2.5	2.5

Total-1000g of the experimental diet

Appendix B

Microscopic Characteristics of *Lactobacillus* specie and *Aspergillus niger*

Lactobacillus sp. under a microscope

Aspergillus niger under a microscope

Appendix C

GENE SEQUENCE OF ISOLATED *LACTOBACILLUS* SPECIES

>MW543941 *Lactobacillus plantarum* strain MerLAB1

AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGC
AAGTC
GAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTG
AGTGGC
GAACTGGTGAGTAACACGTGGGAAACCGCCCAGAAGCGGGGGATAACACC
TGGAA
ACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGTTTGAAA
GATGG
CTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGG
GTAACG
GCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGYAATCGGCCACA
TTGGGA
CTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCA
CAATGG
ACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTC
GTAAAA
CTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACG
GTATTT
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
GTGGCA
AGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAG
TCTGAT
GTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTT
GAGTGC
AGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATAT
GGAAGA
ACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGA
AAGTA
TGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGATG
AATGCT
AAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCAT
TCCGCC
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CACAA
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CTTGAC
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GGT

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GAGCGC
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CGGTGA
CAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGAC
CTGGGC
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AAGCTA
ATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATG
AAGTC
GGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGG
CCTTGT
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GTAACC
TTTTAGGAACCAGCCGCCTAAGGTGGGACAGATGATTAGGGTGAAGTCGT
AACAAG GTA