NUTRITIONAL AND ANTI-DIARRHOEAL POTENTIALS OF PROBIOTICFERMENTED FORTIFIED WEANING BLENDSINMALE WISTAR RATS

BY

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CERTIFICATION

I certify that this work was carried out by **Olaoluwa Kehinde ALAO** (Matric. No.: 158916) in the Department of Microbiology, Faculty of Science, University of Ibadan, Ibadan, under my supervision.

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DEDICATION

I dedicate this work to GOD and every neglected African child.

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ABSTRACT

Complementary foods are used for transition of infants from a mother's breast-milk preparatory to regular foods. Many food blends including costly foreign and locallyproduced ones have been adopted for weaning infants. However, most of the locally produced ones have doubtful nutritional values and could predispose infants to malnutrition and possibly diarrhoea. Hence, this study was designed to develop a nutritious complementary blend with attendant antidiarrhoeal properties from locally sourced materials.

Millet-based gruels containing cowpea, groundnut and *Moringaoleifera*(dried leaves) blends formulated at 70:25:5:0 (F1), 70:24:5:1 (F2), 70:23:5:2 (F3), 70:22:5:3 (F4), 70:21:5:4 (F5) and 70:20:5:5 (F6) were spontaneously fermented for 72 hours and analysed for nutritient composition using standard methods. From spontaneously fermenting formulations, Lactic Acid Bacteria (LAB) were isolated, characterised and screened for probiotic potentials including pathogenic bacterial inhibition using microbiological techniques. The best probiotic starter-fermented blend was prepared and analysed for nutritional composition using standard techniques. Nutritional and antidiarrhoeal potentials of best starter-produced blend were determinedon male Wistar rats (5 per group, weight≈133.46 g) after 28 and 14 days, respectively. In the nutritional experiment, groups 1-4 were fed with Conventional Food (CF), Fermented Blend (FB), Fermented Blend+Probiotics (FBP) and Nutriborn (Control). The weight gain, haematological parameters and liver enzymes were determined using standard techniques. Diarrhoea was induced in rats using castor oil through oral gavage. Groups (1-5) were uninduced rats treated with CF (UCF), induced rats treated with CF (ICF), induced rats treated with CF+Loperamide HCl-Tm (ICFL), induced rats treated with FB (IFB) and induced rats treated with FBP (IFBP). The haematological (blood), oxidative stress and histopathology alterations (colon tissue) were determined in the sacrificed rats using standard procedures. Data were analyzed using descriptive analysis and ANOVA at $\alpha_{0.05}$.

The F4 had the best nutritional quality (50.87 g/100g moisture, 10.27 g/100g crude protein, 1.60 g/100g ash, 30 mg/100g phytic acid and 17.57 GAE/g polyphenol). Of the 128 LAB, *Lactobacillus plantarum*-MCB4, *Lactobacillus plantarum*-MCB18 and *Lactobacillus pentosus*-MCB47 possessed best probiotic potentials. The selected non-haemolytic *L. plantarum*-MCB18 survived 4 hours incubation in 0.50% bile; showed 58.30% cell surface hydrophobicity and high antagonistic activity against selected pathogens. The *Lactobacillus plantarum*-MCB18fermented blend F4 (FB) had 5.11% moisture, 20.17% crude protein, 1.14±0.12 mg/L calcium, 0.70 mg/100g thiamine, 0.42 mg/100g riboflavin, 5.52±0.01 g/100g phenylalanine and 436.71 Kcal/100g metabolisable energy. Groups 3 (16.44%) and 4 (23.72%) had significant increase in weight gain, while haematological variables, aspartate transaminase,alkaline phosphatase and alanine transaminase levels were normal. Packed cell volume (48.00±0.7 and 43.00±0.0%), haemoglobin (16.35±0.46 and 14.23±0.10 g100mm^{-1}), red blood cell count ($\times 10^6$mm^{-1}): 7.95±0.29 and 7.34±0.04 in IFB and IFBP, respectively were normal and significantly higher compared to ICF. Glutathione (29.06 µM/mg protein), catalase (43.76 µM/mg protein) in IFBP and nitrite (5.49 µM/mg protein) in IFB significantly increased relative to ICF. Colon tissues of rats of IFB and IFBP had no remarkable vascular change compared to negative occurrence in group ICF.

Probiotic *Lactobacillus plantarum*-MCB18-fermented millet-based formulation supplied adequate nutrients for healthy growth and conferred therapeutic effect against diarrhoea in male Wistar rats.

Keywords: Millet-based gruel, Probiotic Lactic Acid Bacteria, Diarrhoea.

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CHAPTER ONE

INTRODUCTION

Feeding with breast milk absolutely for the first six months remains the recommendation of World Health Organization (WHO) for infants by hale and hearty well-fed mothers (WHO, 2014). This inexpensive infant formula plays an important role in impacting positively on infants' well-being (American Academy of Pediatrics, 2012).

It becomes necessary to introduce other foods into the infant's diets to supply the nutritional requirement of micronutrients, protein and energy needed for the swift growth, developmentand regulation of body physiological functions when the breast milk starts to get insufficient(Oyarekua, 2011). Weaning is the gradual practice of shift to a semi-solid diet from purely milk based intake in infants once the recommended time for breast feeding lapses or when situation warrants such (WHO, 2014). Commonly after the four to six months timing, weaning begins effectively as the tongue drive reaction ceases at this age so foods introduced will not be pushed out of the infants mouth. Also the gastro-intestinal tract becomes more mature by this time and is less prone to protein permeability through the wall which may trigger allergic responses (Chafen *et al.,* 2010). Complementary foods expected to be nutritionally balanced and of easy digestibility are used at the weaning stage (Ugwuona *et al.,* 2012). Quality complementary foods found at sales points are within the reach of families that are financially buoyant but unavailable to poor and disadvantaged families due to their high acquisition price. Poor families find solace in complementary food prepared from local staples usually cereals which are lacking in some essential amino acids in spite of their good nutritional worth (Achi and Ukwuru, 2015).

Supply of these nutritionally imbalanced complementary food to infants at the weaning age and beyond is an issue of stern concern because of the high incidence of malnutrition

(which could be hidden in the child) which engenders vulnerability to severe or frequent infections (American Academy of Pediatrics, 2012). This is fallout of consuming diets whose nutrient is in the wrong proportions, not hygienically handled and not culturally acceptable. Malnourishment has not been commendably curtailed in under developed parts of the world where a huge bulk of infants are weaned on most occasions with cheap complementary foods large quantity wise but low in terms of nutritional worth (WHO, 2012).

Diarrhoea can be described as a physiological situation where an individual has at least three loose or watery stools within the period of a hundred an forty-four minutes. It is symptomized with higherincidence offluidity offeacesmorethan normalin a person,coming up fromadisparity between intestinal secretory and absorptive capacity which could persists for few days then lead to dehydration owing to loss of fluid via the watery stool which usually starts with the skin showing a loss of its normal colour and stretchy form (WHO, 2013).

Diarrhoea is ofgreatpublichealth significance,because reports confirmit accounts for a shocking percentage of mortality in infants and adolescents indevelopings nations of Africa (WHO, 2012). It might happen due to lactose intolerance or when a person experiencing stability with diet and microbial milieu is unprotected against strange microorganisms mostly bacteria ingested as part of food and water which overpower the defensive effect of the normal flora of the intestine majorly due to poor hygiene (Elmer, 2001).

Chemically synthetic drugs such as Loperamide HCl-Tm is engaged to stabilize the movement of intestinal contents but are not at all times free from adverse effects, hence, the quest for safe as well as more operational agents found in microorganisms, plant materials etc. lacking side effect (Hardman and Limberd, 1992).Diarrhoea could be prevented/controlled by improved sanitation and adequate nutrition but an effective inexpensive probioticcould also be of notable help.

Probiotics are living microorganisms which once ingested in sufficient quantities impacts healthy value on an individual (WHO, 2013). Potential functions of these live

microorganisms include producing and secreting antimicrobial materials, host immune response stimulation then displacement of pathogen colonization as effective ones have demonstrated an ability to prevent and treat some infections restricted to definite body parts besides the gastrointestinal tract upon its ingestion, hence, engendering physiological stability in human system (Kalhoro *et al*., 2019). Bifidobacteria, Lactobacilli, *Streptococcus, Pediococcus* and *Saccharomyces* arerenowned with probiotic effect in the body as they help sustain the balance of the gastrointestinal micro-flora (Denkova and Krastanov, 2012). Achieving a probiotic status requires microorganisms to also fulfill criteria strongly connected to technological and safety qualities (FAO/WHO, 2001). To choose microorganisms with probiotic prospect, they need be preferably isolated from same area in which they are going to be used and should be preferably from same environment, as there are numerous probiotics sold commercially that are less functional because they have been isolated in other regions or countries (Ventura *et al.,* 2000). To be used, probiotics cultures must:own a Generally Regarded As Safe (GRAS) statuswith clinically authenticated documented health effects, long term survival trait, be propagated and integrated into foods easily (Stanton *et al.,* 2003). They must tolerate acid and bile hence permitting selected strains to stay alive, grow, and execute therapeutic and perhaps prophylactic functions in the intestinal tract (Usman and Hosono, 1999), resist digestive enzymes, adhere to intestinal mucosal wall, lower cholesterol effects, enhance bioavailability of food compounds and boost the immune system (Ouwehand *et al.,* 1999).

Lactic acid bacteria were endorsed in food produces due to their apparent outstanding proven health profits and are included as starters in complementary food fermentation, hence, being of immense benefit to infants who consume these foods (Baquerizo *et al*, 2014). As starters they possess good fermentative action, suitable growth and viability in foods, and are of great stability throughout long-term storage; conferring on food products good consistent organoleptic property among other qualities (Saarela *et al.,* 2000). *Enterococcus*, *Lactobacillus casei* and *L. rhamnosus* among others of lactic acid bacteria species have been reported to lessen effectively the sternness and period of diarrhoea among other health benefits in the human body (Kalui *et al.,* 2010).

Fermentation is an ancient method of food processing used to synthesize useful food products by a single microorganism or a combination of two or more microorganisms, principally benefiting as it transforms sugars and other carbohydrates to functional end products (Egwim *et al.,* 2013). The need to improve the nutritive value and other physico-chemical properties of complementary food through fermentation process while sticking to guidelines and regulations linked to hygienic processing cannot be over-stressed (Sanoussi *et al*., 2013). Fermentation is a low cost process, which leads to production of beneficial enzymes, antimicrobial substances and preserved food (Chelule *et al*., 2010). Reduced viscosity and anti-nutrient level in cereal-based food leading to increased nutrient density and extended shelf life have been accomplished through fermentation (Ikujenlola and Adurotoye, 2014).

Other approaches aimed at addressing inadequacies of infant's complementary foods include processing steps like strict hygienic processing, cereal-legume co-fermentation, fortification, malting, dry-milling among others(Wakil and Kazeem, 2012). Wholesomeness and overall quality not separated from upgraded hygiene, safety and quality control in backing of HACCP approaches of cereal-based traditional fermented complementary foods largely depended on by poor family unitscan be and have been improved through usage of starter culture reported as the cause of the loss of diversity in nutritional attributes, flavor, taste but decreases the risk of failed fermentation (Smid and Hugenholtz, 2010).

Supplementation of cereals with cowpea, and soy-bean, bambara-nut, tigernut among others have been reported to contribute immensely to increase cereal-based complementary foods protein value; and its fortification with *Moringa oleifera* leaves have been reported to meet increased energy demands resulting from a balanced supply of protein, carbohydrate, fat, minerals and vitamins (Shiriki *et al*., 2015; Wakil and Olorode, 2018).

1.1 Statement of problem

The consumption of complementary diets with low nutrient and energy density handled non-hygenically due to decreased supply of breast-milk has led to incidence of

malnutrition and diarrhoea which adversely affect infants' wellbeing and accounts for increased percentage of mortality amongst children particularly in developing nations (WHO 2013).Chemically synthetized drugs engaged to stabilize the movement of intestinal contents during episodes of diarrhoea are not at all times free from adverse effects.

1.2 Justification

Formulation of complementary food from cheaper and more readily available food resources fermented with lactic acid bacteria (LAB) with probiotic potentials could provide a way out, as it has the potentials of conferring health benefits beyond nutritional gains and could help ameliorate malnutrition while impacting anti-diarrhoeal prophylaxis in infants (Wakil and Olorode, 2018).

1.3 Aims and objectives

This study aimed at

1 Development of probioticated *Moringaoleifera* fortified cereal-legume based complementary food with anti-diarrhoeal effect.

2 Evaluation of Nutritional qualities and anti-diarrhoeal potentials of probioticated *Moringaoleifera* fortified cereal-legume complementary blend (*in-vitro* and *in-vivo)*.

This was achieved through the following objectives:

1. Formulation of cereal-based complementary blends (Cereal-Legume) fortified with dried *Moringa oleifera* leaf powder and determine the nutritional and antinutritional contents of the spontaneously fermented blends.
2. Isolation, screening and characterization of starter culture with probiotic potential from spontaneously fermenting formulated blends.
3. Investigation of the nutritional and antinutritional quality of starter-fermented blends.
4. Evaluation of the vitamins, amino acid, mineral contents, physico-chemical and organoleptic properties of the starter-fermented best blends from 3 above.
5. Investigation of the nutritional and anti-diarrhoeal potential of the best fermented blend (from 4 above) and lactic acid bacteria with best probiotic potential (from 2 above) using an animal model.

CHAPTER TWO

LITERATURE REVIEW

2.1 Breastmilk

It is suggested by Health professionals that absolute breastfeeding should start from the first hour of a baby's lifetime and continue for at least four to six months by healthy well-nourished mothers (WHO, 2014). Breastfeeding is the practice of feeding milk to infants from the mammary gland of a lactating mother (NICHHD, 2017). This more affordable than artificial infant formula provides the baby required calories, macronutrients (protein, carbohydrates etc.) and micronutrients (minerals, vitamins etc.) (Ballard and Morrow, 2013).

Advantages the breast milk affords the baby are well documented as it is loaded with nutrients in balanced ratios and free from harmful microorganisms as well as diseases they cause e.g. diarrhoea (American Academy of Pediatrics, 2012; WHO, 2012). Studies revealed breast-milk possess balanced quantities of methionine, tyrosine, phenylalanine, taurine, cysteine etc. a composition which averts hurt of the central nervous system and supports neuro-development in infants (Allen *et al*., 2010). The mothers' breast milk preventsinfants'access to unclean source of water and food, and more precisely through its immune-defense mechanism helps a child maintain a healthy status.

As a child grows older after six months, provided breast milk becomes insufficient quantity and quality wise to deliver needed nutritional necessities of aninfant; consequently it becomes obligatory to add-on the breast milk with other appropriate diets promptly (Eka *et al*., 2010). Within this first six months of lifetime, as opined byWHO (2012), growth and maturation takes placespeedily and the demands of the body for essential nutrients are comparatively increased than any other stage of life due to an increase in physiological developments together with increased energy usage. This

physiological transformation in infants demands a transition, during which his/her diet changes in terms of consistency and source (WHO, 2014).

2.2 Weaning

The word weaning originated from the Anglo-Saxon expression "wenian" meaning "to become accustomed to something dissimilar" (Wakil and Olorode, 2018). Weaning could be defined as the process of transition from a purely milk based intake to a semi-solid diet in infants (when extra nutrients becomes needed) to supplement or in some cases swap breastfeeding absolutely (Kids Health from Nemours, 2014).

Weaning is done based on the recommendation that a child should be given a recipe of semi-solid foods and breast milk by healthy and well-nourished mothers to maintain adequate growth after their first six months of age until the baby is a year old at least (WHO, 2013). A mother's milk or any other form of milk is sufficient for a neonate, though its sufficiency for vitamins D and C, iron, trace elements beyond six months of age is doubtful (WHO, 2014). Infants at the weaning stage become vulnerable to risks owing to the loss or watering down of defense properties of a mother's breast milk and the introduction of a developing immune system to contamination in the surroundings (Kids Health from Nemours, 2014).

There exists therefore an expedient need to introduce a balanced mix of semi-solid foods (of low bulk density, free of pathogenic microorganisms, easily swallowed and a host of other required qualities)to supplement infants feeding, hence, delivering a secured nutritional status early in life (Jindal, 2004). This practice could be slow but sure and steady for months up until when the baby is finally familiarized and accustomed to diets of the family (Wakil and Alagbe, 2017). An abrupt weaning process in which infants are accustomed to the family diets generates a nutritional crisis as these infants may not be be able to consume an adequate amount of the adult diet to meet nutritional necessities (WHO, 2014).

The weaning process is fundamental for development, constant growth, and maintenance of a child, hitherto it is a phase when complementary diets are supplied to provide the bulk needed to avert hunger with much insensitivity to the nutritional worth of the food (Valle *et al.,* 2003). Apart from ensuring there is no breach sandwiched between nutrient

necessities and what an infant is capable of consuming to avoid an occurrence of malnutrition, the case of mishandling traced from stages of processing down to delivery of complementary foods to infants is of serious concern, worthy of note (Eka *et al.,* 2010). Some infants are even fed with spoon, cup or feeding bottle that are not sterile. A sizable number of mothers entrenched in tradition use the forced hand feeding technique which may be inappropriate when done, not considering hygiene, leading to an increased risk of infection as seen in cases of diarrhoea (Wakil and Alao, 2013).

2.3 Complementary foods

Complementary food is described as that food meant to provide more concentrated energy, protein and other essential nutrients apart from breast milk (when its sufficiency becomes reduced) introduced to infants of weaning age (Kids Health from Nemours, 2014). Good complementary food should possess good microbial safety status and special qualities in line with nutritional needs to aid mental plus physical improvement during growth and developmental stages, reason being the immune system is evolving and the protection made available by a mother's breast milk comes in short supply (Ugwuona *et al.,* 2012).

Complementary foods are first nutrients providing foods which are adequate for consumption in one feeding session administered alongside a mother's breast milk to infants after six months of life. They are used for the withdrawal of the mothers' breast milk in preparation for acclimatization of infants to the regular food in the family(Wakil and Oriola, 2012).

Nutritious complementary foods available are marketed but priced above the financial strength of majority of poor parents. Moreover, availability of baby foods and ready-made snacks in local markets and remote areas is highly poor; therefore mothers in these areas resort to accessible low-cost foodstuff mixes to wean their infants (Eka *et al*., 2010). They are practicallyexclusively dependent on home-made preparations of low nutrient quality. This is stringed to lack of knowledge about simple processing techniques used to produce nutritious complementary blend formulated with local staples usually cereal grains which may lack essential amino acids like threonine, tryptophan and lysine (Ikujenlola, 2014).

Towards achieving an upgrade of all nutritional qualities of a complementary food at the same time for best positive impact on health outcomes, the following basic considerations are made:

1. Availability of basic raw materials needed for their formulation,
2. Procedural knowledge for mixing these foodstuffs in quantities that supply daily requirement of an infant, as there could be a case of multiple nutrient deficiencies,
3. Cost of local ingredient linked to economic and geographic constraints,
4. Storage and shelf life which are both important to nutrient stability, safety and sustainability (Thathola and Srivastava, 2002).

Traditional weaning formulations could be upgraded by putting together locally accessible food substances that complement one another such that the different amino acid pattern formed by this blend is comparable to that endorsed to positively impact the growth performance and overall development of infants (Sengev *et al*., 2016). Several attempts have been made to come up with formulations in varying proportions comparable to that recommended to supply nutrients required per feeding session. This has involved the use of constituents like legumes, tiger nut, moringa oleifera leaves among other. A need to improve on these has been strongly emphasized (Wakil and Alao, 2013).

2.4 Malnutrition

A child's nutritional status is generally described in relation to measurement of body features like weight relative to height or number of years which is suggestive of a childs level of wasting or underweight (WHO, 2009).

Adequate nutrition in infants is key to sustaining proper formation and functioning of organs, strengthening of the immune system together with sound cognitive, neurological and healthy growth as dietary composition and nutrient supply evidently imparts beneficial or adverse implications on health throughout ones lifespan (Mastorci *et al*., 2017). Malnutrition stems from illnesses, consuming foods in which key nutrients are in excess, lacking, in incorrect amounts etc. (WHO, 2012).

Prevalence of micronutrients deficiency and undernutrition ishigh among growing infants between 6 and 23 months of age causing a great concern of human distress connected with

greater than half of all infants' mortality all over the world (WHO, 2014). It adversely influences the socio-economic progress of a people because a working population depleted physically, socially and mentally will possibly have an underdeveloped human resource regardless of its number, as the interplay between insufficiency, poor nutrition and ill-health impacts a multiplier consequence on overall prosperity. This contributes expressively towards keeping a populace in a sliding trend of lack together with nutritional insecurity (WHO, 2012). If a nation therefore really wants sustained development, it has to supply nutritionally balanced food to her children starting from when they are infants through other developmental stages to build up a formidable work capacity.

In many developing countries, infant malnutrition constitutes mostly in macronutrient and micronutrient undernutrition (which may take place together). This remains a serious problem that causes growth retardation and diseases of all sorts which re-occur frequently (WHO, 2012). Among a list of diseases associated with nutritional deficiencies in infants, marasmus and kwashiorkor which can result to mental retardation in cases of survival symptomized with tissue oedema, bloated abdomen, changes in hair pigmentation, muscle wasting and loss of subcutaneous fat can be developed consequent of low energy with protein intake respectively (Wakil and Onilude, 2010). The infant immune system could also be impaired as a result of the incidence of diarrhoea, dysentery etc. because nutrients absorption is altered (WHO, 2012). Well-nourished persons when exposed to these diseases put up a measure of resistance from their strong immunity gained through a balanced supply of macro and micronutrients for production of antibodies and cells, proper communication of messages in the body, to wrestle infections effectively than the ones already weakened through malnutrition (WHO, 2012).

Factors aiding malnutrition in developing nations include governments negligence to policies on maternal and child health, gender discrimination against women, poor child feeding and care practices, vegetarian diet, low birth weight (resulting from poor maternal status during pregnancy) and unhealthy environment (WHO, 2009). Some palliative measures and preventive strategies that have been employed in tackling malnutrition incidence in infants include: appropriate breastfeeding practices supported with the use of specially formulated foods that provide energy and nutrients when there is a short fall in

breast milk supply, emphasis on social norms, gender equity and maternal access to true education (UNICEF, 2009). Also, in order to reduce high morbidity and mortality due to malnutrition, attention is being focused on the exploitation and utilization of indigenous plant resources (Wakil and Alao, 2013).

2.5 Diarrhoea

This is a health condition characterized with an experience of an abnormal changed movement of bowel symptomized by increased water content or rate of stools in twenty four hours (Banwo*et al*., 2020). Bulk of diarrhoea incidents happen in developing world on average three times a year with more than half of the documented cases occurring in Africa and Asia (WHO, 2013). Among other diseases such as measles, pneumonia etc. connected with sudden deterioration of nutritional status, infant-hood diarrhoea is commonly indicted for infant deaths in environments where proper sanitation is not observed (Afagnigni *et al*., 2017).

Diarrhoea usually come up due to bacterial, viral, or parasitic infection picked up from feaces contaminated food or water or straight from infected persons (WHO, 2014). Studies have shown that bacterial agents are important causes of diarrhoea in children in their early days of life in many developing countries but in most circumstances, stool cultures to confirm the precise causes are not required (DuPont, 2014). From studies of stool cultures however, the most frequently isolated bacteria associated with diarrhoea include *Salmonella*spp., *Escherichia coli*, *Campylobacter*spp, and *Shigella*spp.Particular microorganisms like *Shigella* and *Salmonella* species etc. are constantly connected to the disease whereas others including *Escherichia coli* are commensal flora members and turn out to be pathogenic as soon as they obtain genes coding for a virulence factor on bacteriophages or plasmids (Michael, 2008).

Fluid loss through diarrhoea can result into electrolyte (sodium, potassium, chloride) imbalances as well as dehydration, altering the naturally balanced electrolytes and water levels which could be severe in younger children that are six months old from birth (weaning period) if not treated promptly (Galadima and Okolo, 2014). Frequent episodes

of diarrhoea lead to long term health challenges such as stunted growth and poor intellectual development heavily linked to malnutrition (WHO, 2013).

Non-infectious causes which include irritable bowel syndrome, lactose intolerance, inflammatory bowel disease, non-celiac gluten sensitivity hyperthyroidism and celiac disease etc. could result into diarrhoea as well (Basem and John, 2013). Recinoleic acid an active metabolite in castor oil which increases the biosynthesis of prostaglandin which contributes to pathophysiological functions in the gastrointestinal tract for instance causes irritation of the small intestinal lining, increasing peristaltic movement and bringing about normalcy in a case like constipation but could also lead to digestive discomfort, diarrhoea and other related side effects(Sanders, 1984). In the case of diarrhoea, it brings about a change in the electrolyte permeability of the intestinal mucosal wall (Akudor *et al*., 2011).

Prevention of diarrhoea could be guaranteed by engaging: better-quality sanitation, drinking uncontaminated water, breastfeeding infants for at least six months, hand washing with cleanser etc. To overcome the menace (diarrhoea), affected persons are advised to carry on with consumption of healthy foods, and for affected babies, continuous breast feeding is recommended (WHO, 2013). The use of traditional herbal medicines in developing countries have also been introduced for diarrhoea control, as several African medicinal plants is recounted to be suitable for treating, managing and sustaining its control by traditional healers (Akudor *et al*., 2011). Phytochemicals of plants such as tannins, flavonoids, alkaloids and saponnins have been reported to be effective in managing diarrhoea incidence compared to Loperamide HCl-Tm an antidiarrhoeal drug which effectively delays diarrhoea induced by castor oil through the inhibition of prostaglandin biosynthesis (Awouters *et al*., 1978).

Use of antibiotics have been reported to have notable drawbacks linked to side effects, antimicrobial spectrum, their spread misuse which has resulted into growing clinical resistance etc. (Fischer *et al*., 2001). This birthed the search which led to finding of immune-stimulants that are non-specific as well as vaccines, with an increased curiosity in the usage of probiotic bacteria because of their several helpful health influences observed in humans (Puphan *et al*., 2015). These influences include lessening the possibility of

diarrhoea occurrence in antibiotics consumers together with its' observed signs by twenty-four hours and the probability of its symptoms staying for more than seventy-two hours by sixty percent (Hempel *et al.,* 2012). David and Famurewa (2010) described in their work the therapeutic effect of fermented food (*ogi* or pap produced from maize) as well as the prophylactic or preventive effect they have on consumers of such foods. The potential therapeutic along with prophylactic effect of metabolites of lactic acid bacteria in *ogi* or pap is mainly immunostimulatory and causes a change in the gastrointestinal microflora to suppress the growth of pathogens and also reduces the occurrence of diarrhoea (Kalui *et al.,* 2010).

2.6 Probiotics

The word "Probiotics" is used to describe consumables that are microorganisms (found in the intestine) based, capable of instigating control biologically and effecting regulatory together with trigger properties in the body (Podgorskji, 2002). They can be defined therefore as lively microorganisms that impacts a measurable physiological health gain on host majorly by improving the intestinal microbial balance when administered in suitablequantities (Hill *et al.,* 2014). The term probiotics at first was used as an antonym for the expression antibiotics and was coined from the words in Greek (*pro* and *biotos*) interpreted as "for life" and its first use can be linked to Kollath in the year 1953 who used it to refer to the restoration of malnourished patients health. Probiotic foods are consumables with microorganisms and their metabolites which impacts varying health properties beyond needed nutritional gains in consumers (Mahmoudi *et al.,* 2012).

In the 20th century, stress, eating disorders and environmental deterioration prompted the development and application (practical-wise) of the ideology- 'probiotics' in produced consumables (Astashkina *et al*., 2014). Metchnikoff around this timing suggested that the ingestion of live microorganisms increased the longevity of the consumer and attributed this positive effect observed on the host health to a reduction in spoilage bacteria and or toxin producing bacteria in their gastro-intestinal tract (Junior *et al*., 2015). From dairy products as well as gastro intestinal tract of humans most commercialized and studied probiotics have been isolated (Marcó*et al*., 2017). However, novel strains of probiotic bacteria isolated and characterized from environments yet to be investigated might be

advantageous in exposing taxonomic features and acquiring strains with remarkable new functional qualities that may be beneficial for use (Ortu *et al.,* 2007). Probiotics use require they must be screened and selected having met safety, technological, functional and physiological requirements. Sound *in-vitro* tests endorsed by Food and Agriculture Organization (FAO)for screening bacteria with probiotic potentials have been put to use by researchers (Daliri and Lee, 2015). Probiotic bacteria's source or origin, lack of infectivity, pathogenicity characteristics, and virulence factors including their resistance to antibiotics together with their metabolic activity should be appraised in an assessment of their safety status (Junior *et al*., 2015).With respect to use in human medicine, antibiotic resistance is one area that requires analysis due to the grave concerns about the mounting level of resistance to antibiotics. The absence of resistance activity in these probiotic microorganisms should be a selection criterion (Wakil and Olorode, 2018).

A good probiotic must stay alive while transiting through the stomach which has a pH in the range of 1.5 and 2.0, resist digestive enzyme and bile before reaching onto other parts of the gastrointestinal tract (Thakkar *et al*., 2015). Physiological human bile concentration ranges between 0.1% and 0.5% (Mathara *et al.,* 2008) and its staying time remains estimated to be four hours (Thakkar *et al*., 2015). Agaliya and Jeeveratnam, (2012) reported species of *Lactobacillus* grew in the presence 0.05%, 0.1% 0.3% of bile concentration after four hour of incubation.

Probiotics are required to show antagonism towards opportunistic pathogens in the gastrointestinal tract a trait which is more of prophylactic and bio-therapeutic to anyone who ingests it (Hardy *et al*., 2013). This antagonistic nature is demonstrated in their capacity to impart microbial homeostasis, occupy functional niches left open by endogenous community, compete predominantly for nutrients and physical sites or receptors hence dislocating opportunistic pathogens from same niche, lowering their invasion as well as development in the body by producing organic acids, bacteriocins and hydrogen peroxide (Rattanachaikunsopon and Phumkhachorn, 2010).

Probiotic must possess a good shelf life in food preparations and constitute a large number of viable cells at the stage of consumption to impart health advantage on the host

successfully (Paraschiv *et al*., 2011). A standardized probiotic food as reported by the Food and Agriculture Organization, is required to have a least dose of 10^6 CFU/g alive and active as at the time of ingestion (Rasic, 2003).

Probiotic culture like lactic acid bacteria found in fermented food substances or alone should subdue genetic alterations caused by genotoxic substances formed inside the body as they have been reported to produce butyrate gotten from lactase which inhibits inflammation, cancer development, bolstering of components of the defense mechanism and reduce oxidative stress (Kalhoro *et al*., 2019). A physiological state which come about as an upshot of abnormal increase in reactive oxygen species level compared to capacity of antioxidants in tissue and organ systems (an indication of toxicity) is known as oxidative stress (Renuka *et al*., 2015).

Measurement of biomarkers of oxidative stress through colon tissue,an indicator of a possblephysiological damage leading to increased reactive oxygen speciesas a result of oxidation could be a reliable tool to assess diarrhoea (Mastorci *et al*., 2017). Reactive oxygen species if not neutralized can damage cellular macromolecules susceptible to oxidation (Wang *et al*., 2017). A mechanism in charge of counteracting damage by reactive oxygen species in tissues is important enzymes like glutathione peroxidase, superoxide dismutases alongside catalase which reduces concentration of harmful oxidants (Zadak *et al*. 2009).

Components of the physiological defense system are essential minerals such as copper, manganese and zinc necessary for the formation of these enzymes and other antioxidants from diets (i.e. carotenoids, vitamin E and vitamin C). Reports have it that the depletion of these minerals and vitamins has substantial influence on development of oxidative stress during some nutritional and clinical condition (Zadak*et al*., 2009). Probiotic bacteria are noted to carry out antioxidation through: chelating metal ion, enzyme system, antioxidant metabolites, mediating antioxidant signaling pathway, regulating the enzymes producing reactive oxygen species, regulating the intestinal microbiota etc. (Wang *et al*., 2017).

Suggestions founded on reports from *in vitro* as well as *in vivo* systems have it that probiotic microorganisms can increase immune responses most likely by increasing the

levels of natural killer cells, immunoglobulins, cytokines, and activate macrophages hence boosting the immune system and stopping occurrence of infections (Kailasapathy, 2013). Probiotic bacteria such as *Lactobacillus pentosus*, *Lactobacillus brevis* with the Generally Regarded as Safe (GRAS) status have been reported to reduce incidence and duration of gastroenteritis and bind aflatoxin B1, a potent mycotoxin of various aflatoxins which could be produced in the course of storing cereal grains (Soro-Yao*et al.,* 2014).

2.7 Fermentation

Fermentation of food is one old and low cost food processing methods which evolved due to its practical use to preserve food thereby increasing its shelf life for later use, enhancing properties such as taste, aroma, texture, nutritive value among other positive qualities (Sengev *et al*., 2016). It has over the years become and still forms a segment of traditional and cultural customs among ethnics in Africa (Chelule *et al*., 2010). In food processing, fermentation using yeast together with or bacteria at optimum conditions leads to carbohydrates being transformed into alcohol and carbon dioxide (CO_2) or organic acids (William and Dennis, 2011).

Fermentation is a less labour demanding process which utilizes locally purchased raw material noticeably affecting the worldwide resource of food to humans hence contributing to the daily diets of consumers of such food, directly impacting and effecting curative, preventive and other health benefits (Sengev *et al*., 2016). Fermentation has and continues to suit developing countries socio-economic framework as an affordable technology for food preservation at little or no cost even when refrigerating facility is lacking such that these foods still conform to acceptable standard (Ikujenlola and Adurotoye, 2014).

In food substrates, fermentation serves functions like reduction of: viscosity, bulk density and antinutriets; food substrate enrichment with essential amino acids, vitamins and essential fatty acids; detoxification, improvement of palatability and acceptability biologically to mention a few (Thorat *et al*., 2017). Fermentation of food substrates promotes reduction in percentage of its dry matter, bringing about a concentration of protein, minerals and vitamins which seems to be on an increase through microbial

synthesis and act as a wall against bacteria which are non-acid tolerant which becomes ecologically excluded from the food content (Sengev *et al*., 2016). Worthy of note is its ability to reducefuel use and thecosts for cooking food materials having changed its nature and property in its raw form (Oyewole and Isah, 2012). Aworh (2008) reported that fermentation improved the texture of foods, imparted pleasant sour taste to fermented foods such as garri and ogi adding distinct flavor and aroma constituents atypical to fermented foods making them more sorted after than ones unfermented considering consumer acceptance.

Increase in the nutritive quality of the plant foods comes along with reduced phytochemicals level such as phytic acid [which in cereals has been wholly degraded by adding exogenous phytases commercially produced or by stimulating indigenous phytases after soaking, germinating alongside fermenting (Marero *et al.,* 1991), polyphenols, and oxalic acid (Hotz and Gibson, 2007). Fermenting food substrates due to their acidic nature, boosts microbial enzymes activity during lactic acid fermentation affording an enzymatic degradation of phytic acid present in cereals in the form of complexes with polyvalent cations (such as iron, zinc, calcium, magnesium etc.) at optimum pH conditions (Ikujenlola and Adurotoye, 2014). Fermentation and cooking combined has been found to improve the quality of nutrient of all tested sorghum seeds as well as reduce the content of antinutritional elements to a non-injurious level in contrast with other processing techniques (Adesulu and Awojobi, 2014).

2.8 Fermented food

Fermented foods are staples already exposed to the influence of microorganisms and their enzymes to bring about desired significant change to the raw food (Mesele, 2018). These produce are described as wholesome, palatable possessing inviting flavors, aromas, texture and improved processing and cooking properties and have been consumed even before the start of human civilization (Marcó*et al*., 2017).

Fermented food produced from dairy foods, maize, millet, maize, sorghum amon others help prevent protein needs of inhabitants of West African countries and are mainly important as dietary staples with reduced carbohydrates level together with poly-

oligosaccharides that are non-digestible (Soro-Yao *et al.*, 2014). According to Odunfa (1985), these fermented foods may be grouped according to their starting raw materials. In Nigeria, we have fermented products from maize, millet, sorghum, acha ("masa", "pito", "ogi", "ogi baba", "burukutu", "kunuzarki"), from cassava ("fufu", "lafun", "garri",), from soya beans and African locust beans and soya beans ("dawadawa", "iru"), from oil bean ("ugba"), from melon seed ("ogiri"), from wine etc. (Adesulu and Awojobi, 2014).

Fermented gruel made from these staples and used as complementary food are observed usually to be limited in important nutrients, resulting into protein energy imbalance (malnutrition) prevalence during the period of weaning (Egwim*et al*., 2013). Upon several investigations and discoveries of the nutritional benefit and inadequacies of fermented gruel linked to their physico-chemical properties, attempts channeled at nutrient upgrade through pre-treatment, supplementation and fortification (as seen in mixing with legumes, other suitable food substances like dried leaves of *Moringa oleifera*, pumpkin leaf powder, crayfish, parts of edible fruits at various levels of substitution) have been made (Wakil and Alao, 2013; Sengev *et al*., 2016).

In a number of developing countries, traditionally fermented food taken either as beverage, main dish or condiment are a rich hob of probiotics hence they have gained popularity because they could fall into the category of food known as functional foods due to their ability to confer specific health benefit (through the microorganisms enzyme or other products of their metabolic processes available in the fermented food which may perform as antigens stimulating antibodies production) (Sharma *et al*., 2013).

Probiotic use as starter cultures in place of naturally occurring microorganisms (which do not pose any health risk) to ensure predictable variation (consistency) and desirable qualities with respect to sensory, nutritional as well as rheological properties of final fermenteation product has been explored especially in cases where high level fermentation is difficult to achieve, not predictable with respect to length of fermentation period and quality of products and in the long run conferring a health benefit (Singh *et al*., 2014).

2.9 Lactic acid bacteria

Lactic acid bacteria (LAB) refer to a large bacteria group that are facultative anaerobes strictly fermentative. They occasionally possess highly fastidious nutritional requirement for amino acid and carbohydrates in most food substrates (Mesele, 2018). They are gram-positive, non-motile, non-sporulating, insensitive to oxygen (hence grow and survive in its presence) and produce lactic acid which is a major fermentation product (Balciunas *et al*., 2013). Lactic acid bacteria groups' heterogeneity is expressed well in their morphological characters as their cells appear in single, couple, tetrads and short or long chains of coccal or rod shapes (Settanni *et al*., 2010). They generally lack catalase, in rare cases pseudocatalase can be found though (Schleifer and Ludwig, 1995). A major differentiation between lactic acid bacteria subgroups is the pattern of product formed from the fermentation of sugars as they are grouped as homo-fermentative (i.e. they produce a single fermentation product i.e. lactate), and hetero-fermentative (i.e. they produce other products majorly carbon (IV) oxide, ethanol and lactate) (Yelnetty *et al.,* 2014). The lactic acid bacteria possess peptidoglycan, polysaccharide, and teichoic acid which are all components of cell wall which have been revealed to possess immunostimulatory properties (David and Famurewa, 2010).

In nature, lactic acid bacteria are broadly dispersed with heavy presence usually found in cereal, gastrointestinal flora of man and other animals, meat and meat products, raw vegetables and decomposing plants all very rich in nutrients essential for their sustenance (Yelnetty *et al*., 2014). Lactic acid bacteria, generally known as food grade organisms, demonstrates special positive fermentative effect considered for their selection and application as defensive cultures (Thakkar *et al.,* 2015). Lactic acid bacteria play a vital part in a fermentation process by acidifying a substrate rapidly via production of organic acids and in the process improve stability, reduce flatulence and cholesterolemic effect, increase nutrient value of these fermentation product etc. (Yalnetty *et al*., 2014).

Having been used to slow spoilage process thereby sustaining food preservation, lactic acid bacteria have found applications commercially as starter cultures in food industries as compounds like diacetyl, organic acids, bacteriocins also known as bactericidal proteins

and hydrogen-peroxide produced during lactic fermentations are leveraged on (Achi and Ukwuru, 2015). These qualities are not only focused for the effects they have on food smell, colour, taste, texture and smell but are furthermore engaged in inhibiting harmful microflora, resisting levels of acid, salts and bile salts on the high side in the gastrointestinal tract which is of great value to health, produce amino acid decarboxylase, amylolytic, lipolytic and proteolytic enzymes required to hydrolyze major nutrients in fermenting substrates. These qualities raise the fermented food status to that of a functional type (Nwagu and Amadi, 2013).

Lactic acid bacteria belong to the major representative of safe probiotics due to the proposed positive roles they play in treating and managing metabolic diseases, improving immune system, oxidative status and growth as they are naturally resident in the human gastrointestinal system (Yalnetty *et al*., 2014). Therefore the concept of providing nutritional supplements, pharmaceuticals and notably functional foods which is fast gaining grounds has steered up increased curiosity in the development of novel food containing a significant ratio of probiotic microorganisms with the Qualified Presumption of Safety status (Wang *et al*., 2017).

2.10 Cereals

Cereals are of the monocot family known as Gramineae with stalks that are thin and long (Ugwuona *et al*., 2012). Majorly they constitute a part of African diet and are used to prepare a variety of foodand beverages (Evans *et al.,* 2013). Cereals like barley, sorghum, millet, maize etc. are grown in large amounts, therefore providing food and energy globally than other variety of crop (IDRC, 2016).

For the growth of probiotic microorganisms, cereal grains are good fermentable substrates and in recent years have been accepted as functional food being main sources of energy, dietary fibre, anti-oxidants, protein, vitamins and minerals with beneficial effect on target roles in the human body obligatory for good health (Gernah *et al.,* 2011). Reports have it that grains ingestion are linked with reduced risk of inflammatory bowel disease, delayed gastric emptying, coronary heart disease, blood pressure, some cancers of the large

intestinal bowel etc. which are severe diet-related disease conditions (Kumar *et al*., 2011; Achi and Ukwuru, 2015).

The cereal grains of economic significance are those of the cool season mostly wheat, barley, oats, etc. and the warm season e.g. rice, maize, sorghum, millet, acha etc. which have played foremost role in the improvement of traditional agriculture, nutrition, and indigenous medicines (Ballogou *et al.,* 2013). For so long, these have stayed the chief constituent of man's diet bearing in mind they are staples which are essential and critical to everyday existence of countless individuals above 50% of the World and 77% of Africa (Sarwar *et al*., 2013).

In Africa, a bulk of traditional cereal based foods used importantly as complementary foods for infants and as dietary staples for grown persons are processed through natural or spontaneous fermentation (Wakil and Kazeem, 2012). Fermented cereal foods made from millet, maize, sorghumare either porridge (ogi, mawe) or stiff gels (agidi, banku, kisra, kenkey, injera) in terms of texture (Sanni and Adesulu, 2013).

Cereals are pre-treated depending on its type as well as its desired finished product. Drying, washing, steeping, roasting, wet or dry milling together with sieving are possible pretreatment processing steps applied in preparing fermented cereal foods (Wakil and Alagbe, 2017). The bran (pericarp) and germ of some grains used as human food are removed by milling just to meet up with sensory expectancies of consumers, but this process strips these grains of vital nutritional quality (protein, dietary fiber, vitamins, minerals, phenolics etc.) which are of significant benefit to human health (Awika, 2011).

Cereals protein content are known to be lacking in essential amino acids like lysine, tryptophan and threonine hence are of poor nutritional quality for its consumers (Gernah *et al.,* 2011). Efforts to increase the nutritional status of staples have been carried out in supplementing cereals with legumes to make available these lacking amino acids (Achi and Ukwuru, 2015). The use of tiger-nut and soya bean to produce complementay food of improved nutritional quality has been reported (Abdulkadir and Danjuma, 2015; Wakil and Alagbe, 2017). Also the utilization of *Moringa oleifera* leaves concentrated with higher amounts of vitamins, minerals and phytochemical in synergy with a supplemented

fermented cereal legume blend increased the nutritional value of traditional complementary foods and has been suggested could have unique nutrient composition for feeding and boosting the immune system hence lessening the occurrence of most nutrition based diseases (Wakil and Alao, 2013).

2.10.1 Pearl millet

(*Pennisetum glaucum*) is one of the oldest grown foods reported with a higher protein composition and superior amino acid balance compared with sorghum, maize with other cereals (Oelkel *et al.,* 1990).Its unique nutritional quality is conferred on it by the higher proportion of germ to endosperm (Dendy, 1995). Amongst its wide range of uses are; its consumption in form of alcoholic beverages, breads, boiled or steamed food and porridges in African countries where they are grown (Apaliya *et al*., 2017). Millet seeds are reported to be rich with phytochemicals which has anti-oxidative effects due to their direct free radical scavenging activity as seen in the example of phytic acid which has been linked to cancer and cholesterol reduction (Achi and Ukwuru, 2015).

2.11 Legumes

Legumes of the family *Leguminosae* found in tropical areas of Asia and Africa are a vital source of inexpensive protein for poor persons where they are mainly ingested to address protein malnutrition and other food security concerns as its protein content doubles or triples that of cereals (Okoruwa, 1999). They could be made into several processed semi-finished and finished products (Ojimelukwe, 2009).

The chemical composition of legumes varies depending on their source, variety and species (Annor *et al*., 2014). Protein contained in legumes is rich in lysine for instance which is an amino acid that is essential, making them good supplements for cereals known to lack such, still theyare deficient in sulfur containing amino acids like cystine and methionine (Freidman, 1996). In tropical areas, commonly grown legumes including pigeon pea, kidney bean, cowpea, groundnut, soybean, bambara groundnut, lima bean to mention a few, are known to have higher levels of riboflavin, thiamin, and niacin similar to those obtainable from egg, milk, beef and fish (Ikujenlola and Fashakin, 2005).

With exceptions of groundnut and soybean, majority of legumes are a low fat source but a rich hob of diverse minerals like iron, phosphorus and calcium with improved bioavailability often if subjected to specific processing (Afify *et al*., 2011). A militating factor against legumes especially when it is not adequately and properly processed before use is its inappropriate level of antinutritional constituents like saponin, oxalate, phytic acid, lectins, oxalate, goitrogen, phytic acids, tannin and trypsin inhibitor all of which causes reduced protein digestibility and availability together with flatulence, diarrhoea etc. in infants (Armar-Kelemesu and Wheeler, 1991). Amarowicz and Pegg (2008) reported that some of these phytochemicals however confer health gains as tannin have been observed to have antioxidative activity.

2.11.1 Groundnut

Arachis hypogeae L. is the world's third to fourth most significant source of vegetable protein and edible vegetable oil respectively (Lucas, 1979). It is a good source of crude protein content (of a higher proportion than any tree nut) and niacin hence contributing to brain health and blood flow (Pancholy *et al.,* 1978). They are good source of foliate, fiber, magnesium, vitamin, manganese, phosphorus and naturally free of trans-fats (Adenuga, 2010).

Supplementation of cereals with groundnut has ensued in upgrading the nutritional quality of dietary proteins. An appreciable increase in the nutritional quality of a complementary blend made of millet supplemented with groundnut was reported by Wakil and Alao (2013) while muffins prepared from wheat and peanut flours had substantial protein content which can serve as a high-protein bakery items or snack foods as reported by Ory and Conkerton (1983). Data obtained and presented on peanut flours used in protein fortification have shown significant upgrading in protein quality of akara, puff-puff, chin-chin etc. (Reber *et al.,* 1983). Children placed on millet and rice diets fortified with peanut experienced greater weight growth and height, greater chest and arm development and higher hemoglobin concentration levels than children placed on a different diet in a control group (Devadas *et al.,* 1984).

2.11.2 Cowpea

Vigna unguiculata (L) Walp is a warm seasonal herbaceous leguminous plant indicated to have originated from Africa were it was introduced alongside millet and sorghum (Annor *et al*., 2014).

Cowpea used for human and animal nutrition are good source of proteins as well as carbohydrates (Minka and Bruneteau, 2000). Their proteins are made up of over 30% of essential amino acid with lysine and leucine being predominant (Jirapa *et al*., 2001). Dry cowpea is considered nutritious as it quantitatively has a protein content of about 23%, 1.8% fiber content, 1.3% fat content, 67% carbohydrate content and 9% water content which complements the nutritional content of cereals (Oyarekua, 2011). Cowpea is reported to be high in dietary fiber, selenium, iron, potassium, molybdenum, thiamine, vitamin B6 and folic acid (Choung *et al.,* 2003).

2.12 *Moringa oleifera*

Moringa oleifera is a perennial small, fast growing evergreen tree which usually grows as high as 9m, with a wood soft and white, corky and gummy bark (Mishra *et al.,* 2011). All parts of the Moringa tree are edible and have long been consumed by humans (Fahey, 2005). It has been backed for traditional medicine and industrial uses and known as "Mother's best friend" in some parts of the world (Jed, 2005). Other uses for Moringa include green manure, animal forage,alley cropping, , domestic cleaning agent biogas, , blue dye, fencing, fertilizer, foliar nutrient, gum, honey as well as sugar cane juice clarifier, ornamental plantings, bio pesticides, rope, etc. (Fahey, 2005). Another identified uses of Moringa is the use of its seeds in powdered form: to flocculate contaminants as well as purify drinking water, for tea etc. (Gassenschmidt *et al.,* 1995).

In recent time, the tree has been advocated as an exceptional indigenous source of amino acids, fatty acid, minerals, vitamins suitable for use in developing regions of the world where undernourishment particularly among infants and nursing mothers is a major concern (Aljohani and Abduljawad, 2018). Uncountable instances of live saving nutritional rescue that are attributed to moringa has been reported based on its nutritional qualities which exist in both scientific and popular literatures as it leaves comprisemore

iron than spinach,more calcium than milk,more vitamin A than carrots, more Vitamin C than oranges and more potassium than bananas, and a high phosphorus content and moderate antioxidants concentration (Fuglie, 2000). According to Fahey (2005), moringa leaves protein quality rivals that of milk and eggs. It has been observed by Asian Vegetable Research and Development Centre (AVDRC) scientist that boiled moringa leaves or leaf powder provides at least three times more bio-available iron than raw moringa leaves and that nutrient content was more in mature than younger leaves however the younger shoots is preferred to be eaten (Price *et al.,* 1985). The vitamin A content is more during hot wet season, while iron and vitamin C are more during cool dry season (Price *et al.,* 1985).

Alkaloids, flavonoids, tannin, and other antioxidants found in plants reported to have antimicrobial and antidiarrhoeal activity (Shiriki *et al*., 2015) are present in abundance in *Moringa oleifera*. The biological activities of this plant to strengthen the immune system against various infections as linked to these antioxidants may be done through reduced intensity of lipid peroxidation, inhibition of generation of free radicals and enhanced activities of anti-oxidants enzymes (Aljohani and Abduljawad, 2018).

Air drying has been utilized traditionally as a method for preserving certain vegetables in most developing countries and one such plant is *Moringa oleifera* (Gyamfi, 2011). The basis for drying is to decrease: the moisture content to a level which prolongs shelf life, colonization by microorganisms and loses in nutritional quality (Eklou *et al.,* 2006).

Moringa is particularly promising as a food source in the tropics because the tree has full leaf at the end of the dry season when other foods are typically scares and they can be eaten fresh, cooked, or stored as dried powder for a long period without refrigeration, and reportedly without reducednutritional value (Fahey, 2005).

A need to study the effect of having a synergetic balance of readily available and cheap indigenous based plants materials in a complementary food fermented with microorganisms with probiotic potentials which could impact nutritional and antidiarrhoeal effect in infants at the weaning stage is essential.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample collection

Pearl millet (*Pennisetum glaucum*), Ife brown cowpea (*Vigna unguiculata*(L) walp) and groundnut (*Arachis hypogaea L*) used for this research work were collected from the Institute of Agricultural Research and Training (I. A. R. & T) These were conveyed to the Department of Microbiology laboratory, University of Ibadan, Ibadan, Nigeria and preserved in the refrigerator (4^{o}C) until use.

Fresh *Moringa oleifera* leaves were collected from the Botanical garden of the Department of Botany, University of Ibadan, Ibadan in clean polyethene bags, and transported to the herbarium of same department for authentication (authentication number- U/H-22698) before being transfered to the Microbiology laboratory for further use.

3.2 Processing of cereal, legumes and M*oringa oleifera* leaves samples

Pearl millet(*Pennisetum glaucum*)were sorted manually by separating broken and moldy grains from the main bulk. The grains were washed severally in distilled water to free from dirt. Clean grains were dried in the oven (50^{o}C) for eight hours and dry milled into fine particle size using a sterile blender. To obtain finer particle size, the milled flour was sieved and packed in polyethylene bags that are clean and stored at 4^{o}C for further use.

The cowpea seeds (*Vigna unguiculata*) were freed from dirt by manual separation and washed in distilled water as seeds floating were discarded. Cleaned seeds of cowpea were soaked for 5 minutes in distilled water followed by manual dehulling. The dehulled clean cowpea seeds were oven dried, dry milled using a sterile blender, sieved to obtain a fine particle size, packaged into polyethylene bags that are clean and stored at 4^{o}C pending use.

Groundnut seeds were sorted manually by removing spoilt and moldy seeds, washed severally in distilled water to free from dirt. Clean seeds were air dried, and roasted using an oven. Roasted seeds were dry milled using sterile blender to a fine particle size, and packaged into polyethylene bags that are clean and stored at 4^{o}C for further use.

Moringa oleifera leaves were washed with water and dried in open air at 30 ± 2^{o}C for seven days in an area protected from dust, pests (to prevent contamination) and light to prevent loss of vitamins. Using an electronic blender, dried *M. oleifera* leaves were milled into fine powder, sieved to obtain finer particle size, secured in well labelled polythene bags that are clean for storage at 4^{o}C for subsequent use.

3.3 Formulation of composite blends

Blend formulation: This was done using the modified method of Malleshi *et al*. (1989)

Table 3.1: Formulation of Blends (%)

Sample Code	P	C	G	M
F1	70	25	5	0
F2	70	24	5	1
F3	70	23	5	2
F4	70	22	5	3
F5	70	21	5	4
F6	70	20	5	5

P- Pearl millet, C- Cowpea, G- Groundnut, and M- *Moringa oliefera* leaves

3.4 Fermentation and sampling

The formulations were transferred into sterile flasks separately with appropriate labeling for further study. Using the method of Livingstone *et al*. (1993), reconstitution of the flour blends with sterile distilled water was done at concentration of 30% (w/v) and allowed to ferment spontaneously at room temperature (27 ± 2^{o}C) for 72 hours. During fermentation, samples were obtained from each fermenting formulations to evaluate microbial load, nutritional quality and physico-chemical parameters of the fermenting slurry 24 hourly.

3.5 Chemical analysis of sample

3.5.1 Hydrogen ion concentration (pH)

Monitoring the pH of the fermenting gruel and steeping water was done using a pH meter (Jenway, UK) 24 hourly. Ten milliliter of a mixture of the fermenting gruel and steep water were obtained aseptically from the fermenting blend and its pH was measured using the glass electrode of the pH meter (Wakil and Aalo, 2013).

3.5.2 Total titratable acidity (TTA)

Total Titratable Acidity (stated as percentage lactic acid) of the fermenting slurries was determined using the method of Ojokoh and other (2015) by titrating 10 mL aliquot against 0.1 N NaOH (sodium hydroxide) using 1% phenolphthalein as indicator. A colour change to pink was the endpoint and the titre value was noted(Wakil and Aalo, 2013).
This was carried out in triplicate.
TTA was determined using the equation:

$$\%TTA = V_{NaOH} \times N_{NaOH} \times M.E. \div 100$$

Where,

V_{NaOH}= volume of sodium hydroxide (measured in mL)

N_{NaOH} = molarity of sodium hydroxide

M.E= mass equivalence of lactic acid (approx. 90.08).

3.6 Microbiological analysis

To nine milliliters of sterile distilled water, one milliliter volume of fermenting slurry was added to give 1:10 dilution and was serially dilluted up to 10^{-10}. Aliquot (0.5 mL) of higher dilutions were introduced into appropriate media using pour-plate method described by Harrigan and McCance (1976). De Mann Rogosa and Sharpe (MRS) medium used for lactic acid bacteria (LAB), Nutrient agar for aerobic bacteria/ total viable count (TVC), MacConkey agar for enteric bacteria, Malt Extract agar (MEA) for moulds and yeasts. Plates were incubated at 35 ± 2^{o}C for 24 hours (aerobic and enteric bacteria), and 48 hours (lactic acid bacteria, mould and yeast). Colony growth were observed on plates, counted and randomly selected after the incubation period.

3.7 Obtaining and preserving pure cultures of lactic acid bacteria

Colonies of lactic acid bacteria picked on MRS agar plates were streaked continuously on a MRS agar plate until pure cultures were obtained. Pure cultures were transferred onto sterile MRS agar slants, followed by incubation at 30 ± 2^{o}C until growth became visible and storage in a refrigerator at 4^{o}C. Sub-culturing of the isolates was done every 2 - 3 weeks onto MRS agar medium to maintain viability.

3.8 Microbiological characterization of lactic acid bacteria isolates

3.8.1 Cultural characteristics

Cultural features of lactic acid bacteria (LAB) colonies on the plates were examined for size and shape on growth medium.

3.8.2 Gram's staining

On a grease free glass slide, a thin smear of an 18 - 24 hour old LAB culture was made and heat fixed by way of passing over a burner flame. Each smear was flooded with 2 drops of Crystal Violet solution for 1 - 2 minutes, rinsed with water and Gram's iodine solution was added for sixty seconds. This solution was also washed off the slide with 95% alcohol until no more violet traces were seen and then washed under gentle-running clean water. It was counterstained with two drops of safranin reagent between 1 – 2 minutes, rinsed using clean water, dried by blotting utilizing a filter paper and examined under the microscope utilizing oil-immersion objective. Thebacteria that were Gram positive were identified with purple colour while Gram negative bacteria were identified by their pinkish coloration. This technique was utilized in observing different shapes as well as arrangements of the bacteria cells (Olutiola *et al.,* 1991).

3.9 Biochemical characterization

3.9.1 Catalase test

A thick emulsion of each LAB isolate was made on a clean glass slide. Two drops of 3% H_2O_2 was then added and the reaction was examined. Effervescence revealed a catalase positive reaction(Olutiola *et al.,* 1991).

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

3.9.2 Endospore test

A thin smear of each LAB isolate from 18 - 24 h old culture was made on a thin glass slide and heat-fixed by passing above a burner flame. Malachite green solution was poured on the smears and steamed within 5 - 10 minutes ensuring that the stain does not dry out, after which it was washed carefully with water. Safranin solution was utilized to counterstain the smear for 15 seconds, washed with water, blotted dry and examination under the light microscope using the oil-immersion objective (Olutiola *et al.,* 1991). Spores were identified by their green colouration and vegetative cells were identified by their red colouration.

3.9.3 Motility test

Half strength medium (MRS) used for this test was dispensed into test tubes, sterilized, allowed to cool and set. Isolates from 18 - 24 hour old lactic acid bacteria culture were inoculated on the agar by a gentle straight stab using a sterile inoculating needle followed by incubation at 37^{o}C for 24 hours. Growth along the line of stab indicates a negative result while growth away from the 'line of stab' into the agar indicated a positive result(Olutiola *etal*., 1991).

3.9.4 Oxidase test

Few drops of 1% solution of tetramethyl-p-phenylenediaminedihydrochloride placed onto a piece of whatman filter paper was smeared with some lactic acid bacteria cultures taken with a sterile wire loop. A purple colour developing within 5 to 10 seconds indicates a positive reaction. Colour development however may take up to 10 - 15 seconds in a weak or delayed positive reaction. The absence of purple coloration indicates a negative reaction (Olutiola *etal*., 1991).

3.9.5 Methyl red and Vogues–Proskauer test

Into culture tubes was dispensed 10 mL of glucose phosphate broth and sterilizedat 121^{o}C for 15 minutes. Afterward, the culture tubes containing the sterilized medium were

inoculated with lactic acid bacteria culture. Incubation of culture tubes was done at 37^{o}C for two to five days. Five millilitres of the culture was taken into sterile tubes and few drops of methyl red indicator was added and production of a red colouration was considered positive for methyl red test after incubation. To 1 mL of 6% of ∝-naphtol solution, 1 mL of 10% NaOH was added in a sterile tube. Development of red colouration in the space of 5 minutes was indicative of a positive reaction for Voges-Proskauer's test(Olutiola *etal*., 1991).

3.9.6 Citrate utilization test

Simmons's citrate agar was dispensed into screw cap tubes and sterilized at 121^{o}C for 15 minutes. The tubes were left to solidify and inoculated with the 24 hours old culture of LAB isolates and subsequently incubated for 2 - 3 days at 35 ± 2^{o}C. The growth of the test isolate in the medium signified their ability to utilize citrate in the medium. Appearance of blue coloration indicated a positive result while the initial green coloration was retained for negative results (Olutiola *etal*., 1991).

3.9.7 Indole test

Indole test was done to determine amino acid tryptophan degradation ability of LAB isolates to produce indole. Lactic acid bacteria was inoculated into sterilized screw cap tubes containing 10 mL of 1% peptone broth followed by incubation 35 ± 2^{o}C for 5 - 7 days after which 0.5 mL Kovac's reagent (5 g P-dimethylamino-benzaldehyde in 75 mL amylalcohol and 25 mL concentrated Hydrochloric acid) was introduced and rocked gently. The culture tubes were made to stand for 20 minutes in order to allow the rising of the reagent to top of the medium. A red colour at the reagent layer is indicative of production of indole (Prescott *et al*., 2002).

3.9.8 Starch hydrolysis

MRS agar plus 1% soluble starch was sterilized at 121^{o}C for 15 minutes and poured to set in sterile Petri-dish. Plates containing medium were inoculated by streaking across the surface followed by incubation for 2 - 3 days. Gram's iodine was poured on the plates and observed for blue-black colouration. Non-hydrolyzed starch developed a blue–black

colouration and a positive reaction was seen as a clear zone due to hydrolysis of the starch (Olutiola *et al.,* 1991).

3.9.9 Protein hydrolysis

Test for proteolytic activity of the LAB isolates was carried out on skimmed milk agar plates. Five grams of skimmed milk powder and 1.25 g of agar powder was dissolved in 100 mL of sterile distilled water in a beaker. The solution was autoclaved at 110^{o}C for 10 minutes. The cooled skimmed milk agar was poured in petri-dishes, left to set, inoculated with lactic acid bacteria followed by incubation at 35 ± 2^{o}C for 24 hours. Proteolytic activity of LAB was shown by clear zones of hydrolysis in the medium around the colonies (Harrigan and McCance, 1976).

3.9.10 Lipid hydrolysis

Test for lipolytic activity of the LAB isolates was carried out on agar plate containing peptone (1%), $CaCl_2$. H_2O (0.01%), agar (2%), Tween 80 (1%) and olive oil. Bacterial isolates were inoculated on this medium followed by incubation at 35 ± 2^{o}C for 48 hours. A positive reaction was seen as produced halo around the colony due to hydrolysis of lipid.(Kim *et al*., 2001).

3.9.11 Nitrate reduction

Five millilitres of broth medium consisting of peptone water and 0.1% KNO_3 was dispensed into culture tubes consisting of an inverted Durham tube each. The tubes were covered with a screw-cap, sterilized at 121^{o}C for 15 minutes. The tubes were allowed to cool, inoculated with LAB and incubated at 37^{o}C for 4 days. The addition of 1% suphuric acid in 5 N acetic acid followed by 0.5 mL of 0.6% dimethylnaphthylamineic acid to each tube was done to determine the ability of the lactic acid bacteria to convert nitrate to nitrite and ammonia and finally to free nitrogen through reduction processes. Red colouration of the medium showed that the test organism was able to reduce nitrate present in the medium and a positive result. Presence of gas in the Durham tube showed that nitrogen gas was produced(Olutiola *etal*., 1991).

3.9.12 Ammonia production from arginine

MRS-Arginine broth (modified MRS broth) containing 0.2% sodium citrate instead of triammonium citrate and 0.3% Arginine excluding meat extract and glucose was utilized. MRS broth which had no arginine was used as control. An eighteen to twenty-four 24 hours old culture of LAB isolate was inoculated into culture tube containing 10 mL of sterile broth followed by incubation at 30^{o}C for 5 days. Subsequent introduction of drops of Nessler's reagent into the culture tubes resulting into an observed yellow or orange colour as compared to that produced by a similarly treated control medium indicated the production of ammonia from Arginine (Olutiola *etal*., 1991).

3.9.13 Sugar fermentation test

For determination of the test isolates abilities to ferment sugars, test sugars each were mixed with modified MRS broth containing bromocresol purple indicator to be used as sole carbon source. The lactic acid bacterial test isolates were inoculated into tubes containing basal medium mixed with the sugars and incubated for 5 days at 30^{o}C. A change in colour from purple to yellow indicated a positive reaction (Olutiola *et al.,* 1991).

3.9.14 Gas production from glucose

Carbon dioxide production from glucose according to the method of Bulut (2003) was used in determining heterofermentative and homofermentative lactic acid bacteria isolates. Ten milliliters each of MRS broths which had no citrate was dispensed in glass tubes inserted with inverted Durham tubes, sterilized at 110^{o}C for 10 minutes, cooled, and inoculated with a loopful of overnight fresh cultures of bacterial isolates being tested followed by incubation for 5 days at 37^{o}C. The control comprised of a medium that was not inoculated with the test isolate. The presence of air trapped in the Durham tube shows gas production from glucose for heterofermentative lactic acid bacteria isolates while there were no traces of trapped air in the Durham tubes showing no gas production for homofermenters.

3.10 Molecular identification of lactic acid bacteria

3.10.1 DNA extraction

The DNA extraction was carried out by the method of theInceoglu *et al*.(2010). A 1.5 mL of 12 hours lactic acid bacterial broth cultures were transferred to 1.5 mL micro centrifuge tube and spun for 5 minutes. The supernatant was decanted, and the pellet was drained well onto a Kimwipe. The resuspension of the pellet was done in 520 µL of TE buffer (comprising 10 mMTris-HCl, 1mM EDTA, pH 8.0) by recurrent pipetting. Fifteen microliters of 20% sodium dodecyl sulphate (SDS) and 3.0 µL of 20 mg/mL proteinase K were added, mixed and incubated for 1 hour at 37°C. A hundred microlitres of 5 M NaCl and eighty microlitres of a 10% cetyl trimethylammonium bromide (CTAB) solution in 0.7 M NaCl were added and votexed, incubated for 10 minutes at 65°C and kept on ice for 15 minutes. Appropriate aliquot of equal volumes of phenol/chloroform was added and mixed well but very gently to avoid shearing of the DNA by inverting the tubes until the phases were completely mixed. The DNA/Phenol mixture was carefully transferred into a Phase Lock Gel tube and allowed to spin at 12,000 rpm for 10 minutes. The upper aqueous phase was carefully transferred to a new tube followed by an addition of equal volumes of phenol/chloroform. The upper aqueous phase was again transferred to a new tube followed by an addition of 0.1 volume of 3 M sodium acetate and mixed. Isopropanol (0.6 volumes) was added and mixed gently until the DNA precipitated. DNA was spooled onto a glass rod, washed by dipping end of rod into with 500 µL of 70% ethanol for 30sec and resuspended in 50 µL of TE buffer.

3.10.2 Polymerase chain reaction (PCR)

PCR sequencing preparation cocktail comprised 10 µL of 5x GoTaq colourless reaction, 3.0 µL of 25 mM Magnesium chloride ($MgCl_2$), 1.0 µL of 10 mM ofnucleoside triphosphates containing deoxyribose as the sugar (dNTPs mix), 1.0 µL of 10 pmol for forward (27F 5'- AGA GTT TGA TCM TGG CTC AG-3') and another for reverse (-1525R, 5′-AAGGAGGTGATCCAGCC-3′) primers and 0.3 units of Taq DNA polymerase (Promega, USA) made up to 42 µL with sterile distilled water and 8.0 µL DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem

Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for five minutes; followed by a thirty cycles consisting of 94°C for 30 seconds, 50°C for 60 seconds and 72°C for 90 seconds; and a final termination at 72°C for ten minutes and chilled at 4^{o}C.

3.10.3 Gel integrity

The integrity of the amplified gene fragments was checked on a 1.5% Agarose gel ran to validate amplification. The buffer (1 X TAE buffer) was prepared and thereafter used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for five minutes. The molten agarose was allowed to cool to 60°C and stained with 3.0 µL of 0.5 g/mL ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for twenty minutes to create wells. The 1 X TAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2.0 µL) of 10 X blue gel loading dye (which gives color and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4.0 µL of each PCR product and loaded into the wells after the 100 bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120 V for forty-five minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100 bp molecular weight ladder that was ran alongside experimental samples in the gel(Lee *et al*., 2012).

3.10.4 Purification of amplified product

After gel integrity, amplified fragments were ethanol purified to remove PCR reagents. Very briefly, 7.6 µL of $C_2H_3NaO_2$ (sodium acetate) 3M and 240 µL of 95% ethanol were added to each about 40 µL PCR amplified product in a new sterile 1.5 µL tube eppendorf, mix thoroughly by vortexing and keep at -20°C for not less than thirty minutes. Centrifugation for ten minutes at 13,000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µL of 70% ethanol, mixed and centrifuge for fifteen minutes at 7,500 g and 4°C. Again tubes were inverted on trash to remove all supernatant. Tubes were inverted on paper tissue and

allowed to dry in the fume hood at room temperature (27± 2^{o}C) for ten to fifteen minutes. Prior to sequencing tubes were resuspended with 20 µL of sterile distilled water and kept in -20°C. The purified fragment was checked on a 1.5% agarose gel ran on a voltage of 110 V for about 1 hour, to validate the presence of the purified product and quantify using a nano drop of model 2,000 from Thermo-scientific.

3.10.5 Sequencing

Using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual and the sequencing kit (BigDye terminator v 3.1 cycle sequencing kit), the amplified fragments were sequenced. Using a Bio - Edit software and MEGA 6 genetic analysis was carried out.

3.10.6 Phylogenetic tree/evolutionary distance and Nucleotide sequence accession number

A computation of the evolutionary distances were done using the Maximum Composite Likelihood method described by Tamura *et al*. (2004). The nucleotide sequence data of identified isolates were registered in the Genbank Nucleotide Sequence database under serially allotted accession numbers.

3.11 Probiotic screening tests

3.11.1 Temperature sensitivity

The temperature sensitivity test was carried out by the method of Tambekar and Bhutada (2010). A 0.1 mL of overnight culture of bacterial isolates were inoculated onto MRS plates using pour plate method which was followed by incubation at various temperature level ranging between 25^{o}C - 45^{o}C for 48 hours. Temperature tolerant isolates were labeled based on their growth on the medium.

3.11.2 Acid tolerance

Ten milliliters of MRS broth with different acidic level ranging from pH 2.0 – 5.0 were dispensed separately into screw capped tubes and sterilized at 121^{o}C for 15 minutes. Lactic acid bacteria isolates were inoculated in the medium containing the acid and

incubated at 37°C for 24 hours. Bacterial growth estimation was done using UV Visible spectrophotometer at 600 nm optical density (Tambekar and Bhutada, 2010).

3.11.3 Growth of isolates at different sodium chloride (NaCl) concentration

MRS broth containing NaCl (4%, 6% and 8%) was dispensed separately into screwed-capped tubes and sterilized at 121°C for 15 minutes. Isolates were inoculated into the sterile medium and incubated at 37°C for 48 – 96 hours. Increase in turbidity of the broth was compared with uninoculated test tube containing MRS broth with varying NaCl concentrations as mentioned above serving as control in determining the presence or absence of growth (Olutiola *et al.,* 1991).

3.11.4 Bile salt tolerance

An aliquot of 0.5 mL of freshly prepared cell suspension of overnight cultures adjusted to 0.5 McFarland's standard were inoculated into MRS broth containing bile salt No. 3 (Oxoid, England) in the following concentrations 0.3, 0.5, 0.8 and 1% (w/v) in glass tubes. After four hours, a 0.1 mL from each tube was inoculated onto sterile MRS agar plates followed by incubation at 37°C for 24 hours. Bacterial growth was enumerated by plate counts while tube without bile salt served as control (Oluwajoba *et al*., 2013)

3.11.5 Microbial adherence to hydrocarbon (MATH) test

In MRS broth, lactic acid bacteria strains were grown at 37°C for 24 – 48 hour and centrifugedat 4,000 rpm for 15 minutes. Bacterial cells were washed two times in PBS (phosphate buffer saline pH 7.0), dissolved in PBS and the Optical Density (OD) determined at 600 nm. A 3.0 mL volume of the suspension of bacterial cells was added to a 1.0 mL volume of hydrocarbon (xylene, chloroform, benzene), vortex ("mrc" VORTEX MIXER) at speed 10 for 120 secondsand incubated at 37°C for 30 minutes. After separation or partitioning of the aqueous and organic phase, 1.0 mL volume of the lower aqueous phase was removed carefully using a syringe followed by determination of its optical density at 600 nm(Rosenberg *et al*., 1980).

Calculation of hydrophobicity (%) was done as follows

$$\%\, Hydrophobicity = \frac{[OD_{initial} - OD_{final}]}{OD_{initial}} \times 100$$

Where OD= optical density

$OD_{initial}$ and OD_{final} are the absorbance before and after extraction with hydrocarbon.

3.11.6 Antagonistic activity of LAB

Agar well diffusion method was utilized for testing antagonistic activities of the LAB isolates. Test organisms (*Escherichia coli, Salmonella* sp.*, Pseudomonas* sp.*, Bacillus* sp.*, Klebsiella* sp.*, Listeria* sp. *and Staphylococcus aureus*)were inoculated into sterile nutrient agar medium by spread plate method with the use of sterile swab sticks. After solidifying, 8 mm diameter wells were bored in the medium using a sterile cork borer. Cell free supernatants of LAB isolates in MRS brothcentrifuged at 4,000 rpm for 15 minutes were introduced in the well, allowed to diffuse into the agar followed by overnight incubation under aerobic conditions at 30^{o}C for 24 hour. LAB antagonistic effect was recorded after measuring the zone of inhibition around the well (Mahnaz *et al*., 2012).

3.11.7 Antibiotic sensitivity test

Disc diffusion method described by Pundir *et al*. (2013) was used. On MRS agar, LAB isolates were grown respectively for 24 - 48 hours. Cell suspension in sterile distilled water was made of LAB isolates followed by adjustment to 0.5 McFarland's standard (approximately 1.5×10^{8} CFU/mL). Sterile cotton swabs were used to make lawns of the LAB isolates on MRS agar. Even placement of antibiotic disc on agar surface in plates utilizing a sterile forceps was done followed by incubation at 35 ± 2^{o}C for 24 hours. Inhibition zone was measured using a ruler. The zones in diameter were compared with the standard zone diameter imperative chart (adapted from nutraceutical clinical laboratories international (NCLL) standard by BBL). Results were reported as resistant (≤ 10 mm) and sensitive (≥ 13 mm).

The Rapid Lab IVD CM — 12 — 8PR 100 used for the LAB isolates contain the following antibiotics: Ceftazidime (CAZ) – 30 µg, Gentamycin (GEN) – 10 µg, Cefuroxime (CRX) – 30 µg, Ceftriaxone (CTR) – 30 µg, Cloxicillin (CXC) – 5 µg,

Erythromycin (ERY) – 30 µg, Ofloxacin (OFL) – 5 µg and Amoxycillin/ Clavulinate (AUG) – 30 µg.

3.11.8 Determination of upper gastric transit tolerance

Method described by Charteris *et al*. (1998) was adopted.Simulated gastric juice was freshly prepared, by adding pepsin (3.0 gL^{-1}) to sterile saline (0.5% w/v), adjusted to pH 2.0 ± 0.1 with 0.1 N HCl and passed through a 0.22 µm sterile membrane filter for sterilization.Simulated small intestine juice was made by adding pancreatin to 0.5% (w/v) sterile saline solution to the concentration of 1 g/L, with or without 0.3% bile salt. Both types of small intestinal juice were adjusted to pH value8 with 0.1 N NaOH.

Preparation of washed cell suspension: In MRS broth, LAB isolates were grown for 18 – 24 hours. Bacterial cells suspension was spun as cells were collected and washed two times in PBS (pH 7.0 ± 0.2). A complete viable count of cells already washed was estimated by plating 0.1 mL of cell suspension prior to assay for transit tolerance.

Determination of transit tolerance: Aliquot(0.2 mL) of already washed cell suspension contained in a 2.0 mL microfuge tubes were admixed one millilitre of simulated gastric juice as well as 0.3 mL NaCl (0.5 w/v). This mixture was vortexed with the aid of mrc Vortex mixer at speed 5 for 10 seconds and then incubated at 37^{o}C in the incubator. During the assay, 0.1 mL of the aliquot was removed at 1 minute, 90 minutes and 180 minutes and inoculated on plates containing solidified MRS agar followed by incubation for 24 – 48 hours at 37^{o}C to determine the lactic acid bacteria isolates viable count.

3.11.9 Lactose utilization

Production of acid by cultures of selected lactic acid bacterial was identified by observing the test medium for colour change. Sterile fermentation medium [peptone (10 g), phenol red (0.018 g), NaCl (15 g), lactose (5 g), distilled water (1.0 L)] with pH 7.0 was inoculated with the bacterial cultures followed by incubation at 35^{o}C for 24 hours. Production of acid was indicated by colour change from red to yellow (Ahmed and Kanwal, 2004).

3.12 Safety assessment

3.12.1 DNase test

Production of DNase (deoxyribonuclease) enzyme was determined using the method of Gupta and Malik (2007). Lactic acid bacterial isolates were inoculated on DNase agar medium (Oxoid) and incubated for 48 hours at 30^{o}C. Observed clear pinkish zone around the bacterial colonies indicated DNase production.

3.12.2 Gelatine hydrolysis

Determination of activity of gelatinase was carried out using the method described by De La Cruz and Torres (2013). On nutrient gelatine agar (Oxoid, England), spot inoculation of young lactic acid bacterial culture was done. This was followed by anaerobic incubation for 48 hours at 37°C. Flooding of inoculated plates with ammonium sulphate solution was done and observed for zones of clearing around the colonies indicating positive reaction for gelatine hydrolysis.

3.12.3 Exopolysaccharide production (EPS) assay

Point inoculation of lactic acid bacteria was done on supplemented MRS agar containing 0.2 g/L of sodium azide (N_3Na), 2% (w/v) sucrose and bromocresol purple. The inoculated plates were incubated at 37^{o}C for 24 – 48 hours. Plates with yellow coloration indicated EPS production (Sobrun *et al*., 2012).

3.12.4 Hemolytic activity

Hemolytic activity was determined using Gerhardt *et al*. (1981) method. Actively growing lactic acid bacteria cultures were inoculated onto sterile blood agar. Preparation of blood agar was done by adding sheep blood (7%) preserved in ethylene diaminetetra acetic acid into sterile blood agar base. Inoculated plates were incubated anaerobically for 24 – 48 hours at 37°C followed by observation for α and β hemolysis shown by a zone of clearing around the LAB colonies.

3.12.5 Lecithinate test

The white and embryo part of cracked fresh eggs was cautiously separated and transferred into a beaker that is sterile. The intact egg yolk material was centrifuged using an ultracentrifuge. By cutting through the cellulose nitrate tube, the clear yellow viscous fluid (egg yolk lipoprotein) composed primarily of lecithin contained in the upper portion was removed carefully and transferred into another sterile beaker. MRS agar media containing the egg yolk phospholipid (lecithin) were point inoculated with 16 hours old culture of LAB isolates and incubated for 48 hours at 37°C. The release of diglycerides that is seen as an area of opacity around the bacterial colonies indicated positive result (Sharaf *et al*., 2014)

3.13 Controlled fermentation of sample

3.13.1 Cultures and preparation of inocula

Lactic acid bacterial cultures were subcultured on fresh MRS agar then incubated overnight at 30 ± 2^{o}C to get young cultures. Subsequently,cell suspension in sterile distilled water was made of young cultures of LAB isolates followed by adjustment to 0.5, 1.0 and 2.0 McFarland's standard (1.5×10^{8} CFU/mL, 3.0×10^{8} CFU/mL and 6.0×10^{8} CFU/mL respectively). An aliquot of 1.0 mL of freshly prepared cell suspension of overnight cultures of lactic acid bacterial isolates adjusted to 0.5, 1.0 and 2.0 McFarland's standard were used.

3.13.2 Fermentation of 3 g *M.oleifera* supplemented blend (Sample F4) using lactic acid bacteria isolates with best probiotic potentials

The 3 g *M. oleifera* supplemented formulated blend (sample F4) was reconstituted at 30% (w/v) (Livingstone *et al*., 1993) with sterilized distilled water and then aseptically inoculated with 1.0 mL of the starter culture. The inoculated slurries were mixed manually by gentle shaking, then left to ferment for 48 hours at room temperature (27 ± 2^{o}C). An uninoculated sample was included as a control (Vieira-Dalode *et al*., 2008).

Sampling was done every 12 hours for 48 hours aseptically for the determination of pH, total titratable acidity (TTA), proximate, phytochemicals, mineral, vitamin, amino acid quality, bulk density and metabolisable energy.

3.14 Proximate analysis of samples

3.14.1 Crude protein determination

Chemical analysis of the complementary food samples was done following the methods described by the Association of Official Analytical Chemist (A.O.A.C., 2005). The determination of the crude protein content of the samples was done by the routine semi-micro Kjeldahl procedure/technique comprising of the following steps: (1). Digestion, (2). Distillation and (3). Titration.

Digestion: Weight of finely ground samples of dried food (0.5 g of each) was transferred into the Kjeldahl digestion tubes followed by the addition of a Kjeldahl catalyst Tablet then ten milliliters of concentrated H_2SO_4. In a fume cupboard these tubes were fixed in suitable hole of the digestion block heaters where digestion occurred for 4 hours leaving a clear colourless solution. Into a 100 mL volumetric flask the cooled digest was carefully and thoroughly rinsed and made up to mark using distilled water.

Distillation: A five milliliter volume of the digest was pipetted through the small funnel aperture into a vessel of a Markham Distillation Apparatus. Five milliliter of 40% (w/v) NaOH was added to the digest via the same opening using a five milliliter pipette. Resulting mixture was steam-distilled into a 50 mL conical flask holding ten milliliter of Boric acid (2%) plus mixed indicator solution positioned at the receiving tip of the condenser for two minutes. A colour change from purplish pink to bluish-green observed of the boric acid mixed with the indicator solution indicated all the ammonia liberated have been trapped.

Titration: The obtained solution, bluish green in colour was titrated against 0.01 N HCl held in a fifty milliliter burette. At the end point the purplish pink colour changed to bluish-pink colour. This shows that all the trapped nitrogen in ammonium borate $[(NH_4)_2BO_3]$ have been separated as ammonium chloride (NH_4Cl).

Calculation of percentage nitrogen in this analysis was calculated using the formula:

$$\%N = \text{Titre value} \times \text{Atomic mass of Nitrogen} \times \text{Normality of HCl used} \times 4$$

or

$$\%\,N = \text{Titre value} \times \text{Normality/Molarity of HCl used} \times \text{Atomic mass of N} \times \text{Volume of flask containing the digest} \times 100$$

Determination of content of crude protein (CP) is done multiplying percentage Nitrogen by a constant (6.25).

$$\rightarrow \%\,CP = \%\,N \times 6.25.$$

3.14.2 Ether extract determination

Using the A.O.A.C. (2005) method, determination of the crude fat protein content existing in the samples was done by weighing one gram of dried food sample into a fat free extraction thimble plugged with cotton wool afterward. The extraction thimble was positioned in an extractor, fitted up with a reflux condenser attached to a soxhlet flask (250 mL) already dried, cooled and weighed. Seventy-five percent of the soxhlet flask is thereafter filled with petroleum ether (with boiling point 40ºC – 60ºC). The extractor together with the condenser apparatus is positioned on the heater for 6 hours with water running constantly from the tap for ether vapour condensation. This set-up is observed constantly to check ether leaks and to ensure the ether boils moderately the heat source is adjusted appropriately. Until the ether is short of siphoning it is allowed to siphon ten to twelve times. After this, into the ether stock bottle it is observed that any ether content of the extractor is drained carefully. The thimble which has the blend sample is thereafter removed and placed on a clock glass positioned on the bench top where it is dried. Replacement of the extractor, flask and condenser is done and until the flask is dry, the distillation process continues. The flask containing the fat or oil is removed, cleaning of its exterior is done and dried in the oven to a constant weight. Percentage fat/oil is determined using the formula below:

$$\text{Percentage fat} = \frac{W_1 - W_0}{\text{weight of Sample taken}} \times \frac{100}{1}$$

Where;

W_o is initial weight of dry soxhlet flask

W_1 is final weight of oven dried flask + oil/fat.

3.14.3 Dry matter and moisture determination

Finely ground dried complementary food samples were analyzed chemically for dry matter and moisture content using the official methods of analysis of A.O.A.C. (2005). Into a previously weighed crucible, 2 g of food blend was weighed, transferred into the oven set at 100°C for 24 hours to dry and obtain a constant weight. After 24 hours, the crucible containing the sample was retrieved from the oven and placed in a desiccator where it was cooled for 10 minutes then re-weighed. Percentage dry matter and the percentage moisture were determined using the formula below:

$$\text{Percentage Dry matter (\%DM)} = \frac{W_3 - W_0}{W_1 - W_0} \times \frac{100}{1}$$

$$\text{Percentage Moisture (\%M)} = \frac{W_1 - W_3}{W_1 - W_0} \times \frac{100}{1} \text{ OR Percentage Moisture} = 100 - \%DM$$

Where, weight of empty crucible $=W_o$,
weight of crucible together with sample $=W_1$
weight of crucible together with oven-dried sample $=W_3$

3.14.4 Determination of ash

A determination of ash content of finely ground dried complementary food samples were carried out according to the official methods of analysis described by A.O.A.C. (2005).
Into a clean dry porcelain crucible, two grams of blend sample was transferred and placed in a muffle furnace set at 550°C for 4 hours until the sample turned to white ash. In a desiccator, the crucible and its content were cooled to 100°C and weighed. Calculation of percentage ash was done using the formula below:

$$\text{Ash Content: } \frac{\text{Weight of ash}}{\text{Original weight of Sample}} \times \frac{100}{1}$$

3.14.5 Determination of fibre

Determination of fibre content of finely ground dried complementary food sample was carried out using the official methods of analysis of A.O.A.C. (2005).

Into a fibre flask, 2.0 g of the food sample was weighed and 100 mL volume of 0.255 N H_2SO_4 was added. Using the heating mantle, the mixture was heated under reflux for one hour. The hot mixture was filtered via a fibre sieve cloth and the filtrate gotten was discarded as the trapped residue was transferred into the fibre flask. A 100 mL of 0.313 N NaOH was added and heated using the heating mantle for another one hour under reflux. The obtained mixture was filtered via a fibre sieve cloth and to dissolve any organic constituent present in it, ten millilitre of acetone was added. Washing of the residue was done using about fifty milliliter of hot water on the sieve cloth followed by its final transfer into the crucible for oven-drying at 105^{o}C overnight to expel moisture. Using a desiccator, the oven-dried crucible holding the residue was cooled and weighed to get the weight (W_1). The weighed crucible was returned into the muffle furnace for ashing for four hours at 550^{o}C after which content of the crucible turned to grey ash. Using a desiccator, the carbonaceous free material was cooled and weighed to obtain W_2. The weight of fibre was obtained from difference between W_1 and W_2 while the percentage fibre was calculated using the formula below:

$$\text{Percentage Fibre} = \frac{W_1 - W_2}{\text{weight of Sample taken}} \times \frac{100}{1}$$

3.15 Metabolisable energy

Using the Atwater factor, formulated blend metabolisable energy was obtained.

Metabolisable energy (Kcal/100g) = (Protein×4)+(Fat×9)+(Carbohydrate×4)

3.16 Determination ofphytochemical contents of the formulated blends

The phytochemicals in the dried complementary food samples determined are tannin, polyphenol and phytic acid, oxalate, alkaloid, flavonoid, saponnin.

3.16.1 Determination of tannin content

To extract tannin, 1.0 g of the food sample was weighed and soaked for 5 hours with 25 mL solvent (80 mL of 10% glacial acetic acid) in a beaker. This mixture was sieved via a filter paper of double layers to separate the filtrates. A preparation of tannic acid standard solution was done which ranged between 10 - 50 ppm. The standard solutions and the

filtrates absorbance were read at 500 nm wave length on Spectronic 20 then used for a standard graph plotting (standard curve). The extrapolation of the content of tannin of the blend sample from the standard curve was carefully done (A.O.A.C., 2005).

3.16.2 Determination of polyphenol content

Into a 250 mL conical flask, one gram of complementary food sample was weighed then 20 mL of deionized water volume was added. Into a test tube, filtrate of 4 days old mixture of sample was measured and 3 mL of 0.01 N of ferric chloride was added. Three milliliter of 0.08 N hexacyanoferrate (III) was added to the mixture. The content was further diluted with water to reduce its heaviness. At 600 nm after 10 minutes, using a Spectronic 20 spectrophotometer, the absorbance was read (A.O.A.C., 2005).

% Total polyphenol=Absorbance multiplied by average gradient multiplied by dilution factor all divided by weight of sample multiplied by 10,000.

3.16.3 Determination of phytic acid content

Into 250 mL conical flask, 2.0 g of the complementary food sample was weighed and soaked with 100 mL of 2% HCl for 3 hours. This was followed by filtration using a hardened filter paper of double layer. Fifty milliliter of filtrate was transferred into a 250 mL Erlenmeyer flask, and to obtain an appropriate acidity, distilled water of 100 mL volume was added. As indicator, 10 mL of ammonium thiocyanate solution (0.3%) was introduced into the solution. Using a standard 0.1 M iron (III) chloride solution containing 0.00195 g/mL of iron, the solution was filtered and an end point marked with a slightly brownish-yellow colouration which stayed for five minutes (A.O.A.C., 2005). Percentage phytic acid was determined thus

$$\%Phytic\ Acid\ Content = \frac{X \times 1.19}{Weight\ of\ Sample} \times 100$$

Where X = Titre value × 0.00195.

3.16.4 Determination of oxalate content

Into a 100 mL beaker, five gram of complementary blend was weighed. 20 mL of Hydrochloric acid (0.30 N) was added, stirred and warmed at 40^{o} – 50^{o}C with the aid of a magnetic hot plate for one hour. Extraction of this sample into a 100 mL volumetric flask was done three times having added 20 mL of HCL acid (0.30 N) and filtered. Dilution of combined extracts up to the 100 mL mark of the volumetric flask was done and oxalate was estimated after pipetting 5.0 mL of diluted extract into a conical flask then turned alkaline using 1.0 mL of 5 N ammonium hydroxide (NH_4OH). To determine the alkaline regions of this mixture, an indicator paper was dropped in the conical flask while glacial acetic acid was added in drops to make it acidic followed by addition of 1.0 mL of calcium chloride ($CaCI_2$). The mixture was centrifuged for 15 minutes at 3,000 rpmafter 3 hours. Washing of the precipitate was done three times with heated water after mixing thoroughly and centrifuging each time while the supernatant was discarded. Precipitates in tubes were dissolved by heating in a water bath (70^{o} – 80^{o}C) having added 2.0 mL of 3 N H_2SO_4 (sulfuric acid). Into a clean conical flask, the content of the tubes were transferred carefully and titrated with 0.05 M potassium permanganate ($KMnO_4$) until the first pink colour showed up and the solution turned colourless. This solution was gently heated between 70^{o} – 80^{o}C then titrated until a persistent permanent pink colour was observed (A.O.A.C., 2005).

3.16.5 Determination of alkaloid content

To 1.0 mg of food sample, 1 mL of 2 N hydrochloric acid (HCl) was added, filtered and filtrate was transferred into a separating funnel to which an addition of 5 mL of bromocresol green solution as well as 5 mL of phosphate buffer were made. Resultant mixture was vigorously shaken, collected in a 10 mL volumetric flask and diluted with chloroform. Preparation of set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/mL) was made and using an ultraviolet/Visible spectrophotometer, the absorbance at 470 nm for test and standard solutions were determined against the reagent blank (Rao *et al*., 2012).

3.16.6 Determination of total flavonoid content

The aluminum chloride ($AlCl_3$) colorimetric assay was used in quantifying total flavonoid content. Reaction mixture consisting one milliliter of food sample together with 4 mL volume of distilled water was held in a 10 mL volumetric flask to which 0.30 mL of sodium nitrite (5 %) was added followed by subsequent addition of 0.3 mL of aluminum chloride (10 %) after five minutes. After 5 minutes, two milliliter of 1 M NaOH (Sodium hydroxide) was treated followed by its dilution with distilled water to 10 mL. Preparation of set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were made then using an ultraviolet visible spectrophotometer, the absorbance at 510 nm for test and standard solutions were determined against the reagent blank (Har and Ismail, 2012).

3.17 Determination of mineral content in formulated blends

Mineral analysis of all blends was done starting with ashing 5 g of the ground dried food blend for two hours at temperature 550^{o}C in a muffle furnace. The ash was cooled and washed in 2.0 mL of HNO_3 (Trioxonitrate V acid). The residue obtained was further heated for 30 minutes, dissolved in 40 mL of hydrochloric acid and digested for 4 hours on hot plate magnetic stirrer. 20% diluted HCl was further added then boiled. This was filtered with filter paper Whatman filter No. 4 washed with HCl, then with distilled water and the volume was made up to 100 mL.

Potassium (K) levels of the samples were ascertained using a flame emission photometer with NaCl and KCl as standard. Using atomic absorption spectroscopy methods, other minerals were analyzed. The mean signal responses of presented sample were recorded for each of element with their corresponding wave length. Calculation of concentration of each element was determined using the formula below

$$Concentration/100ml = \frac{[Std\ Concentration \times Sample\ Abs \times 100 \times d.f]}{Std.Abs}$$

Where,

d.f is the dilution factor

Std Concentration is the Standard concentration

Std Abs is the Standard absorbance

Concentration per 100 mL = Concentration (mg)/100 mL.

Magnesium (Mg), zinc (Zn), Iron (Fe), manganese (Mn), calcium (Ca), potassium (K), sodium (Na) and phosphorus (P) were the minerals analyzed in the samples (A.O.A.C., 2005).

3.18 Determination of bulk density

The gravimetric method described by Okaka and Porter (1979) was employed. Five gram of the food sample was transferred into a calibrated measuring cylinder and the volume it occupied was noted. Repeated tapping of the bottom of the cylinder onto a pad that is firm on a laboratory bench was done until a constant volume was detected (i.e. no further reduction was possible). The packed volume was recorded in each case; the density was obtained both as loose and as packed as a ratio of the weight of sample to the volume it occupied.

$$Bulk\ Density\ (g/ml) = \frac{Weight\ of\ Sample(g)}{Volume\ of\ Sample\ (ml)}$$

3.19 Determination of viscosity

Preparation of the porridges were made in a beaker by mixing thirty grams of flour and 200 mL of water. The resulting mixture of water and flour was cooked at 96^{o}C for ten minutes. The gruel was placed in a water bath maintained at 40°C (heating temperature) and its viscosity was measured at this temperature. The paste viscosity was measured using a Brookfield Viscometer (Model DVII Rheometer V2.0 RV; Middleboro, Massachusetts, USA). The cooked gruel was poured into the viscometer beaker, cooled to 40^{o}C and viscosity was measured (in centipoises, cP) using spindle number 52 at a shear rate of 6 revolution per minute. In the space of 2 minute, the average of the maximum and minimum viscosity reading was obtained (Ikujenlola, 2014).

3.20 Determination of vitamin content

Finely ground dried complementary food samples were retrieved from a 4^{o}C refrigerator and positioned on a bench to attain room temperature (27± 2^{o}C)in the laboratory. For water-soluble vitamin extraction, into a 100 mL volumetric flask, 0.100 g of food sample

was weighed and transferred then water was added to the volumetric flask mask after an ultrasonic extraction which lasted for 15 minutes. While 0.125 g of ground food sample was weighed and transferred into a ten milliliter volumetric flasks followed by the introduction of eight milliliter of methanol methylene chloride (CH_3OH-CH_2Cl_2) (1:1 v/v) for fat-soluble vitamins. Solution of sample prepared then stored in the dark; and diluted if required. The solution of samples were filtered through a 0.2 µm filter (Millex-GN) prior to injection. Water and fat soluble vitamins were then separated concurrently using chromatography at optimized conditions in conjunction with valve switching, double injection, envelope-injection, and then wavelength switching. For fat soluble vitamin- 3 µm, 120 A, 3.0×150 mm column was used while for water soluble vitamin- C18 3 µm, 120 A, 3.0×150 mm column was used. The column temperature was 25^{o}C. The water-soluble vitamin determination was done using 5 mM Phosphate buffer and CH_3CN-Mobile Phase A (7:3 v/v). Fat-soluble vitamin determination was done using CH_3OH-CH_3CN (8:2 v/v) and Methyl tert-butyl ether (MTBE). The Injection Volume was 10 µL (A.O.A.C., 2005).

3.21 Amino acid profiling of fermented food

Amino acid analysis was carried out using a validated Reversed-Phase High Performance Liquid Chromatographic Method [Agilent 1100 RP-HPLC Systems (Agilent Technologies, Palo Alto, CA) which consisted of a diode array detector (DAD) (G1321A)]. The fermented complementary food sample was dried, pulverized and freed of water in the laboratory by observing a constant weight over a specific time. Half of a gram of dried fermented food blend was weighed and transferred into a 250 mL conical flask. By extracting the fat content of the food sample using 30 mL of petroleum spirit three times with the aid of a soxhlet extractor equipped with thimble, the defatting of sample was done. For whole hydrolysis to be accomplished for total amino acid recovery, this sample was hydrolyzed thrice. At this phase, the sample was soaked in 30 mL of 1 M KOH (potassium hydroxide) solution then in a hermetically closed borosilicate glass container, was incubated at 110^{o}C for 48 hour. After the alkaline hydrolysis, neutralization of the hydrolysate was carried out to obtain a pH ranging between 2.5 and 5.0 then using a cation-exchange solid phase extraction, the solution was purified and derivatised using

ethyl chloroformate ($C_3H_5ClO_2$). Nitrogen gas was used for proper mop up of the excess derivatization reagent after which the derivatised amino acid was made up to 1.0 mL in a vessel for chromatographic separation in an Agilent Zorbax Eclipse AAA column with dimensions of (4.6 mm X 150 mm, 5 μm). The gradient elution started with 100% A in 1.9 minutes; ramped at 57% B within next 18.1 minutes; ramped at 100% B in 18.6 minutes and kept with 100% A till 26 minutes. The column was operated at $40^{O}C$ and the flow rate of the method was set at 2mL per minute all through the routine. The Zorbex AAA guard columns (4.6 nm X 12.5 mn were used to prolong the duration of the analytical column (A.O.A.C. 2005).

3.22 Evaluation of sensory qualities of selected fermented formulated blend

Organoleptic attributes of the formulated blends were evaluated by 40 panelists of nursing mothers who are untrained in Iyanna environ, Akinyele Local Governement Area, Oyo state. These panelists were all in sound health and familiar with aroma, taste, texture, colour of complementary food. Already cooked complementary food to be assessed were prepared and served for assessment of sensory qualities and over-all acceptability. Panelists were asked to drink water before and after evaluating each cooked sample. Using a 7 point hedonic scale ranging from 7 to 1 (like extremely to dislike extremely respectively), these samples were assessed.

3.23 Nutritional study

Seventy (70) Albino rats (*Rattus norvegicus*) of 6 - 8 weeks, weighing 140 -160 g were obtained for this study from the breeding section of the Central Animal House, University of Ibadan and transported in plastic cages to the Department of Physiology Animal House, University of Ibadan. Male Wistar rats were used because they do not under go hormonal changes as observed in their female counterpart. This is expected to ensure no other physiological function, but, the one induced, interfere with proceedings of the experiment.

3.23.1 Acclimatization and Experimental Groupings for *in-vivo* Nutritional Study

Twenty (20) Albino rats(*Rattus norvegicus*) were randomly allocated into four groups (A - D) containing 5 animals each. During a period of one week acclimatization in metallic

cages positioned in the Department of Physiology Animal House, University of Ibadan under optimized conditions (photoperiod: twelve hours natural light and twelve hours dark; temperature: 25 ± 2°C; humidity 40 - 45%), rats was fed with known proportion of commercial feed (Ladokun commercial pellet) and water for drinking *ad libitum*.

3.23.2 Experimental Set – Up

Animals were allocated into four sets.

Group CF Animals fed with commercial feed (Positive control)

Group FB Animals fed with fermented food

Group FBP Animals fed with fermented food + probiotics (skimmed milk + microorganism: dose 10^6 CFU/mL twice daily)

Group CWF Animals fed with branded complementary food (Negative control)

Having starved the animals overnight before the test but allowed access to drinking water after the period of acclimatization for one week, animals of Group B – C were treated with fermented food and Group C was given probiotics (dose 10^6 CFU/mL twice daily) together with fermented diet using orogastric cannula (Doherty, 1981) for 28 days.

At the end of each weekly protocol schedule, animals were examined for, **feed intake**, **feed conversion ratio**, **body weight changes andprotein efficiency ratio** as well as **hematological parameters** and **liver function tests** to check the level of toxicity of the fermented food.

3.23.3 Feed intake (grams)

Animals were fed twice daily after which feed residues were retrieved, dried in the oven for 48 hours at 80°C and weighed with the aid of a weighing scale. Feed intake was gotten from the differences between the quantity of food supplied (g) and the quantity of food left over

3.23.4 Body weight changes (grams)

Initial live weight of animals was taken with the aid of a weighing scale at the inception of the experiment then subsequently weighed every week for 35 days study period. Weights

were recorded based on the treatment group. The gained weight was arrived at by evaluating the difference between the initial weight and final weights of animals.

3.23.5 Feed conversion ratio (FCR)

This is the degree of an animal ability to transform mass of feed into upturns of an output desired. This is calculated, dividing feed intake (g) by weight gained (g).

$$F.C.R = Feed\ intake\ (g) \div Weight\ gained\ (g)$$

3.23.6 Protein efficiency ratio (PER)

This is centered on the ratio of weight gain of animal to its protein consumption. It is calculated using the formula below:

$$P.C.R = Weight\ gain\ (g) \div Protein\ intake\ (g)$$

On the 15th day of protocol schedule, animals were anaesthetized and then sacrificed by cervical dislocation. Briefly, 4.0 mL of blood was collected using the cardiac puncture bleeding procedure into plain and Ethylene Diamine Tetra Acid (EDTA) bottles and taken for hematology test.

3.24 Hematological Studies

The assessment of haematological parameters a satisfactory and applicable technique in evaluating health status served as a guide to determining possible alterations in the levels of metabolic products, heamatology, normal functioning of organs in the body, to mention a few as blood is integral in nutritional, physiological as well as pathological functions of living organism (Nordqvist, 2017). Hematological determination was carried out on blood collected into EDTA bottles. The blood was centrifuged and the plasma collected for determination of hematological parameters.

3.24.1 Packed Cell Volume (PCV):

This was measured using the microhematocrit method. Three quarter of a capillary tube was filled with blood that is well mixed then using a crystal seal, one end was blocked.

For six minutes at a high speed, the tube was spun in microhematocrit centrifuge then using a microhematocrit reader with a sliding cursor the packed cell volume was read.

3.24.2 Hemoglobin (HB) analysis:

This is a test used to measure the concentration of hemoglobin in whole blood. The cyanomethyglobin method was used (Larsen and Snieszko, 1961). A four milliliter volume of Drabkin solution was introduced into 20 µL of blood, allowed to stand for ten minutes at temperature 35±2^{o}C for complete conversion to cyanomethylglobin. In a buffered solution of ferricyanide ($C_6FeN_6^{-3}$) and potassium cyanide (CKN) to yield cyanomethemoglobin the blood sample was diluted. The potassium cyanide converted the hemoglobin to methemoglobin, which was further converted to cyanomethemoglobin by the action of potassium cyanide.

3.24.3 White Blood Cell (WBC) count:

This was carried out using a hemocytometer and neubauer counting chamber. Mirrored slide which had an improved Neubauer ruling with a cover slip fixed to ensure on both sides the Newton rings were visible were used. Accurate dilution of blood sample in ratio 1:20 with Turck fluid was carried out using an automatic pipette. Thoroughly mixed sample was allowed to stand for ten minutes as the stain was moved into the cells then using a capillary (PCV) tube, the chamber of the hemocytometer was filled. To calculate the total WBC count × 10^9 / L, number of cells present in the grids four large corner squares were completely counted and divided by twenty.

3.24.4 White Blood Cell Differentials:

To determine the number of leucocytes, neutrophils, monocytes and eosinophils, on a slide, smear of blood was made, air dried, properly labeled then fixed in methyl alcohol (CH_4O) for 30 minutes. It was slanted to dry and then stained with Giemsa's stain for thirty minutes. Thereafter it was washed again, slanted to get dried then observed using an oil immersion microscope to count the leucocytes, neutrophils, monocytes and Eosinophlis. To calculate absolute WBC differential count;

Lymphocte = Lymphocyte/100 × WBC

Neutrophils= Neutrophils/100 × WBC

Monocytes= Monocytes/100 × WBC

Eosinophils= Eosinophils/100 × WBC

3.24.5 Red Blood Cell:

A ten microliter volume of blood was introduced into one milliliter of Dacie fluid. The neubauer chamber was filled with blood and the total number of cell was counted using the hemocytometer.

3.24.6 Mean Cell Hemoglobin (MCH)

This was calculated as reported by Cheesbrough (2006), using the formula:

$$\frac{\text{Hemoglobin concentration}}{\text{Value of red blood cell count}}$$

3.24.7 Mean Cell Hemoglobin Concentration (MCHC)

The mean cell heamoglobin concentration was calculated as reported by Cheesbrough (2006), using the formula:

$$\frac{\text{Hemoglobin concentration}}{\text{Packed cell volume}}$$

3.24.8 Mean Cell Volume (MCV)

The mean cell volume was calculated as reported by Cheesbrough (2006) using the formula:

Ratio of the packed cell volume to that of the red blood multiplied by the factor of ten.

3.24.9 Biochemical analysis for nutritional study

In plain bottles, blood samples were collected and left to clot for 10 minutes at temperature 35 ± 2^{o}C then centrifuged for 10 minutes at 3,000 rpm. Using a clean pasture pipette, separated serum was retrieved and stored frozen until needed for further use. Biochemical analysis of Aspartate Transaminase (AST), Alanine Transaminase (ALT) and

Alkaline Phosphatase (ALP) were determined from the collected blood samples. Activities of AST, ALT and ALP were analyzed as follow:

In phosphate buffer with pH 7.4, 0.2 mL of the serum and 1.0 mL of aspartate and α-ketoglutarate for AST; alanine and α- ketoglutarate for ALT was incubated for an hour in case of AST and 30 minutes for ALT. Thereafter, one milliliter of the solution 2, 4 dinitrophenylhydrazine (DNPH) was introduced to halt the action of these enzymes and kept back for twenty minutes at 35 ± 2^{o}C. One milliliter of 0.4 N NaOH was introduced into the mixture and after incubation, at 540 nm absorbance was observed using a spectrophotometer (Reitman and Frankel, 1957).

3.25 Antidiarrhoeal study

3.25.1 Acclimatization and experimental groupings for diarrhoea inhibition studies

Random allocation of fifty (50) Albino rats was done into ten groups each having five animals.

In the first one week, animals were acclimatized in clean metallic cages placed in the Department of Physiology Animal House, University of Ibadan with optimum condition (photoperiod: twelve hours natural light and twelve hours dark; temperature: 25 ± 2^{o}C; humidity 40 - 45%), rats were fed with known proportion of commercial feed (Ladokun commercial pellet) and water for drinking *ad libitum*.

At the expiration of the first week, rats were starved for 12 hours, then fed with experimental diets for another one week and water *ad libitum* before the anti-diarrhoeal study. Cleaning of cages was done on regular basis.

3.25.2 Induction of Diarrhoea (Castor Oil-Induction):

Diarrhoeal-like condition was induced in the rats by oral administration of castor oil (1.0 mL/animal).

Based on the different treatments, ten groups were evaluated for 6 hours and 24 hours post diarrhoea induction analysis.

Group UCF (A & B) Healthy animals treated with conventional food (Normal control)

Group ICF (A & B) Diarrhoea induced animals treated with conventional food (Diarrhoea control)

Group IFB (A & B) Diarrhoea induced animals treated with fermented food

Group IFBP (A & B) Diarrhoea induced animals treated with fermented food + probiotics (skimmed milk +microorganism: dose 10^6 CFU/mL twice daily)

Group IFCL (A & B) Diarrhoea induced animals treated with commercial feed +Loperamide HCl-Tm (5.0 mg/kg)

Diarrhoea Induction Reaction Was Monitored In All Experimental Groups As Follow:

Post diarrhoea induction; Animals of all groups were examined for the presence of diarrhoea confirmed by defecation of watery stool from the anus. The animals were observed for 6 hours for the presence of characteristic diarrhoea droppings recorded using a predetermined scoring index (Di Carlo *et al.,* 1993) as follows: (++) for copious, (+) for mild and 0 for lack of diarrhoea.

Six hours and twenty four hours post diarrhoea induction; Physiological changes in four rats of Groups: 1A – 5A (for six hours) and 1B -5B (for twenty four) hours respectively, were checked for hematological, histological and biochemical parameters after being sacrificed.

3.26 Biochemical assay

Preparation of colon tissue for biochemical assays: On the 15th day of protocol schedule, animals having been anaesthetized then sacrificed as stated above, their gastrointestinal tract was opened and the colon tissue was harvested, rinsed with ice cold isotonic saline potassium chloride (KCl) solution, weighed and then kept in ice before being transferred into the freezer at -20°C in a phosphate buffer saline (PBS).

Homogenization: Colon tissue samples were homogenized using a Teflon homogenizer using 10 times (w/v) cold 0.1 M PBS (pH 7.4). For fifteen minutes, the homogenate was spun at 10,000 rpm to obtain post mitochondrial fraction (supernatant). The supernatant was collected and separated into different portions which were kept refrigerated for Protein, Catalase, Glutathione and Nitrite assays (Gornall *et al.,* 1949).

Preparation of Buffers:

1. Homogenizing buffer (0.1 M phosphate buffer saline)
    a) In 200 mL of distilled water, 7.1628 g of sodium dihydrogen phosphate monohydrate ($Na_2PO_4.2H_2O$) (Mol weight 358.22) was dissolved.
    b) In 100 mL of distilled water 1.5603 g of $NaH_2PO_4.2H_2O$ (156.00) was dissolved.

    The preparation of phosphate buffer (0.1 M) was thus prepared by adding 200 mL of (a) to 100 mL of (b) then adjustment of the pH to 7.4 was achieved using drops of HCl or NaOH as the case may be.
2. Perfussion buffer (4% phosphate buffer formalin)
    a) A 40 mL of formalin was added in a cylinder
    b) A 960 mL of distilled water was measured into "(a)"
    c) Next, 4 g of $Na_2PO_4.2H_2O$ and 6.5 g of sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.2H_2O$) were also added to "(a)" and stirred properly
3. Saline was used for rinsing colon tissue (1.15% of KCl solution)
    a) Dissolution of potassium chloride (11.5 g) in one hundred milliliter of distilled water then storage at 4°C was done.

3.26.1 Total protein content determination

Using the Biuret method the protein concentration of various homogenates was evaluated. Amino groups of proteins reduce cuprous ions to the cupric form which then form a blue-colored complex with the proteins that absorbs maximally at 540 nm in alkaline solution. Sodium potassium tartrate ($KNaC_4H_4O_6$) is added to stabilize the complex. Fifty microliter volume of the supernatant was introduced into 1.950 mL distilled water followed by an addition of 3 mL Biuret reagent in a test tube. The absorbance of the whole sample already incubated at temperature 35±^{o}C for 30 minutes was examined at 540 nm and from the standard curve, the concentration was determined (Gornall *et al.,* 1949).

3.26.2 Determination of Glutathione (GSH) Activity:

Glutathione level was estimated using the method described by Beutler *et al*. (1963). Test sample/supernatant (0.1 mL) was diluted in phosphate buffer (0.9 mL) followed by the addition of 20% Trichloroacetic acid (TCA) ($C_2HCl_3O_2$) (1.0 mL). After 20 minutes the mixture was spun for another 10 minutes at 10,000 rpm. An introduction of phosphate

buffer (0.75 mL) into the supernatant (0.25 mL) was done mixed followed by introduction of 2 mL two milliliter of 0.0006 M of 2, 2-Dithiobis (5-nitropyridine) (DTNP) was introduced and thereafter incubated for 10 minutes. At 412 nm, absorbance was read using a spectrophotometer.

3.26.3 Determination of Catalase (CAT) Inhibition Activity

Using a colorimetric assay described in detail by Goth, (1991), catalase activity was determined. The inhibition of catalase was studied as follows. Briefly, a 50 µL reaction mixture containing 50 mM H_2O_2 and various concentrations of ionic liquids (ILs) in 0.2 M phosphate buffer (pH 7.4) was prepared in a 96 well microtiter plate then incubated for 5 minutes at 37°C after which 50 µL catalase solution (50 µg mL^{-1} in, 0.2 M phosphate buffer (pH 7.4) was introduced and incubated for another 5 min at 37°C. Using 100 µL ammonium molybdate [$(NH_4)_2MoO_4$] (64.8 mM), the enzymatic reaction was halted and the absorbance was measured in a microplate reader at 405 nanometer using a spectrophotometer.

3.26.4 Nitric oxide (NO) activity determination

Using the method of Ebrahimzadeh *et al.* (2009), the effect of nitric oxide radical in tissue of colon was measured. The technique is founded on the premise that sodium nitroprusside ($C_5FeN_6Na_2O$) in aqueous solution naturally produces nitric oxide which interpalys with oxygen to produce nitrite ions (NO^{-2}) at a physiological pH which could be estimated utilizing the Griess reagent. Nitric oxide scavengers contend with oxygen, resulting to decreased formation of nitrite ions.

Dissolution of 2 mL of sodium nitroprusside (SNP) (10 mM) in 0.5 mL volume of phosphate buffer (PBS) having a pH of 7.4 was done. This was added to the test sample (0.5 mL) at varying concentrations, mixed together and incubated for 150 minutes at 25°C. The incubated sample (0.5 mL) was mixed with equal amount of Griess reagent, incubated for thirty minutes at 35±2°C followed by reading of its absorbance at 548 nm. Nitrite estimation was done from the standard curve generated form sodium nitrite solution

3.27 Histological Studies

After the sacrifice, animals underwent cardiac perfusion with 4% formalin from each group, colon tissue of animals in each group were then harvested for histological study. Histological study was carried out as described by Avwioro (2010), and this was done as follows:

Grossing: The tissues were observed, cut into small pieces of not more than 4 mL thick into cassettes pre-labeled then immersed in 10% formalin saline for 24 hours for its fixation.

Tissue processing: This was done automatically using an automatic tissue processor. For the purpose of dehydration, tissues were passed through several reagents such as: station one and two which contained 10% formalin saline, station three to station seven then alcohol of 70%, 80%, 90% and 95%. For the purpose of clearing, tissues were passed through station eight and station nine which contained two changes of xylene as well. Then for infiltration or impregnation tissues were transferred into three wax baths already programmed to run for twelve hours indicating in each station, these tissues stayed for one hour.

Embedding: Using a semi-automatic tissue embedding center, tissues already processed were submerged and oriented into a metal mold containing molten paraffin wax to give the tissues a solid support. Thereafter, cassettes pre-labeled was positioned and on them, relocated to a cold plate for solidification then formed tissue block were detached from the mold.

Microtomy: To expose the tissue surface, the blocks were trimmed with the aid of a rotary microtome at 6 micrometer, then tissue surfaces were kept on ice before sectioning at four micrometer (ribbon section)

Floating: Using labelled slides that are clean, floating of picked tissue sections was done on water bath set at 55°C.

Drying: The slides were dried on a hotplate set at 60°C four 1 hour

Staining: Heamatoxylin and Eosin technique stain and Cresyl fast violet (Nissl stainning) were used.

Heamatoxylin and Eosin technique: this method was done to examine the architecture and integrity of the colon tissues.

Procedures for Heamatoxylin and Eosin (H & E) technique:

1. Section was ewaxed in xylene for 15 minutes, and through absolute alcohol, 95% and 70% Alcohol
2. Section staining in Harris heamatoxylin for five minutes was done after it rinsing with water.
3. Further rinsing in water was done followed by differentiation in 1% acid alcohol briefly.
4. It was rinsed again in water, and in ten minutes was made blue under a running tap water.
5. It was counterstained for two minutes using 1% aqueous Eosin afterward, then rinsed in water.
6. Lastly, in ascending grades of alcohol dehydration was done followed by clearing in xylene then mounting in DPX machine was done.
7. The nuclei appeared blue and the cytoplasm appeared pink.
8. Using Olympus digital camera, photomicrographs of sections from each group that appeared good were signed then documented.

3.28 Analysis of data

The experimental data was analysed using Analysis of variance and T-test to determine the mean, Standard Error and standard deviation using SPSS version 23.The level of significance was set at $P \leq 0.05$.

CHAPTER FOUR

RESULTS

4.1 Chemical analysis of spontaneously fermented blends

Theresults of the analysis of chemical properties of formulated blends (Table 3.1) shows the pH of all the fermenting samples decreasesas fermentation period increased (Figure 4.1).The highest pH value was recorded in sample F6 (which contained 5 g *Moringaoleifera* supplemented blend) by 0 hour and the lowest by 72 hours in sample F1 (without *M.oleifera* supplementation).

The titratable acidity of the samples as shown in Figure 4.2 increasedwith increase in fermentation time.Highest lactic acid content was recorded by 72 hours in 3 g *M.oleifera* supplemented blend (sample F4) and the lowest was recorded in sample F1 (without *M.oleifera* supplementation) by 0 hour.

The observed increase in pH and TTA values with increase in the quantity of *M. oleifera* added to the blends is as shown on table 4.1. A significant effect ($P \leq 0.05$) onthe pH and TTA contentwas observed with *M. oleifera*supplementation compared to the blend without*M. oleifera* supplementation (sample F1) as presented on the table. The pH values however were not different significantly ($P \leq 0.05$) as *M. oleifera*supplementation increased except for sample F2(1g *M.oleifera* supplementation). TTA values were different significantly ($P \leq 0.05$) as *M. oleifera*supplementation increased except for 2 g, 4 g and 5 g *M.oleifera* supplementation in samples F3, F5 and F6 respectively. The pH value ranges from 4.83 obtained in sample F3 (with 2 g *M.oleifera* supplementation) to 3.96 obtained in sample F1 (without *M.oleifera* supplementation) while the TTA value was observed to be highest (0.55 mg/mL) in sample F4 (with 3 g *Moleifera* supplementation) and lowest (0.41 mg/mL) in sample F1 (without *M.oleifera* supplementation).

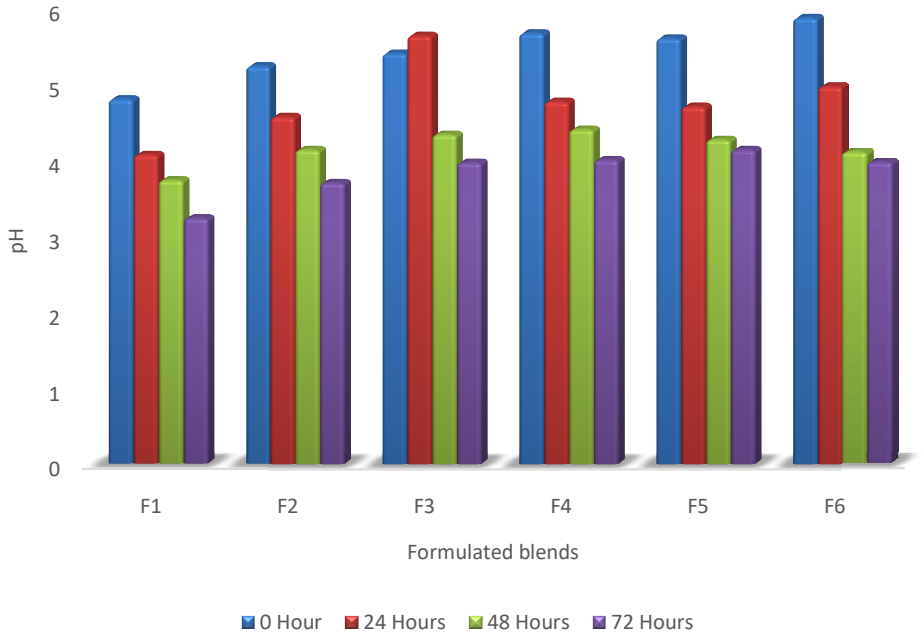

Figure4.1:Effect of fermentation timeon pH of fermented blends

Key:

Sample Code	P	C	G	M
F1	70	25	5	0
F2	70	24	5	1
F3	70	23	5	2
F4	70	22	5	3
F5	70	21	5	4
F6	70	20	5	5

P- Pearl millet, C- Cowpea, G- Groundnut, and M- *Moringa oliefera* leaves

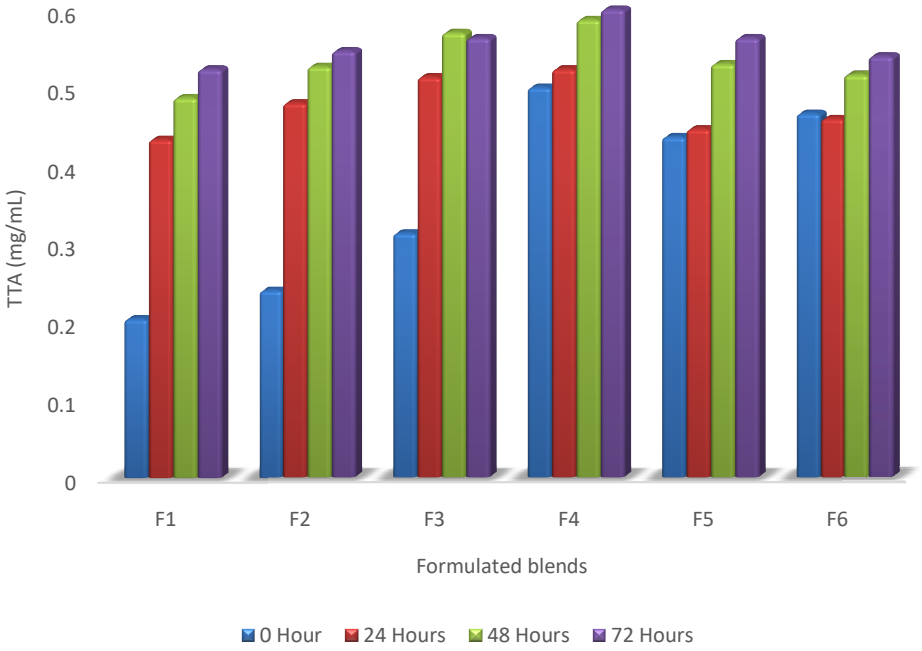

Figure4.2:Effect of fermentation timeon total titratable acidity (TTA) of fermented blends

Key: As presented in figure 4.1.

Table4.1: Effect of supplementation with *M. oleifera* on the chemical properties of blends.

Sample code	pH	TTA(mg/mL)
F1	3.96 ± 0.17^{a}	0.41 ± 0.04^{a}
F2	4.41 ± 0.17^{b}	0.45 ± 0.04^{b}
F3	4.83 ± 0.21^{d}	0.49 ± 0.03^{c}
F4	4.71 ± 0.19^{cd}	0.55 ± 0.01^{d}
F5	4.68 ± 0.19^{c}	0.49 ± 0.02^{c}
F6	4.73 ± 0.23^{cd}	0.50 ± 0.01^{c}

Values are presented as mean ± standard deviation recorded from triplicate readings.
At 95% confidence level, means with similar / different superscripts along the same column are not significantly different/ significantly different from one another.Duncans Multiple Range Test (DMRT).
Key: As presented in figure 4.1, page 62.

4.2 Microbiological analysis

The total lactic acid bacteria count (LogCFU/mL) on De Mann Rogasa Sharpe agar (MRS) increased over the period of fermentation in all samples as shown in Figure 4.3a – 4.3f. Samples F3, F4, F5 and F6 (with 2 g, 3 g, 4 g and 5 g *M.oleifera* supplementation respectively) had no growth by 0 hour of fermentation time. The highest lactic acid bacteria count was recorded in sample F6 (5 g *M.oleifera* supplementation) by 72 hours of fermentation time and lowest in unfermented sample F1 (without *M.oleifera* supplementation).

As shown in Figure 4.3a – 4.3f, aerobic bacteria count (LogCFU/mL) on nutrient agar (NA) increased as fermentation time increase as observed in all samples except in 1 g *M.oleifera* supplemented sample where a decrease was observed by 72 hours of fermentation time. Highest aerobic count was recorded in 4 g *M.oleifera* supplemented blend (sample F5) by 72 hours of fermentation time while the lowest recorded in unfermented 5 g *M.oleifera* supplemented blend (sample F6).

The result of microbiological analysis as revealed on Figure 4.3a – 4.3f shows that the total plate count (LogCFU/mL) on plate count agar (PCA) increased in all samples with progress in fermentation time. Highest plate count was obtained in 2 g *M.oleifera* supplemented blend (samples F3) and 4 g *M.oleifera* supplemented blend (sample F5) by 72 hours of fermentation time and lowest was obtained in unfermented 3 g *M.oleifera* supplemented blend (sample F4).

Microbiological analysis result as shown on Figure 4.3a – 4.3f shows the total enteric count (LogCFU/mL) on Mac Conkey agar (MAC). From the table, there was no observable growth over the period of fermentation time except in the unfermented form of *M.oleifera* non-supplemented blend (sample F1) which had 2.30 LogCFU/mL.

The total fungalcount (LogCFU/mL) on malt extract agar (MEA) as shown in Figure 4.3a – 4.3f showed fungal countincreased as fermentation time progressed in sample F1 while in other samples, there was increase in the first 24 hours of fermentation and decrease as fermentation time increased. The highest fungal count was recorded in sample F1 and the lowest was recorded in 5 g *M.oleifera* supplemented blend (sample F3) by 48 hours of fermentation time.

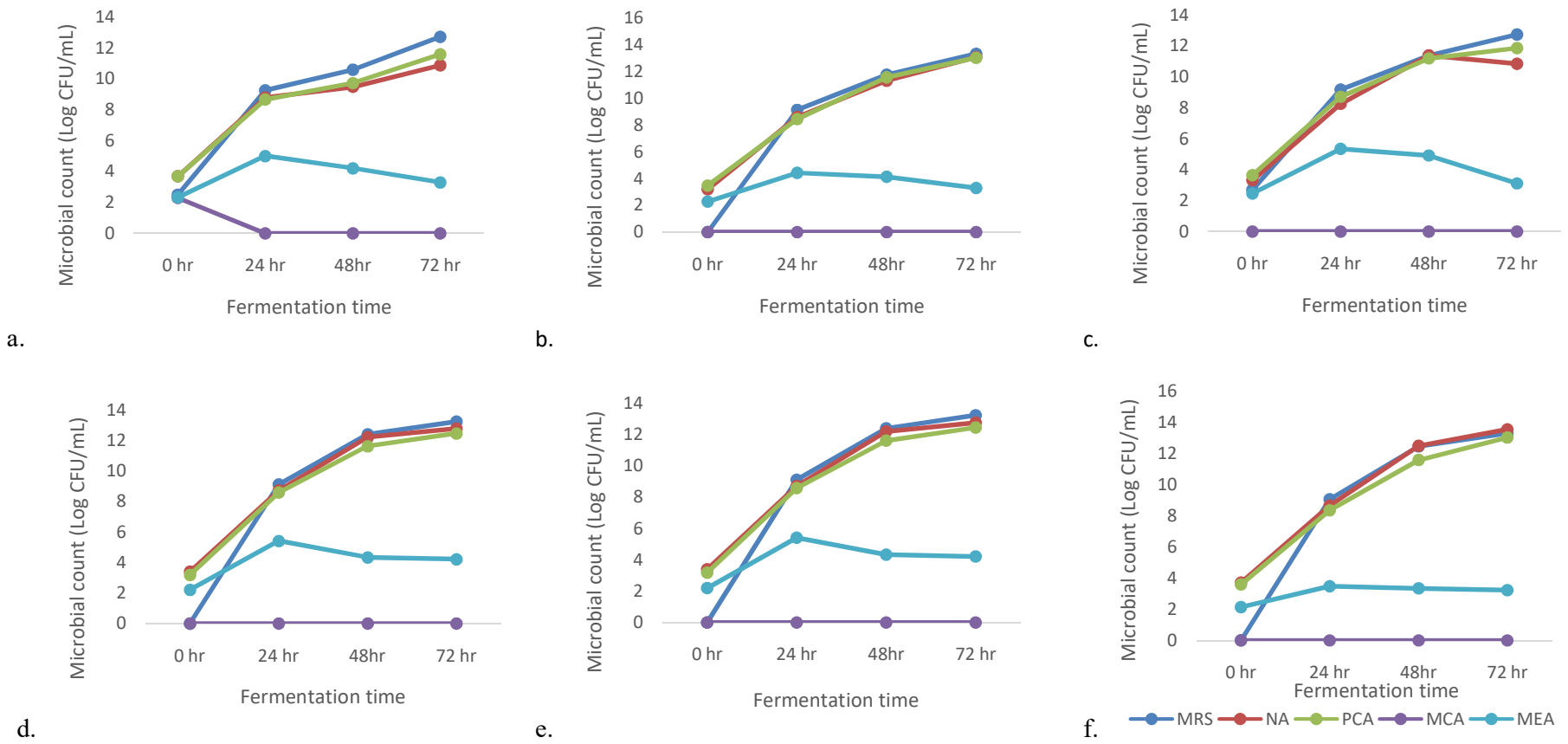

Figure 4.3: Microbial load (Log CFU/mL) of spontaneously fermented a. Sample F1, b. Sample F2, c. Sample F3 d. Sample F4, e. Sample F5, f. sample F6.MRS (de Mann Rogosa and Sharpe), NA (Nutrient Agar), PCA (Plate Count Agar), MAC (MacConkey Agar), MEA (Malt Extract Agar).Key: As in table 3.1.

4.3 Proximate composition of spontaneously fermented formulated blends

The results of effect of fermentation time on proximate composition of *M. oleifera* formulated blends is as shown in table 4.2. From the table, the crude protein, crude fibre and carbohydrate contents of non-supplemented *M. oleifera* blend (sample F1) increased as fermentation time increased while the moisture content decreased as fermentation time progressed. The crude fat and ash contents decreased as recorded by 24 hours and increased as fermentation increased. In sample F1, the highest and lowest crude protein, crude fibre and carbohydrate contents were obtained by 72 hours and 0 hour respectively. The moisture content of sample F1 ranges between 53.73 g/100g recorded by 0 hour and 50.40 g/100g obtained by 72 hours fermentation time. Crude fat and ash content values were obtained lowest by 24 hourswhile their highest values were obtained by 72 hours fermentation time in sample F1. Fermentation time had significant effect ($P \leq 0.05$) on the proximate composition of sample F1 except its crude fat content only.

As shown in table 4.2, the proximate composition of 1 g *M.oleifera* supplemented blend (sample F2) revealed a reduction in the moisture content as fermentation time increased while carbohydrate content increased as fermentation time progressed. Crude fibre and ash contents increased up till 48 hours and reduced by 72 hours fermentation time. Crude fat content increased within the first 24 hours and reduced as fermentation time increases. The moisture and carbohydrate contents ranged from 54.66 g/100g and 37.53 g/100g respectively by 0 hour to 50.63 g/100g and 33.96 g/100g respectively by 72 hours of fermentation time. The highest crude fibre and ash contents were obtained at 48 hours while their lowest were recorded at 0 hour. The highest crude fat content and lowest was recorded by 24 hours and 72 hours respectively. Statistical analysis shows fermentation time had significant effect ($P \leq 0.05$) on the proximate composition of sample F2.

The result of analysis of proximate composition of 2 g *M. oleifera* supplemented blend (sample F3) during the period of fermentation as shown in table 4.2reveals the moisture content decreased up till 48 hours and increased at 72 hours while the ash and carbohydrate contents increased up till 48 hours and reduced by 72 hours fermentation time. The crude fibre and crude protein contents increased as fermentation time increased. The crude fat increased by 24 hours and decreased with increase in fermentation time. The ash as well as carbohydrate contents were recorded highest by 48 hours of fermentation time while their

lowest, were obtained by 0 hour.Moisture content of sample F3 was recorded highest by 0 hour and its lowest by 48 hours. The highest crude protein and highest crude fibre contents were recorded by 72 hours fermentation time and their lowest were obtained by 0 hour. The crude fat content ranged from 1.10 g/100g recorded by 0 hour to 1.00 g/100g obtained by 72 hours. Fermentation time had significantly effect ($P \leq 0.05$) on the proximate quality of sample F3 except for its crude fat content.

As shownin table 4.2, the result of the analysis of proximate composition of the blend supplemented with 3 g *M.oleifera* (sample F4) over 72 hours fermentation periodshows increase in the crude fibre, crude protein, and ash contents up till 48 hours and decrease by 72 hours while moisture content decreased up till 48 hours and increased by 72 hours fermentation time. As fermentation time increased, there was an observed decrease in crude fat content while carbohydrate content increased with increase in fermentation time. Highest crude protein, crude fibre and ash contents were recorded by 48 hours fermentation time while their lowest were recorded at 0 hour. Crude fat content was recorded highestand lowest at 0 hour and 72 hours respectively however the moisture content ranged between 54.46 g/100g obtained by 0 hour and 50.87 g/100g recorded at 48 hours. The carbohydrate content was recorded lowestand highest at 0 hour and 72 hours fermentation time respectively.Fermentation time had significant effect($P \leq 0.05$)on the proximate quality of sample F4.

In table 4.2, the effect of fermentation time on the proximate composition of 4 g *M.oleifera* supplemented blend (sample F5) was shown to have resulted in the reduction of the crude fat content as fermentation time increased while the crude fibre content increased with increase in fermentation time. The ash content increased by 24 hours fermentation time and decreased as fermentation time increased.The highest and lowest crude fat content was recorded at 0 hour and 72 hours respectively but the highest and lowest crude fibre content value was obtained at 72 hours and 0 hour respectively. Fementation time had significant effect ($P \leq 0.05$) on the proximate quality of sample F5 based on statistical analysis.

Analysis of proximate content of the fermented 5 g *M.oleifera* supplemented blend (sample F6) over the period of fermentation is presented in table 4.2. The moisture content as well as crude fat content reduced as fermentation time progressedwhile the ash contents increased as fermentation timeprogressed. The carbohydrate content increased up till 48

hours andreduced at 72 hours. The crude protein decreased by 48 hours and increased as fermentation time progressed. The moisture content ranged between 50.17g/100g obtained by 72 hours fermentation time and 55.1g/100g obtained by 0 hour. Crude fat content ranged from 1.33g/100g obtained by 0 hour to 1g/100g obtained by 48 hours. Itsash content ranged between 1.6g/100g obtained by 72 hours fermentation time and 1.2g/100g obtained by 0 hour. Highest carbohydrate content was recorded by 48 hours fermentation time and lowest by 0 hour. The crude protein content ranged between 10.4g/100g and 9.1g/100g obtained by 72 hours and 48 hours fermentation time correspondingly. Fermentation time had significant effect ($P \leq 0.05$) on the proximate composition of sample F6.

Generally, the moisture content and crude fat decreases as fermentation time increased except for formulations F3 and F4 which increases after 48 hours fementation. The crude protein, ash and carbohydrate contents increases with increased fermentation time in all blends except for formulations F2 and F4 which had their highest crude protein and ash at 48 hours fermentation time.

Table4.2: Effect of fermentation time on proximate content of formulated blends

Sample code	FT (Hour)	Proximate Composition (g/100g)					
		Moisture Content	Crude Protein	Crude Fat	Crude Fibre	Ash	Carbohydrate
F1	0	53.73^{d}	6.13^{a}	0.83^{a}	1.27^{a}	1.13^{a}	36.90^{a}
	24	52.45^{c}	6.33^{ab}	0.73^{a}	1.47^{ab}	1.20^{ab}	37.97^{b}
	48	51.43^{b}	6.47^{bc}	0.77^{a}	1.57^{b}	1.33^{bc}	38.43^{c}
	72	50.40^{a}	6.60^{c}	0.84^{a}	1.57^{b}	1.43^{d}	39.17^{d}
F2	0	54.66^{d}	7.80^{a}	1.10^{a}	1.30^{a}	1.13^{a}	33.96^{a}
	24	53.63^{c}	8.23^{b}	1.07^{ab}	1.57^{b}	1.27^{ab}	34.27^{b}
	48	52.23^{b}	8.27^{b}	1.03^{b}	1.73^{b}	1.40^{b}	35.33^{c}
	72	50.63^{a}	7.90^{a}	0.97^{ab}	1.60^{b}	1.30^{ab}	37.53^{d}
F3	0	53.33^{c}	7.13^{a}	1.10^{a}	1.33^{a}	1.10^{a}	36.00^{a}
	24	51.83^{b}	7.50^{b}	1.07^{a}	1.50^{b}	1.13^{ab}	36.97^{b}
	48	51.10^{a}	7.80^{c}	1.03^{a}	1.63^{b}	1.30^{c}	37.13^{b}
	72	51.76^{b}	8.53^{d}	1.00^{a}	1.63^{b}	1.27^{bc}	35.80^{a}
F4	0	54.46^{c}	8.47^{a}	1.27^{b}	1.43^{a}	1.20^{a}	33.17^{a}
	24	52.76^{b}	8.67^{a}	1.07^{a}	1.50^{a}	1.27^{a}	34.73^{b}
	48	50.87^{a}	10.27^{c}	1.07^{a}	1.83^{b}	1.60^{b}	34.37^{b}
	72	51.10^{a}	9.40^{b}	1.00^{a}	1.80^{b}	1.50^{b}	35.20^{c}
F5	0	52.57^{c}	9.90^{b}	1.37^{b}	1.57^{a}	1.30^{a}	33.30^{a}
	24	51.47^{a}	9.97^{b}	1.30^{b}	1.77^{b}	1.53^{b}	33.97^{b}
	48	52.60^{c}	9.50^{a}	1.07^{a}	1.80^{c}	1.50^{ab}	33.57^{ab}
	72	51.87^{b}	10.00^{b}	1.03^{a}	1.87^{c}	1.47^{ab}	33.77^{b}
F6	0	55.10^{d}	9.33^{ab}	1.33^{d}	1.50^{a}	1.20^{a}	31.53^{a}
	24	53.87^{c}	9.10^{b}	1.20^{bc}	1.70^{b}	1.40^{b}	32.20^{b}
	48	51.60^{b}	9.53^{a}	1.00^{a}	1.50^{a}	1.47^{c}	35.40^{c}
	72	50.17^{a}	10.40^{c}	1.07^{ab}	1.87^{b}	1.60^{d}	34.90^{c}

Values are presented as mean recorded from triplicate readings.At 95% confidence level, means with similar / different superscripts along the same column are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).Key: As presented in figure 4.1, page 62, FT= Fermentation time.

Effect of supplementation with *M. oleifera* on proximate content of the fermented formulated blends is presented in table 4.3. *M. oleifera*supplementation increased the proximate quality of fermented samples relative to the unsupplemented sample (sample F1). There was increase in ash content as quantity of *M. oleifera*added increased except in 2 g *M.oleifera* supplemented blend (sample F3) where a decrease was recorded. Carbohydrate content decreased as *M. oleifera* supplementation increased.

The least moisture content observed in sample F3 was not significantly different ($P \leq 0.05$) from the values observed for samples F1 and F5 while the highest moisture content recorded in sample F2 was not significantly different ($P \leq 0.05$) from the moisture content of 5 g *M. oleifera* supplemented blend (sample F6). The highest crude protein, crude fibre, crude fat and ash contents which were significantly different ($P \leq 0.05$) from the blends was recorded in 4 g *M. oleifera* supplemented blend (sample F5) except for ash content which was not significantly different ($P \leq 0.05$) from 5 g *M. oleifera* supplemented blend (sample F6). The least crude protein, crude fibre and crude fat was observed in 2 g *M. oleifera* supplemented blend (sample F3).

Table4.3: Effect of fermentation and supplementation with *M. oleifera* on the proximate composition of formulated blends

Sample code	Proximate Content (g/100g)					
	Moisture Content	Crude Protein	Crude Fibre	Crude Fat	Ash	Carbohydrate
F1	52.02±0.38^{a}	6.38±0.06^{a}	1.47±0.05^{a}	1.01±0.10^{a}	1.27±0.04^{a}	38.12±0.25^{e}
F2	52.79±0.46^{c}	8.05±0.07^{c}	1.56±0.05^{bc}	1.16±0.06^{bc}	1.28±0.04^{a}	35.27±0.43^{c}
F3	52.01±0.25^{a}	7.74±0.16^{b}	1.53±0.04^{ab}	1.04±0.02^{ab}	1.20±0.03^{a}	36.48±0.18^{d}
F4	52.30±0.44^{b}	9.20±0.22^{d}	1.63±0.06^{cd}	1.10±0.04^{abc}	1.39±0.05^{b}	34.37±0.23^{b}
F5	52.13±0.15^{a}	9.84±0.07^{f}	1.75±0.04^{f}	1.19±0.047^{d}	1.45±0.04^{b}	33.65±0.09^{a}
F6	52.68±0.58^{c}	9.59±0.15^{e}	1.66±0.05^{e}	1.15±0.04^{bc}	1.42±0.06^{b}	33.52±0.50^{a}

Values are presented as mean ± standard deviation recorded from triplicate readings.
At 95% confidence level, means with similar / different superscripts along the same column are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).
Key: As presented in figure 4.1, page 62.

4.4 Phytochemical composition of spontaneously fermented blends

As shown in table 4.4, the analysis of phytochemical composition revealed the phytic acid content of all complementary blends decreased as fermentation time increased except in 5 g *M.oleifera* supplemented blend (sample F6) in which it decreased up till 48 hours and increased slightly at 72 hours.

The highest phytic acid content in: non-supplemented *M.oleifera* blend (sample F1), 1 g *M.oleifera* supplemented blend (sample F2), 2 g *Moleifera* supplemented blend (sample F3), 3 g *M.oleifera* supplemented blend (sample F4), 4 g *M.oleifera* supplemented blend (sample F5) and 5 g *M.oleifera* supplemented blend (sample F6) were recorded by 0 hour. The lowest phytic acid content in: samples F1, F2, F3, F4, and F5 were recorded by 72 hours of fermentation time and in sample F6 by 48 hours.

The phytochemical analysis of the formulations shows tannin content as presented in table 4.4 in sample F2 decreased up till 48 hours and increased at 72 hours while it decreased as fermentation time increased in sample F1. In samples F3, F5 and F6 the tannin content increased by 24 hours of fermentation time and decreased as fermentation time increased. At 0 hour, non-supplemented *M.oleifera* blend (sample F1) and 1 g *M.oleifera* supplemented blend (sample F2) recorded highest value. Samples F3, F5and F6 recorded highest value by 24 hours fermentation time. The lowest tannin content was recorded by 72 hours fermentation time in samples F1, F3, F5 and F6 while sample F2, had the lowest at 48 hours of fermentation time.

The analysis of the phytochemical content shows decrease in the saponin content as fermentation time increased in samples F1, F2 and F3 (table 4.4). Same trend was observed for sample F5 except for the observed increase by 24 hours fermentation time. In 5 g *M.oleifera* supplemented blend (sample F6),the value was observed to decrease up till 48 hours and increase by 72 hours.

The highest saponin content was recorded in samples F1, F2 and F3 respectively at 0 hour. Samples F5 and F6 recorded highestcontent by 24 hours of fermentation time. The lowest value was observed for samples F1 and F2 by 72 hours and sample F3 by 48 hours

res. The lowest saponin content in samples F5 and F6were recorded by 72 hours and 48 hours respectively.

The alkaloid content of the formulated blends decreased as fermentation time increased in samples F2, F4, F5 and F6 (table 4.4). Alkaloid content was recorded highest in samples F2, F3, F5 and F6 before fermentation commenced. Alkaloid content was observed to the same through the period of fermentation in sample F1 and same in sample F4 from 0to 48 hours fermentation time. The lowest alkaloid content at 0 hour in samples F2 and F3; at 72 hours of fermentation timein samples F4, F5 and F6 was also observed.

Polyphenol content of the formulated blends as shown in figure 4.4erevealed that non-supplemented *M.oleifera* blend (sample F1), 1 g *M.oleifera* supplemented blend (sample F2), 2 g *M.oleifera* supplemented blend (sample F3), and 5 g *M.oleifera* supplemented blend (sample F6) increased as fermentation time increased. It also increased up till 48 hours and reduced by 72 hours in 3 g *M.oleifera* supplemented blend (sample F4). From the figure, the lowest polyphenol content in samples F1, F2, F3, F5 and F6 was observed before commencement of fermentation (0 hour). The highest polyphenol contentin sample F1, F2, F3 and F6 was recorded by 72 hours. By 48 hours, the highest polyphenol content was recorded in 3 g *M.oleifera* supplemented blend (sample F4).

The flavonoid content increased as fermentation time increased in non-supplemented *Moleifera* blend (sample F1) and 4 g *M.oleifera* supplemented blend (table 4.4). In 2 g *Moleifera* supplemented blend the flavonoid content decreased by 24 hours and increased as fermentation time progressed. The lowest flavonoid content in sample F1 and F5 was recorded at 0 hour while at 24 hours of fermentation time in sample F3. The highest flavonoid content in non-supplemented *M. oleifera* blend, 4 g *M. oleifera* supplemented blend and 2 g *M. oleifera* supplemented blend was obtained by 72 hours of fermentation time.

The effect of supplementation with *M.oleifera* on phytochemical content of the formulated blends is presented in table 4.5. Analysis of these formulated complementary blends for their phytochemical composition shows the phytic acid content was highest in

sample F5 (4 g *M.oleifera* supplementation) and lowest in samples F1 (blend without *M.oleifera*

Table 4.4: Effect of fermentation time on phytochemicals content of spontaneously fermented blends

Sample code	FT (Hour)	Phytochemical composition (mg/100g)					
		Phytic acid	Tannin	Saponnin	Alkaloid	Polyphenol (GAE/g)	Flavonoid
F1	0	33.33^{d}	155.00^{b}	8.33^{a}	6.67^{a}	11.13^{a}	11.67^{a}
	24	26.67^{c}	155.00^{b}	8.33^{a}	6.67^{a}	11.83^{b}	11.67^{a}
	48	20.00^{b}	135.00^{a}	6.67^{a}	6.67^{a}	12.27^{c}	20.00^{a}
	72	11.67^{a}	128.33^{a}	5.00^{a}	6.67^{a}	16.20^{d}	33.30^{b}
F2	0	36.67^{c}	188.33^{b}	31.67^{d}	11.67^{a}	13.87^{a}	65.00^{a}
	24	28.30^{b}	171.67^{c}	18.33^{c}	10.00^{a}	14.63^{b}	41.67^{ab}
	48	23.33^{a}	150.00^{a}	11.67^{b}	10.00^{a}	15.27^{c}	50.00^{b}
	72	18.33^{a}	153.33^{a}	5.00^{a}	6.67^{a}	15.36^{c}	40.00^{c}
F3	0	35.00^{c}	175.00^{c}	40.00^{b}	8.33^{b}	13.27^{a}	68.33^{c}
	24	35.00^{c}	178.33^{c}	10.00^{a}	5.00^{b}	14.40^{b}	30.00^{a}
	48	25.00^{b}	155.00^{b}	5.00^{a}	6.67^{b}	14.53^{b}	35.00^{a}
	72	13.33^{a}	140.00^{a}	10.00^{a}	6.67^{b}	16.20^{c}	51.67^{b}
F4	0	41.67^{c}	205.00^{c}	28.33^{a}	15.00^{a}	14.53^{a}	50.00^{a}
	24	31.67^{b}	185.00^{b}	13.33^{a}	15.00^{a}	15.23^{b}	45.00^{a}
	48	30.00^{b}	188.00^{b}	23.33^{b}	15.00^{a}	17.57^{c}	81.67^{b}
	72	11.67^{a}	168.33^{a}	10.00^{a}	8.33^{a}	16.27^{d}	68.33^{c}
F5	0	48.33^{c}	193.33^{c}	16.67^{a}	21.67^{b}	16.27^{a}	30.00^{a}
	24	45.00^{c}	206.67^{d}	33.3^{b}	21.67^{b}	17.00^{b}	68.33^{b}
	48	30.00^{b}	183.00^{b}	15.00^{a}	13.33^{a}	16.53^{a}	75.00^{b}
	72	21.67^{a}	173.33^{a}	13.33^{a}	11.67^{a}	17.77^{c}	86.67^{c}
F6	0	43.30^{c}	188.33^{b}	20.00^{ab}	16.67^{a}	15.37^{a}	38.33^{a}
	24	35.00^{b}	205.00^{c}	23.33^{b}	15.00^{a}	16.43^{b}	63.33^{b}
	48	23.33^{a}	175.00^{a}	13.33^{a}	11.67^{a}	15.57^{a}	56.67^{b}
	72	23.33^{a}	175.00^{a}	13.33^{a}	11.67^{a}	18.13^{c}	85.00^{c}

Values are presented as mean recorded from triplicate readings.

At 95% confidence level, means with similar / different superscripts along the same column are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT). Key: As presented in figure 4.1, page 62, FT= Fermentation time

supplementation) and sample F2 (1 g *M.oleifera* supplementation). Tannin was highest in the sample F5 and lowest in sample F1. Alkaloid content was observed to be highest in sample F5 and lowest in sample F1. Polyphenol was highest in sample F5 and lowest in sample F1. Saponin content was highest in sample F5 and lowest in sample F1 (blend without *M.oleifera* supplementation). The highest flavonoid content was recorded in 4 g *M. oleifera* supplementation and lowest in blend without *M. oleifera* supplementation. Polyphenol contents was different significantly ($P \leq 0.05$) among all formulated blends.

Generally the least phytochemical contents which was significantly different ($P \leq 0.05$) from other blends was recorded with non-supplemented *M. oleifera* blend (sample F1) and the highest content with sample F5 (4 g supplementation) except for alkaloid contents of samples F1, F2, F3 and saponin contents of samples F2, F4, F5 and F6 which were not significantlydifferent ($P \leq 0.05$) from each other.

Table4.5: Effect of fermentation and supplementation with *M oleifera* on phytochemicals content of blends

Sample code	Phytic acid (mg/100g)	Tannin (mg/100g)	Alkaloid (mg/100g)	Polyphenol (GAE/g)	Saponnin (mg/100g)	Flavonoid (mg/100g)
F1	22.92±2.57^{a}	143.33±3.76^{a}	6.67±0.71^{a}	12.86±0.18^{a}	7.08±0.74^{a}	21.92±3.70^{a}
F2	26.67±2.16^{b}	165.83±4.72^{c}	9.58±0.96^{a}	14.77±0.18^{c}	16.67±3.04^{bc}	49.17±0.19^{b}
F3	27.08±2.85^{b}	162.08±4.83^{b}	8.33±1.42^{a}	14.60±0.32^{b}	16.25±4.31^{b}	46.25±0.65^{b}
F4	28.75±3.38^{bc}	186.67±4.10^{e}	13.33±1.44^{b}	15.90±0.35^{d}	17.92±2.08^{bc}	61.25±4.53^{c}
F5	36.25±3.43^{d}	189.17±3.79^{f}	17.50±1.69^{c}	16.89±0.18^{f}	19.58±2.57^{c}	65.00±6.48^{c}
F6	31.25±2.69^{c}	185.83±3.84^{d}	13.75±1.09^{d}	16.38±0.33^{e}	17.50±1.57^{bc}	60.84±0.18^{c}

Values are presented as mean ± standard deviation recorded from triplicate readings.
At 95% confidence level, means with similar / different superscripts along the same column are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).
Key: As presented in figure 4.1, page 62.

4.5 Isolation and microbiological screening of Lactic acid bacteria (LAB)

A total of twenty-eight (28) out of one hundred and twenty eight (128) LAB isolateswere obtained from fermenting formulated blends. As presented in table4.6,microscopic observation show lactic acid bacteria isolates are Gram positive short rods while biochemical tests show they are: catalase negative in a preliminary screening exercise; negative for: endospore formation, oxidase, Voges Proskauer, arginine, citrate, hydrogen sulphide, motility tests and positive for methyl red test. These LAB isolates grew and survive temperatures 25^{o}C, 30^{o}C, 37^{o}C and 40^{o}C.These isolates were thereafter subjected to screening for probiotic potentials.

4.6 Screening of LAB isolates for probiotic potentials

4.6.1 Antimicrobial activity of LAB isolates against selected pathogens

The antimicrobial activity of theisolates is presented in table 4.7. The selected pathogens are: *Listeria* sp.*,* and *Klebsiella* sp.from environmental source, *Bacillus* sp.(FS) and *Salmonella* sp.from food source, *Pseudomonas* sp., *Eschericia coli*,*Staphylococcus* sp., and *Bacillus* sp.(CS) from clinical source. From thetable, almost 93% of the LAB isolates released substances which inhibited the growth of *Listeria* sp., with highest zone of inhibition recorded in isolate MCB18 and the lowest, in MCB43. *Bacillus* sp.(FS) was susceptible to substances produced by 97% of the LAB isolates with an inhibition zone ranging from 2.30 mm observed in MCB54 to 6.37 mm observed in MCB20. *Klebsiella* sp. was susceptible to substances produced by 75.9% of the LAB isolates with zones of inhibition ranging from 2.03 mm in MCB30, MCB58 and MCB62 to 6.17 mm in MCB2. Approximately 7% of lactic acid bacteria isolates did not produce substances that inhibited growth of *Pseudomonas* sp. The highest inhibition zone was observed in isolate MCB20 as well as MCB31 and the least in MCB55. *Escherichia coli* showed susceptibility to substances produced by 55% of LAB isolates. The inhibition zone ranged between 10.40 mm in MCB45 and 1.80 mm in MCB54. It was observed that *Staphylococcus* sp. was susceptible to substances produced by 62.1% of the LAB isolates. The zone of inhibition was highest in MCB24 and lowest in MCB55. *Bacillus* sp. (CS) was only susceptible to 35% of the LAB isolates and the inhibition zone ranged from 8 07

mm in MCB6 to 4.10 mm in MCB45. Seventy-six percent(76%) of lactic acid bacteria isolates were observed to have produced substances that inhibited *Salmonella* sp.with highest inhibition zone being recorded in MCB18 and lowest in MCB54.

Table4.6: Morphological and biochemical characterization of lactic acid bacteria isolates from fermenting blends

Isolate Code	Grams Reaction	Shape	Catalase	Oxidase	Endospore	V P	Arginine	Citrate	H_2S	Methyl Red	Motility	Growth at 25°C	30°C	37°C	40°C
MCB2	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB4	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB5	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB6	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB10	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB15	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB17	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB18	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB20	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB21	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB22	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB24	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB30	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB31	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB32	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB33	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB35	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB43	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB44	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB45	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB47	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB49	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB51	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB52	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB56	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB58	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB59	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB62	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+
MCB64	+	Rod	-	-	-	-	-	-	-	+	-	+	+	+	+

Key: -:Negative reaction, +: Positive reaction, V.P: Voges Proskauer

4.6.2 Antibiotic susceptibility of isolated lactic acid bacteria

The result of the susceptibility test carried out on the LABstrains to eight different antibiotics is as shown in table4.8. The result showsthe LAB isolates were resistant to allthe antibiotics having an inhibition zone of less than 10 mm in diameter in their presence according to N.C.L.L standard.

4.6.3 Tolerance ability of Lactic acid bacteria to Sodium chloride (NaCl).

The tolerance ability of LAB isolates to 2%, 4%, 6%, 8% as well as 10% (w/v) NaCl concentration incubated at room temperature(27± 2^{o}C) after 24 hours is presented in table 4.9. The growth of LAB strains decreases with increase in the salt concentration. Tolerance and growth was highest between 2%, to6% NaCl, for all LAB isolates. At 8% NaCl concentration, isolates MCB15, MCB22 and MCB33 showed no growth and slightly turbid growth was observed in isolates MCB2, MCB4, MCB6, MCB10, MCB18, MCB20, MCB21, MCB24, MCB30, MCB31, MCB32, MCB35, MCB45, MCB50, MCB51, MCB52, while at 10% NaCl concentration growth was observed in isolates MCB2, MCB5, MCB10, MCB18, MCB20, MCB24, MCB31, MCB32, MCB35, MCB43, MCB44, MCB45, MCB47, MCB49 and MCB52.

4.6.4 Effect of different hydrogen ion concentration (pH) on survival of lactic acid bacteria.

The growth of LAB isolates subjected to different acidic condition after a period of 24 hours was observed generally to increase with increase in pH (Table 4.10). The lowest Percentage survival was recorded for MCB35 at pH 2 while the highest was observed for MCB45 at pH 5.

4.6.5 Bile salt tolerance assay

Survival of the lactic acid bacteria isolates to bile salt of different concentration (0.3%, 0.5% and 0.8%) after 4 hours of holding time is shown in table 4.11. There was a decrease in colony count (survival rate) as the bile salt concentration increases.

With 0.3% bile salt concentration, isolates MCB4, MCB43, MCB47 had the highest growth and least growth in MCB45. Growth rate in 0.5% bile salt concentration

Table4.7: Antimicrobial activity of lactic acid bacteria against selected pathogens

Isolate Code	Diameter of Zone of Inhibition (mm)							
	Listeria sp.	***Bacillus sp***	***Klebsiella sp.***	***Pseudomonas sp.***	***Escherichia. coli***	***Staphylococcus sp.***	***Bacillus sp***	***Salmonella sp.***
MCB2	6.20±0.04	4.17±0.02	6.17±0.06	6.27±0.02	3.33±0.02	2.40±0.04	6.13±0.02	4.13±0.24
MCB4	3.23±0.02	6.07±0.02	3.27±0.02	6.07±0.02	3.33±0.05	4.13±0.05	0.00±0.00	3.13±0.24
MCB5	5.30±0.00	5.07±0.02	4.03±0.02	8.13±0.05	3.33±0.05	4.13±0.05	0.00±0.00	3.13±0.24
MCB6	4.10±0.00	4.20±0.00	4.30±0.00	7.10±0.00	3.10±0.00	2.27±0.02	8.07±0.02	3.20±0.00
MCB10	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.37±0.24
MCB15	4.20±0.00	0.00±0.00	0.00±0.00	7.20±0.00	4.20±0.00	2.40±0.00	6.20±0.00	4.10±0.00
MCB17	3.40±0.00	0.00±0.00	2.10±0.00	6.30±0.00	3.30±0.00	2.10±0.00	0.00±0.00	3.10±0.00
MCB18	6.30±0.00	6.07±0.02	2.50±0.00	7.40±0.00	4.10±0.00	4.20±0.00	6.40±0.00	4.17±0.24
MCB20	6.03±0.02	6.37±0.02	3.17±0.02	9.07±0.05	5.20±0.00	3.10±0.00	0.00±0.00	3.13±0.24
MCB21	3.17±0.02	3.20±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
MCB22	2.10±0.00	0.00±0.00	0.00±0.00	3.30±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.80±0.00
MCB24	2.27±0.02	0.00±0.00	0.00±0.00	5.17±0.02	0.00±0.00	5.10±0.00	0.00±0.00	2.07±0.24
MCB30	0.00±0.00	0.00±0.00	2.03±0.02	2.07±0.02	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
MCB31	5.10±0.04	5.13±0.05	6.07±0.02	9.07±0.02	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
MCB32	2.17±0.02	4.10±0.00	2.10±0.00	4.20±0.00	3.20±0.00	4.10±0.00	0.00±0.00	2.07±0.24
MCB33	0.00±0.00	0.00±0.00	2.20±0.00	6.10±0.00	0.00±0.00	3.20±0.00	4.17±0.02	2.17±0.24
MCB43	2.10±0.00	4.20±0.00	2.10±0.00	6.30±0.00	0.00±0.00	2.70±0.00	0.00±0.00	2.10±0.00
MCB45	4.30±0.00	5.27±0.05	6.10±0.00	7.37±0.02	10.40±0.00	4.10±0.00	4.10±0.00	4.20±0.00
MCB47	5.20±0.00	5.20±0.00	5.13±0.02	4.17±0.02	9.17±0.02	3.07±0.02	5.67±0.02	4.13±0.24
MCB49	5.07±0.02	5.07±0.02	4.87±0.02	8.17±0.02	9.47±0.02	4.17±0.02	6.07±0.02	4.10±0.00
MCB50	2.40±0.00	0.00±0.00	0.00±0.00	4.30±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
MCB51	2.20±0.00	0.00±0.02	2.10±0.00	6.20±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.20±0.00
MCB52	2.20±0.00	0.00±0.02	2.10±0.00	8.07±0.02	0.00±0.00	0.00±0.00	0.00±0.00	2.10±0.00
MCB54	2.13±0.02	2.30±0.02	2.20±0.00	6.30±0.00	1.80±0.00	0.00±0.00	0.00±0.00	1.13±0.24
MCB55	0.00±0.00	6.10±0.02	0.00±0.00	1.80±0.00	0.00±0.00	1.67±0.02	0.00±0.00	0.00±0.00
MCB56	0.00±0.00	0.00±0.02	2.10±0.00	2.40±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
MCB58	3.17±0.02	3.10±0.02	2.03±0.02	4.33±0.05	8.40±0.00	0.00±0.00	0.00±0.00	0.00±0.00
MCB62	5.30±0.00	4.20±0.02	2.03±0.02	7.07±0.02	8.50±0.00	3.10±0.00	5.17±0.02	3.20±0.00
MCB64	6.10±0.00	6.27±0.02	2.20±0.00	7.10±0.00	8.10±0.00	2.87±0.02	6.07±0.02	4.13±0.02

Values are given as mean ±standard deviation from triplicate readings.

Isolate Code	Diameter of Zone of Inhibition (mm)							
	CAZ (30 µg)	CTR (30 µg)	GEN (10 µg)	CRX (30 µg)	CXC (5 µg)	ERY (30 µg)	AGU (30 µg)	OFL (5 µg)
MCB2	R	R	R	R	R	R	R	R
MCB4	R	R	R	R	R	R	R	R
MCB5	R	R	R	R	R	R	R	R
MCB6	R	R	R	R	R	R	R	R
MCB10	R	R	R	R	R	R	R	R
MCB15	R	R	R	R	R	R	R	R
MCB17	R	R	R	R	R	R	R	R
MCB18	R	R	R	R	R	R	R	R
MCB20	R	R	R	R	R	R	R	R
MCB21	R	R	R	R	R	R	R	R
MCB22	R	R	R	R	R	R	R	R
MCB24	R	R	R	R	R	R	R	R
MCB30	R	R	R	R	R	R	R	R
MCB31	R	R	R	R	R	R	R	R
MCB32	R	R	R	R	R	R	R	R
MCB33	R	R	R	R	R	R	R	R
MCB35	R	R	R	R	R	R	R	R
MCB43	R	R	R	R	R	R	R	R
MCB45	R	R	R	R	R	R	R	R
MCB47	R	R	R	R	R	R	R	R
MCB49	R	R	R	R	R	R	R	R
MCB50	R	R	R	R	R	R	R	R
MCB51	R	R	R	R	R	R	R	R
MCB52	R	R	R	R	R	R	R	R
MCB54	R	R	R	R	R	R	R	R
MCB55	R	R	R	R	R	R	R	R
MCB56	R	R	R	R	R	R	R	R
MCB58	R	R	R	R	R	R	R	R
MCB62	R	R	R	R	R	R	R	R
MCB64	R	R	R	R	R	R	R	R

Table4.8: Antibiotic susceptibility of lactic acid bacteria

Key: Ceftazidime = CAZ, Gentamycin = GEN, Cefuroxime = CRX, Ceftriaxone =CTR, Cloxicillin = CXC, Erythromycin = ERY, Ofloxacin = OFL and Amoxycillin/ Clavulinate = AUG, R = Resistant (≤ 10mm), S = Sensitive (≥13mm)

Table4.9: Growth of lactic acid bacteria isolates at different sodium chloride (NaCl) concentration

Isolate	NaCl Concentration (%)				
Code	**2**	**4**	**6**	**8**	**10**
MCB2	++	++	++	+	+
MCB4	++	++	++	+	-
MCB5	++	++	++	++	+
MCB6	++	++	++	+	+
MCB10	++	++	++	+	+
MCB15	++	++	+	-	-
MCB18	++	++	++	+	+
MCB20	++	++	+	+	+
MCB21	++	++	+	+	-
MCB22	++	++	+	-	-
MCB24	++	++	++	+	++
MCB30	++	++	++	+	-
MCB31	++	++	++	+	++
MCB32	++	++	++	+	++
MCB33	++	++	++	-	-
MCB35	++	++	+	+	+
MCB43	++	++	++	++	+
MCB44	++	++	++	++	+
MCB45	++	++	++	+	+
MCB47	++	++	++	++	+
MCB49	++	++	++	++	+
MCB50	++	++	+	+	-
MCB51	++	++	+	+	-
MCB52	++	++	+	+	+
MCB56	++	++	++	++	-
MCB58	++	++	++	++	-
MCB59	++	++	++	++	-
MCB64	++	++	++	++	-

Key: -= No growth, + = Weak growth, ++ = Strong growth.

Table4.10:Effect of hydrogen ion concentration (pH) on survival (%) of LAB isolated from fermenting blends

Isolate	Hydrogen Ion Concentration (pH)			
Code	2	3	4	5
MCB2	79.91±0.00^{r}	80.09±0.00^{w}	81.19±0.00^{q}	88.98±0.00^{x}
MCB4	78.79±0.00^{q}	80.47±0.00^{x}	85.32±0.00^{x}	89.58±0.00^{y}
MCB5	81.03±0.00^{s}	82.33±0.00^{z}	84.60±0.00^{v}	87.21±0.00^{r}
MCB6	73.08±0.00^{n}	82.26±0.00^{y}	85.18±0.00^{w}	87.63±0.00^{s}
MCB10	52.81±0.00^{d}	51.94±0.00^{b}	55.15±0.00^{a}	66.04±0.00^{b}
MCB15	75.57±0.00^{p}	76.45±0.00^{s}	83.39±0.00^{u}	83.79±0.00^{m}
MCB18	54.10±0.00^{f}	62.22±0.00^{m}	81.85±0.00^{r}	86.67±0.00^{q}
MCB20	68.70±0.00^{l}	51.55±0.00^{a}	75.85±0.00^{m}	81.23±0.01^{i}
MCB21	51.84±0.00^{c}	55.58±0.00^{d}	59.48±0.00^{b}	65.13±0.00^{a}
MCB22	52.91±0.00^{e}	55.66±0.00^{e}	74.11±0.05^{l}	82.68±0.09^{l}
MCB24	59.12±0.00^{i}	60.89±0.01^{l}	64.08±0.01^{d}	73.46±0.02^{e}
MCB30	51.83±0.00^{c}	60.44±0.01^{j}	65.84±0.01^{e}	81.44±0.01^{j}
MCB31	71.16±0.00^{m}	74.14±0.01^{r}	82.46±0.01^{t}	77.80±0.01^{g}
MCB33	54.72±0.01^{g}	55.75±0.01^{f}	78.73±0.01^{o}	87.86±0.01^{t}
MCB35	51.14±0.01^{b}	57.35±0.02^{h}	70.17±0.01^{j}	73.10±0.00^{d}
MCB43	54.81±0.02^{g}	60.70±0.01^{k}	66.23±0.01^{f}	79.74±0.01^{h}
MCB44	61.00±0.05^{j}	73.52±0.02^{q}	87.02±0.04^{z}	88.44±0.01^{v}
MCB45	49.95±0.10^{a}	78.48±0.02^{u}	86.35±0.01^{y}	90.09±0.02^{z}
MCB47	75.02±0.09^{o}	77.36±0.02^{t}	78.79±0.01^{p}	88.44±0.01^{w}
MCB49	68.75±0.02^{l}	79.47±0.01^{v}	82.06±0.04^{s}	85.29±0.01^{o}
MCB51	55.49±0.01^{h}	60.31±0.01^{i}	68.33±0.02^{h}	84.89±0.01^{n}
MCB52	63.84±0.01^{k}	52.43±0.02^{c}	67.22±0.01^{g}	81.71±0.01^{k}
MCB56	61.17±0.01^{k}	64.60±0.01^{n}	69.02±0.04^{i}	77.64±0.01^{f}
MCB58	65.28±0.01^{k}	70.36±0.01^{p}	78.40±0.01^{n}	87.94±0.01^{u}
MCB59	54.77±0.09^{g}	57.03±0.01^{g}	62.46±0.01^{c}	66.80±0.01^{c}
MCB62	61.03±0.01^{j}	57.03±0.01^{o}	72.61±0.01^{k}	85.77±0.09^{p}

Values are presented as mean ± standard deviation recorded from triplicate readings.
At 95% confidence level, means with similar / different superscripts along the same column are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).

ranges from 40% obtained in MCB47 to 6.25% obtained in MCB5 and MCB49 while no growth was observed in other isolates. At 0.8% bile salt concentration, MCB18 and MCB47 had the highest growth rate and no growth was recorded in other isolates.

4.6.6 Gastric juice tolerance assay

Tolerance to simulated gastric juice of LAB isolates for 90 minutes and 3 hours is as shown in table 4.12. Initial count of Lab isolates ranged from 6.61 LogCFU/mL in MCB44 to 6.08 LogCFU/mL in MCB24. No growth was observed for MCB21 after 90 minutes and for MCB6, MCB21, MCB22, MCB24, MCB35, MCB51 and MCB52 after 180 minutes. At 90 minutes, the colony count was recorded highest in MCB43 and lowest in MCB51. After 180 minutes, colony count was obtained highest in MCB44 and lowest in MCB64. Tolerance pattern of the lactic acid bacteria isolate to simulated gastric juice at the time intervals differ significantly ($P \leq 0.05$) among the isolates.

4.6.7 Cell surface hydrophobicity assay of the isolated lactic acid bacteria

Table4.13 shows the results of the hydrophobicity test carried out on the lactic acid bacteria isolates. The outcome of this test varied among LAB isolated from the fermenting blends. Microbial adherence to hydrocarbon (MATH) of the lactic acid bacteria isolate ranges from 3.00% in MCB33 to 59.4% in MCB4 for xylene; 2.50% in MCB20 to 82.83% in MCB47 for chloroform and 1.57% in MCB2 to 66.53% in MCB30 for benzene.Hydrophobicity values for only few lactic acid bacteria isolates were high and selected.

After careful consideration of the *in-vitro* tests carried out across board, a total of five isolates (MCB4,MCB15, MCB18, MCB47 and MCB49) out of all LABobtained in the spontaneously fermenting blend hadthe most promising probiotic potential.

Isolates MCB4, MCB15, MCB18, MCB47 and MCB49 showed good antimicrobial activity against the selected test pathogens, susceptibility to antibiotics, adherence to hydrocarbon, tolerance to: acidic condition, high salt concentration, bile salt and simulated gastric juice. Hence they were selected for safety assessment tests.

4.6.8 Safety assessment test of LAB Isolates

The safety assessment of the lactic acid bacteria isolates is presented in table 4.14. From the table, all LAB isolates were negative for the production of the enzyme DNAse (deoxyribonulease) and gelatinase and positive for exopolysaccharide production.

The pathogenicity test carried out on the isolate reveals all lactic acid bacteria isolates were negative for lecithinate and heamolytic test.

Lactic acid bacteria isolates MCB4, MCB18 and MCB47 all utilized lactose, produced amylase, protease and lipase enzymes. These enzymes are essentially required to hydrolyze major nutrients in food substrate hence increasing the nutritional qualities of fermented food.

Table4.11: Total viable counts of lactic acid bacteria isolates (LogCFU/mL) in bile salt after four hours holding time

Isolate	LAB Growth (%)/Bile salt concentration(%)		
Code	0.3	0.5	0.8
MCB2	34.80	NG	NG
MCB4	48.30	25.7	11.8
MCB5	37.8	6.25	NG
MCB6	31.80	NG	NG
MCB10	46.4	11.8	NG
MCB15	42.3	28.6	NG
MCB18	51.6	34.8	16.70
MCB20	28.6	NG	NG
MCB21	11.8	NG	NG
MCB22	21.10	NG	NG
MCB24	46.4	11.80	NG
MCB30	11.8	NG	NG
MCB31	16.70	NG	NG
MCB32	11.80	NG	NG
MCB33	31.80	NG	NG
MCB35	28.60	NG	NG
MCB43	48.30	28.60	6.25
MCB44	31.80	NG	NG
MCB45	6.25	NG	NG
MCB47	48.30	40.00	16.70
MCB49	34.80	6.25	NG
MCB50	21.10	NG	NG
MCB51	11.80	NG	NG
MCB52	25.0	NG	NG
MCB56	11.8	NG	NG
MCB58	16.70	NG	NG
MCB59	44.40	6.25	NG
MCB64	16.70	NG	NG

Values are presented as mean oftriplicate readings

Key: NG – No Growth.

Table4.12: Total viable count of lactic acid bacteria isolates (LogCFU/mL) insimulated gastric juice

Isolate Code	LAB Count (LogCFU/mL)/Time of Assay (Minutes)		
	0 min	90 mins	180 mins
MCB2	6.36±0.01^{defg}	6.23±0.012^{gh}	5.57±0.09^{d}
MCB4	6.58±0.01^{j}	6.15±0.012^{gh}	5.85±0.01^{fhi}
MCB5	6.40±0.12e^{fghl}	6.22±0.061^{gh}	5.30±0.12^{c}
MCB6	6.28±0.01^{cde}	5.85±0.012^{f}	0.00±0.00^{a}
MCB10	6.11±0.01^{j}	6.28±0.012^{hi}	5.60±0.12^{de}
MCB15	6.58±0.01^{j}	6.20±0.12^{gh}	5.83±0.11^{fh}
MCB18	6.46±0.01^{fghij}	6.23±0.01^{gh}	6.04±0.01^{i}
MCB20	6.38±0.01^{defgh}	6.28±0.01^{hi}	5.30±0.12^{c}
MCB21	6.13±0.18^{abc}	0.00±0.00^{a}	0.00±0.00^{a}
MCB22	6.26±0.01^{bcde}	5.48±0.01^{d}	0.00±0.00^{a}
MCB24	6.08±0.01^{a}	5.85±0.01^{f}	0.00±0.00^{a}
MCB30	6.57±0.01^{j}	6.15±0.02^{gh}	5.85±0.01^{fhi}
MCB31	6.32±0.02^{def}	6.18±0.01^{gh}	5.96±0.02^{hi}
MCB33	6.36±0.01^{defg}	6.23±0.01^{gh}	5.28±0.01^{c}
MCB5	6.23±0.01^{abcd}	5.30±0.12^{c}	0.00±0.00^{a}
MCB43	6.53±0.01^{hij}	6.29±0.01^{hi}	5.78±0.01^{efh}
MCB44	6.61±0.01^{j}	6.41±0.01^{i}	6.04±0.01^{i}
MCB45	6.51±0.01^{ghij}	6.23±0.01^{gh}	5.70±0.01^{def}
MCB47	6.54±0.01^{ij}	6.28±0.01^{hi}	5.90±0.12^{fhi}
MCB49	6.57±0.01^{j}	6.17±0.15^{gh}	5.95±0.01^{hi}
MCB51	6.32±0.01^{def}	4.77±0.12^{b}	0.00±0.00^{a}
MCB52	6.26±0.01^{bcde}	5.48±0.01^{d}	0.00±0.00^{a}
MCB56	6.30±0.01^{de}	6.08±0.01^{g}	5.85±0.01^{fhi}
MCB58	6.57±0.01^{j}	6.15±0.01^{gh}	5.95±0.01^{hi}
MCB59	6.40±0.12^{efghi}	6.26±0.01^{ghi}	5.60±0.13^{de}
MCB64	6.11±0.04^{ab}	5.67±0.08^{e}	3.50±0.13^{b}

Values are presented as mean ± standard deviation recorded from triplicate readings.
At 95% confidence level, means with similar / different superscripts along the same column are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).

Table4.13 Microbial adherence of LAB isolates to hydrocarbon

Isolate Code	Cell hydrophobicity (%)/Hydrocarbons		
	Xylene	Chloroform	Benzene
MCB2	17.53±0.20^{e}	32.60±0.21^{i}	1.57±0.07^{b}
MCB4	59.40±0.17^{o}	21.70±0.06^{i}	4.57±0.07^{f}
MCB5	22.23±0.18^{fg}	11.93±0.15^{cd}	30.37±0.12^{n}
MCB6	10.67±0.09^{c}	21.30±0.15^{i}	4.90±0.16^{g}
MCB10	21.67±1.20^{f}	29.30±0.12^{k}	53.17±0.15^{r}
MCB15	43.70±0.12^{m}	54.40±0.12^{m}	33.87±0.12^{p}
MCB18	58.30±0.10^{o}	29.57±0.09^{k}	58.77±0.09^{t}
MCB20	33.57±0.20^{j}	2.50±0.00^{a}	20.43±0.03^{l}
MCB21	7.43±0.033^{b}	12.20±0.10^{cd}	8.20±0.06^{i}
MCB22	13.23±0.09^{d}	11.33±0.12^{c}	8.20±0.067^{i}
MCB24	1.57±0.088^{a}	62.73±0.09^{n}	56.23±0.09^{s}
MCB30	24.33±0.12^{g}	12.47±0.12^{d}	66.53±0.15^{u}
MCB31	36.60±0.12^{k}	11.70±0.12^{cd}	26.30±0.12^{m}
MCB33	3.00±0.00^{a}	10.27±0.12^{b}	3.43±0.03^{e}
MCB35	19.13±0.15^{e}	24.27±0.12^{j}	5.67±0.19^{h}
MCB43	31.57±0.09^{i}	10.30±0.12^{b}	2.90±0.12^{d}
MCB44	30.77±0.09^{i}	12.20±0.12^{cd}	4.40±0.00^{f}
MCB45	22.93±3.22^{fg}	13.77±0.09^{e}	30.77±0.09^{o}
MCB47	49.40±0.12^{n}	82.83±0.15^{p}	2.45±0.09^{c}
MCB49	23.30±0.12^{fg}	65.67±1.21^{o}	30.70±0.12^{o}
MCB51	26.30±0.12^{h}	17.27±0.18^{h}	11.40±0.12^{j}
MCB52	34.57±0.15^{j}	21.90±0.12^{i}	11.70±0.10^{k}
MCB56	11.07±0.09^{c}	17.67±0.88^{h}	45.43±0.15^{q}
MCB58	33.93±0.15^{j}	14.90±0.12^{f}	5.40±0.12^{h}
MCB59	26.77±0.09^{h}	14.33±0.15^{ef}	36.7±0.07^{a}
MCB64	38.87±0.18^{i}	15.90±0.12^{g}	3.53±0.09^{e}

Values are presented as mean ± standard deviation recorded from triplicate readings. At 95% confidence level, means with similar / different superscripts along the same column are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).

Table4.14: Safety assessment tests for LAB isolates

Isolates code	Exopoloysaccharide	Gelatinase	Dnase	Haemolysis	Lecitinate	Lactose Tolerance	Amylase	Protease	Lipase
MCB4	+	-	-	-	-	++	+	++	+
MCB15	+	-	-	-	+	++	+	++	++
MCB18	+	-	-	-	-	++	++	++	++
MCB47	+	-	-	-	-	++	+	++	++
MCB49	+	-	-	-	+	++	+	++	++

Key: ++: Strong activity +: Weak activity, -: No activity

4.6.9 Sugar fermentation pattern of selected LAB isolates

Table 4.15 shows the sugar fermentation ability of LAB isolate MCB4, MCB15, MCB18, MCB47 and MCB49 observed to have probiotic potentials. All the five isolates with considerable probiotic properties fermented glucose with gas production in isolates MCB4, MCB15 and MCB49, while none of these isolates fermented inositol, rhaffinose and rhamnose. All the isolates hydrolyzed galactose, lactose, fructose, sucrose, sorbital, L-Arabinose, D- Arabinose, mannitol and maltose. Isolates MCB18 and MCB49 did not fermented xylose.

Based on these results with reference to the Bergey's manual of systemic bacteriology (Vos *etal*. (2009), the most probable identity of the LAB isolates were: *Lactobacillus plantarum* – MCB4*, Lactobacillus species* – MCB15*, Lactobacillusplantarum* – MCB18, *Lactobacillus plantarum* – MCB47 and *Lactobacillus plantarum* – MCB49.

4.7 Molecular identification of selected LAB isolates

The molecular identification of bacterial isolates is shown in table 4.16. The nucleotide sequence obtained and analyzed from 16SrRNA of isolatesMCB 49, MCB47, MCB4, MCB 15 and MCB18 yielded strong homologies of up to 99%, 99%, 99%, 99% and 95% similarities respectively with those of several cultivated strains *Lactobacillus* and were confirmed to be *Lactobacillus plantarum*-MCB49, *Lactobacillus pentosus*-MCB47, *Lactobacillus plantarum*-MCB4, *Lactobacillus pentosus*-MCB15 and*Lactobacillus plantarum*-MCB18 with accession numbers of MH984841, MH984842, MH984843, MH984844 and MH984845 respectively.

Using MEGA version 7.0.21 with neighbor-joining techniques as well as Jukes-Cantor distance matrices respectively, phylogenetic and molecular evolutionary analyses as well as phylogenetic relationship between the bacterial isolates and nearest *Lactobacillus* strain identified by BLAST analysis were conducted as shown in figure 4.5

Table4.15: Sugar fermentation pattern of selected LAB isolates

Sugar	Lactic acid bacteria Isolate				
	MCB4	**MCB15**	**MCB18**	**MCB47**	**MCB49**
Glucose	+G	+G	+G	+	+G
Ducitol	-	-	-	-	-
Rhaffinose	-	-	-	-	-
Rhamnose	-	-	-	-	-
Galactose	+	+	+	+	+
Lactose	+	+	+	+	+
Fructose	+	+	+	+	+
Sucrose	+	+	+	+	+
Sorbitol	+	+	+	+	+
L-Arabinose	+	+	+	+	+
D- Arabinose	+	+	+	+	+
Maltose	+	+	+	+	+
Xylose	-	+	-	+	-
Mannitol	+	+	+	+	+
Adonitol	-	-	-	-	-
Inositol	-	-	-	-	-
Most probable identity	*Lactobacillus plantarum*	*Lactobacillus sp*	*Lactobacillus plantarum*	*Lactobacillus sp*	*Lactobacillus plantarum*

Key: +: Positive, -: Negative, G: Gas production

Table4.16: Molecular identification of LAB isolates.

Isolate Code	Length of Nucleotide sequence	Accession number of nearest homology	Percentage Similarity (%)	Name of organism
MCB49	1379	MH984841	99	*Lactobacillus plantarum*
MCB47	1507	MH984842	99	*Lactobacillus pentosus*
MCB4	1501	MH984843	99	*Lactobacillus plantarum*
MCB15	1481	MH984844	99	*Lactobacillus pentosus*
MCB18	1341	MH984845	95	*Lactobacillus plantarum*

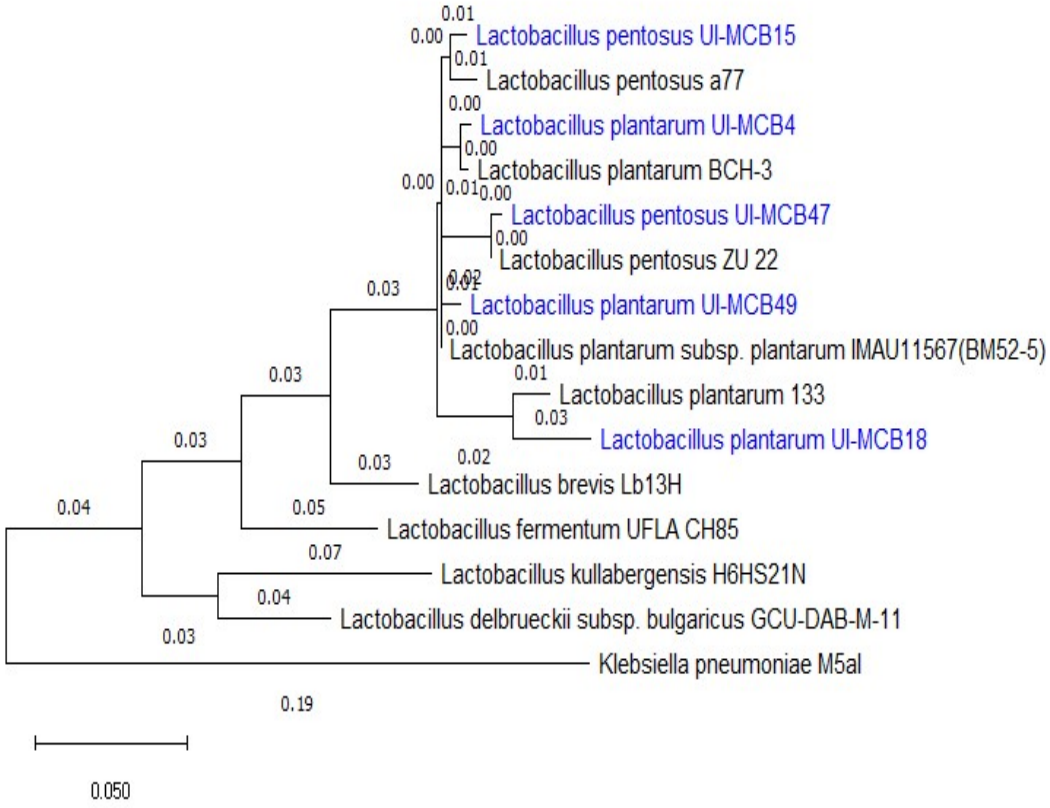

Figure4.4: Phylogenetic relatedness of selected and sequenced LAB isolates

4.8 Controlled fermentation of formulated blend

The 3 g *M.oleifera* supplemented formulated blend (sample F4) with recorded good proximate and phytochemical compositions of all the spontaneously fermented samples was selected for the production of the starter developed complementary food. The lactic acid bacteria isolates with identity: *Lactobacillus plantarum*-MCB18 and *L. pentosus*-MCB47 were selected as starter and used as starter culture of varying inoculum sizes as both had the best overall probiotic potential from *in-vitro* analysis in this study(Table 4.17). The results of the physico-chemical and sensory qualities of starter-developed complementary blends using varying inoculum sizes is shown in figures and tables.

4.8.1 pH of starter-fermented blend

The results of chemical analysis over the period of fermentation as presented in figure 4.5shows the pH of sample F4 fermented with varying inoculum sizes of *L. plantarum* and *L. pentosus* (samples FA1, FA2, FA3, FB1, FB2,FB3andsample FC). There was acidic fermentation as the hydrogen ion concentration was observed todecrease as fermentation time increased while the pH of same blend fermented with a combination of *L. plantarum* (1.5 x 10^8 CFU/mL)and *L. pentosus* (6.0 x 10^8 CFU/mL) (sample FC) decreased by 36 hours and increased again by 48 hours. The highest pH value was recorded in sample F4before commencement of fermentation (0 hour) while the lowest was recorded in sample F4 fermented with *L. pentosus* (6.0 x 10^8 CFU/mL) (sample FA3) by 48 hours.

4.8.2 Total titratable acidity (TTA) of starter-fermented blend

As presented in figure 4.6, total titratable acidity over the period of fermentation shows the TTA of samples FA1, FA2, FA3, FB1, FB2 and FB3 increases as fermentation time increases while the TTA of sample FC increased uptill 36 hours and decreased by 48 hours. The highest content of lactic acid was obtained in blend sample FA3 by 48 hours of fermentation time and lowest was recorded in the blend before fermentation commenced.

Table4.17: Starter culture used as inoculum for controlled fermentation of blend

Sample Code	Isolate name	Inoculum size used (x 10^8 CFU/mL)
FA1	*Lactobacillus pentosus*-MCB47	1.5
FA2	*Lactobacillus pentosus*-MCB47	3.0
FA3	*Lactobacillus pentosus*-MCB47	6.0
FB1	*Lactobacillus plantarum*-MCB18	1.5
FB2	*Lactobacillus plantarum*-MCB18	3.0
FB3	*Lactobacillus plantarum*-MCB18	6.0
FC	*Lactobacillus pentosus*-MCB47	6.0
	Lactobacillus plantarum-MCB18	1.5

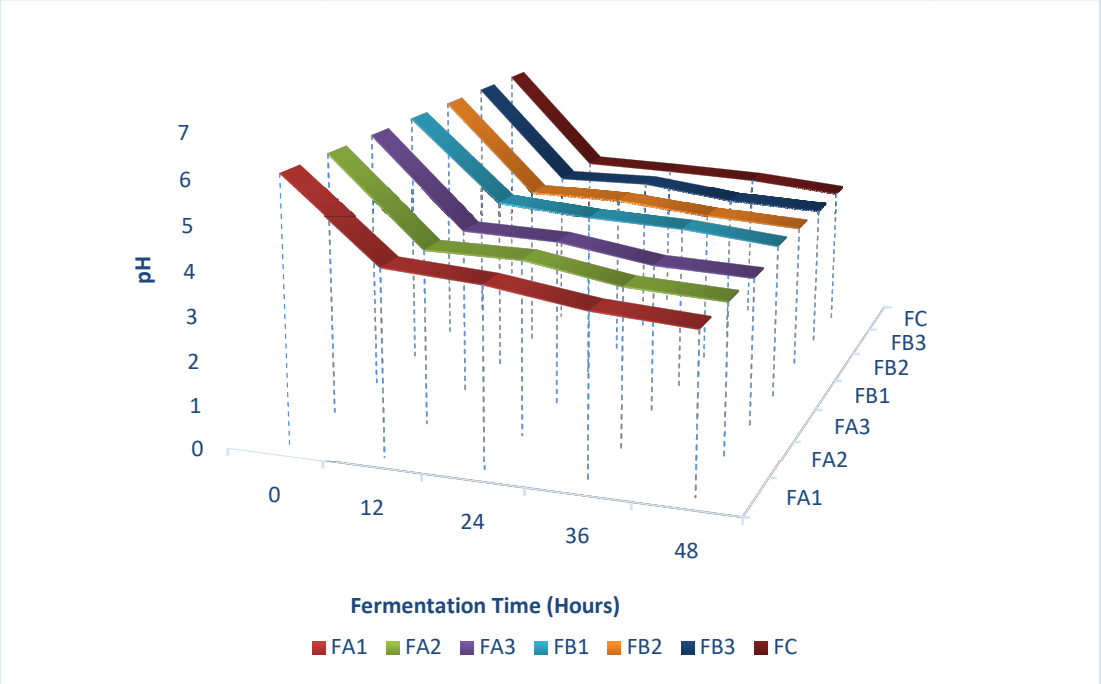

Figure4.5: Effect of fermentation time on the pH of the starter-fermented blends

Key:

Sample Code	Isolate name	Inoculum size used (x 10^8 CFU/mL)
FA1	*Lactobacillus pentosus*-MCB47	1.5
FA2	*Lactobacillus pentosus*-MCB47	3.0
FA3	*Lactobacillus pentosus*-MCB47	6.0
FB1	*Lactobacillus plantarum*-MCB18	1.5
FB2	*Lactobacillus plantarum*-MCB18	3.0
FB3	*Lactobacillus plantarum*-MCB18	6.0
FC	*Lactobacillus pentosus*-MCB47	6.0
	Lactobacillus plantarum-MCB18	1.5

:

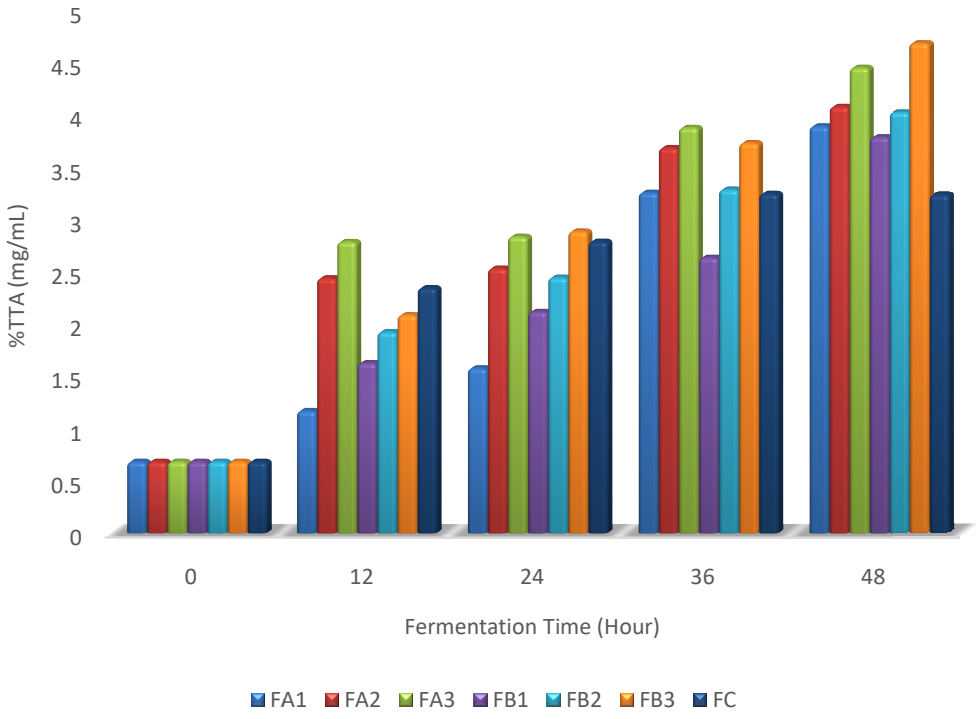

Figure4.6: Effect of fermentation on the titratable acidity of the starter-fermented blends

Key:

Sample Code	Isolate name	Inoculum size used (x 10^8 CFU/mL)
FA1	*Lactobacillus pentosus*-MCB47	1.5
FA2	*Lactobacillus pentosus*-MCB47	3.0
FA3	*Lactobacillus pentosus*-MCB47	6.0
FB1	*Lactobacillus plantarum*-MCB18	1.5
FB2	*Lactobacillus plantarum*-MCB18	3.0
FB3	*Lactobacillus plantarum*-MCB18	6.0
FC	*Lactobacillus pentosus*-MCB47	6.0
	Lactobacillus plantarum-MCB18	1.5

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Table 4.18 shows the effect of using varying inoculum sizes of the isolates on pH and titratable acidity of the fermentedblend. The result shows significant decrease ($P \leq 0.05$) in the hydrogen ion concentration and significant ($P \leq 0.05$) increase in TTA values with increase in inoculum size of both LAB isolates used. The result shows sample FC had the lowest pH relative to the pH values obtained for samples FA1, FA2, FA3, FB1, FB2 and FB3. Sample FC had the lowest pH value while it was highest in sample FA1. The TTA value in sample FB1 was lowest and highestin sample FA3.

Table4.18: Effect of using varying inoculum sizes on chemical analysis of starter-fermented blend

Sample code	pH	TTA (mg/mL)
FA1	4.33 ± 0.24^{g}	2.11 ± 0.33^{a}
FA2	4.25 ± 0.25^{e}	2.68 ± 0.33^{e}
FA3	4.18 ± 0.26^{c}	2.92 ± 0.34^{g}
FB1	4.32 ± 0.24^{f}	2.17 ± 0.28^{b}
FB2	4.21 ± 0.25^{d}	2.47 ± 0.31^{d}
FB3	4.15 ± 0.26^{b}	2.80 ± 0.37^{f}
FC	4.14 ± 0.26^{a}	2.46 ± 0.25^{c}

Values are presented as mean ±standard deviation recorded from triplicate readings.
At 95% confidence level, means with similar / different superscripts along the same column are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).
Key:

Sample Code	Isolate name	Inoculum size used (x 10^8 CFU/mL)
FA1	*Lactobacillus pentosus*-MCB47	1.5
FA2	*Lactobacillus pentosus*-MCB47	3.0
FA3	*Lactobacillus pentosus*-MCB47	6.0
FB1	*Lactobacillus plantarum*-MCB18	1.5
FB2	*Lactobacillus plantarum*-MCB18	3.0
FB3	*Lactobacillus plantarum*-MCB18	6.0
FC	*Lactobacillus pentosus*-MCB47	6.0
	Lactobacillus plantarum-MCB18	1.5

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4.8.3 Proximate or nutritional analysis of the starter-fermented blend

Analysis of proximate composition of sample F4 fermented with *L. pentosus*(1.5 x 10^8 CFU/mL) (sample FA1)during the fermentation period is presented in table 4.19. From the figure, the moisture content increased by 12 hours of fermentation time and decreased as fermentation time increased while crude protein, crude fibre, ashand crude fat contents decreased between 12 and 24 hours and increased as fermentation time increased.

Highest and lowest moisture content value was obtained by 12 hours and 48 hours of fermentation time respectively. Highest crude protein, crude fibre, crude fat contents were obtained by 48 hours of fermentation while their lowest were recorded at 12 hours. Ash content ranges between 2.35g/100g obtained by 48 hours of fermentation and 1.11g/100g obtained by 24 hours.

Effect of fermentation time on 3.0 x 10^8 CFU/mL *L. pentosus* fermented(sample FA2)over a period of 48 hours is presented in table 4.19. The result shows the contents ofproximate composition deceases within the first 12 and 24 hours and increases as fermentation progressedtill 48 hours.

The crude protein content ranges between 19.77% obtained by 48 hours of fermentation time and 11.32g/100g obtained by 12 hours. Highest crude fibre content was obtained by 48 hours and the lowest obtained by 12 hours. Crude fat content ranges from 12.35g/100g obtained by 48 hours of fermentation time to 3.08g/100g obtained by 12 hours. The highest ash content was obtained by 48 hours of fermentation time while the lowest was recorded by 24 hours.

Table 4.19shows result of analysis of the proximate composition of 6.0 x 10^8 CFU/mL *L. pentosus* fermented (sample FA3) fermented for 48 hours. The result showed the moisture, crude protein, and ash contents increased by 12 hours of fermentation time, decreased within 24 and 36 hours while they increased as fermentation time progressed. The crude fat as well as crude fibre decreased within the first 24 hours and increased as fermentation time progressed while carbohydrate content increases within 24 hours and decreased as fermentation time progressed.

The highest moisture content was recorded by 12 hours of fermentation time while the lowest was obtained by 36 hours of fermentation time. Protein content ranges between 20.55g/100g obtained by 48 hours of fermentation time and 11.35g/100g obtained by 12

hours. Highest crude fibre content was obtained by 48 hours while its lowest was obtained by 24 hours. Crude fat was in the range of 13.58g/100g obtained by 48 hours of fermentation time to 2.91g/100g obtained by 12 hours. Ash content was recorded highest by 48 hours of fermentation time and recorded lowest by 24 hours.

Table 4.19 reveals the proximate composition of sample F4 fermented with 1.5 x 10^8 CFU/mL *L. plantarum* (sample FB1) over a period of 48 hours. As shown in the result, the crude fat, crude fibre and ash contents decreased within 12 hours and 36 hours of fermentation time and increased as fermentation time progressed while crude protein increased up till 24 hours, reduced by 36 hours and increased by 48 hours.
Highest protein content was obtained by 48 hours while its lowest was recorded by 0 hour. The crude fibre content was recorded highest by 48 hours of fermentation time and lowest by 12 hours of fermentation time. Crude fat content ranged from 13.35g/100g obtained by 48 hours of fermented time to 3.11g/100g obtained by 24 hours of fermentation time. Ash content ranges from 2.39g/100g obtained by 48 hours of fermentation time to 1.30g/100g recorded by 36 hours of fermentation time.

Presented intable 4.19 is the result of the proximate composition of sample FB2 over 48 hours of fermentation. The result showed moisture, crude protein and ash contents increased by 12 hours, decreased by 36 hours of fermentation and increased by 48 hours while crude fat decreased till 36 hours and increased as fermentation time increased.
Moisture content ranged between 8.81g/100g obtained by 12 hours of fermentation time and 5.73g/100g obtained by 36 hours of fermentation time. Crude protein content was recorded highest by 48 hours of fermentation time and lowest before fermentation started. The crude fat and ash contents were obtained by 48 hours while their lowest were recorded by 24 hours.

Table 4.19 shows result of analysis of the proximate composition of the blend FB3 over a period of 48 hours. The result revealed the crude protein, crude fat, moisture and ash contents decreased within 12 and 36 hours of fermentation time and increased as fermentation time increased while carbohydrate increased up till 24 hours and decreased as fermentation time increased.
Highest moisture content was recorded in the unfermented form and lowest recorded by 36 hours of fermentation time. Protein and crude fat contents were recorded highest by 48

hours of fermentation time and lowest by 24 hours. The ash content ranged from 2.40g/100g obtained by 48 hours of fermentation time to 1.11g/100g obtained by 36 hours of fermentation time.

Carbohydrate content ranges between 77.68g/100g obtained by 24 hours of fermentation time and 59.84g/100g obtained by 48 hours.

Table 4.19 shows the result of the analysis of proximate composition of Sample F4 fermented with the combination of *L. plantarum*(1.5 x 10^8 CFU/mL) and *L. pentosus*(6.0 x 10^8 CFU/mL) (sample FC). The result showed the crude fat, crude protein and ash contents decreased by 12 hours and increasedas fermentation time increases while moisture and carbohydrate contents increased as fermentation time progressed.

Moisture content was in the range of 11.82g/100g obtained by 0 hours to 9.91g/100g recorded by 48 hours of fermentation time. The highest crude protein and crude fat content was obtained by 48 hours of fermentation and their lowest was recorded by 12 hours of fermentation time. The highest ash content was obtained in its unfermented form and its lowest by 12 hours. Carbohydrate content ranged between 74.57g/100g recorded by 12 hours of fermentation time and 67.96g/100g obtained by 48 hours.

Table 4.19: Effect of using varying inoculum sizes on proximate composition of starter-fermented blend

Sample code	FT (Hour)	Proximate Composition (g/100g)					
		Moisture Content	Crude Protein	Crude Fat	Crude Fibre	Ash	Carbohydrate
FA1	0	8.59^{c}	11.82^{a}	3.21^{c}	1.60^{b}	1.63^{c}	72.28^{c}
	12	8.71^{c}	11.79^{a}	3.00^{a}	1.59$_{b}$	1.39^{b}	74.90^{e}
	24	8.39^{c}	12.70^{b}	3.10^{b}	1.26a	1.11^{a}	74.19^{d}
	36	6.36^{b}	14.96^{c}	5.20^{d}	2.22^{c}	1.60^{c}	70.31^{b}
	48	5.08^{a}	19.31^{d}	12.35^{e}	2.81^{d}	2.35^{d}	60.14^{a}
FA2	0	8.59^{c}	11.82^{b}	5.69^{d}	1.60^{d}	1.63^{d}	72.28^{b}
	12	7.02^{b}	11.35^{a}	3.00^{a}	1.02^{a}	1.41^{c}	77.23^{e}
	24	8.77^{d}	12.29^{c}	3.10^{b}	1.25^{b}	1.11^{a}	74.72^{b}
	36	5.23^{a}	12.67^{d}	5.20^{c}	1.41^{c}	1.26^{b}	75.65^{c}
	48	5.57^{a}	19.77^{e}	12.35^{e}	3.40^{e}	2.39^{e}	60.21^{d}
FA3	0	8.59c	11.82^{b}	5.69^{c}	1.59^{c}	1.63^{c}	72.28^{b}
	12	8.96^{d}	11.85^{b}	2.91^{a}	1.45^{b}	1.71^{c}	74.56^{d}
	24	8.95^{d}	11.35^{a}	3.30^{b}	1.02^{a}	1.25^{a}	75.15^{e}
	36	4.80^{a}	14.01^{c}	6.19^{d}	2.20^{d}	1.41^{b}	73.58^{c}
	48	5.46^{b}	20.55^{d}	13.58^{e}	4.79^{e}	2.38^{d}	58.03^{a}
FB1	0	8.59^{e}	11.82^{a}	5.69^{d}	1.59^{b}	1.63^{d}	72.28^{b}
	12	6.22^{c}	11.85^{a}	3.60^{b}	1.01^{a}	1.59^{c}	76.74^{e}
	24	7.91^{d}	12.67^{c}	3.11^{a}	2.02^{c}	1.37^{b}	74.87^{c}
	36	5.78^{b}	12.33^{b}	4.40^{c}	2.21^{d}	1.3^{a}	76.19^{d}
	48	5.11^{a}	20.17^{d}	13.35^{e}	3.80^{e}	2.39^{e}	58.97^{e}
FB2	0	8.59^{d}	11.82^{a}	5.69^{c}	1.59^{b}	1.63^{c}	72.28^{c}
	12	8.81^{e}	13.98^{c}	3.89^{b}	1.61^{b}	1.89^{d}	71.43^{b}
	24	6.89^{c}	11.75^{a}	3.30^{a}	2.24^{c}	1.21^{a}	76.85^{d}
	36	5.73^{a}	12.33^{b}	3.33^{a}	1.25^{a}	1.35^{b}	77.26^{e}
	48	6.18^{b}	17.07^{d}	9.66^{d}	2.81^{d}	2.40^{e}	64.66^{a}
FB3	0	8.59^{e}	11.82^{b}	5.69^{c}	1.60^{b}	1.63^{d}	72.28^{b}
	12	8.01^{d}	11.75^{b}	3.49^{b}	1.27^{a}	1.31^{c}	75.44^{d}
	24	6.37^{c}	11.41^{a}	3.29^{a}	1.96^{d}	1.25^{b}	77.68^{e}
	36	5.10^{a}	13.06^{c}	5.89^{d}	1.80^{c}	1.11^{a}	74.84^{c}
	48	6.18^{b}	17.98^{d}	13.60^{e}	2.22^{e}	2.40^{e}	59.84^{a}
FC	0	11.82^{d}	11.82^{c}	5.69^{d}	1.60^{e}	1.63^{e}	72.28^{c}
	12	10.56^{c}	11.41^{a}	2.36^{a}	0.98^{b}	1.11^{a}	74.57^{d}
	24	10.51^{b}	11.79^{b}	3.01^{b}	1.33^{c}	1.20^{b}	73.48^{cd}
	36	9.94^{a}	14.48^{c}	4.90^{c}	1.41^{d}	1.29^{c}	69.40^{b}
	48	9.91^{a}	14.84^{d}	6.70^{e}	0.89^{a}	1.58^{d}	67.96^{a}

Values are presented as mean recorded from triplicate readings.
At 95% confidence level, means with similar / different superscripts along the same column are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).
Key: as in Table 4.18, Page 101.

Table4.20 showsfermentation effect using varying inoculum loads of lactic acid bacteria isolates individually and in combination on the proximate content of Sample F4. Crude protein, crude fibre, crude fat as well as ash contents were obtained highest in Sample F4 fermented with the LAB isolates singly while the moisture content was obtained highest in Sample F4 fermented with a combination of these isolates.

Highest moisture content was obtained in sample FC and lowest was obtained in sample FB1. The highest protein content was recorded in sample FA1 and the lowest was recorded in sample FC. The highest crude fibre content was obtained in sample FA3 and lowest was obtained in sample FC. Crude fat content ranges between 6.42g/100g obtained in sample FA1 and 4.53g/100g obtained in sample FC. Ash content ranges from 1.70g/100g obtained in the blend fermented with *L. plantarum*(3.0×10^8 CFU/mL) (sample FB2) to 1.36g/100g obtained in the blend fermented with a combination of *L. plantarum*(1.5×10^8 CFU/mL) and *L. pentosus*(6.0×10^8 CFU/mL) fermented blend (sample FC). Carbohydrate content was highest in sample FB2 and lowest in sample FA1. The use of varying inoculum sizes significantlyaffected ($P \leq 0.05$)the proximate composition of sample F4.

Table4.20: Effect of varying inoculum sizes on proximate composition of starter-fermented blend

Sample Code	Proximate composition (g/100g)					
	Moisture	Crude Protein	Crude Fibre	Crude Fat	Ash	Carbohydrate
FA1	7.42±0.39^{f}	14.12±0.76^{g}	1.90±0.15^{d}	6.42±0.92^{f}	1.61±0.11^{c}	70.37±1.43^{a}
FA2	6.97±0.41^{c}	13.58±0.84^{d}	1.73±0.23^{b}	5.87±0.91^{c}	1.56±0.12^{b}	72.02±1.64^{d}
FA3	7.35±0.49^{e}	13.92±0.92^{f}	2.21±0.36^{f}	6.33±1.03^{e}	1.69±0.11^{e}	70.72±1.72^{b}
FB1	6.72±0.35^{a}	13.77±0.86^{e}	2.13±0.25^{e}	6.03±1.01^{d}	1.66±0.10^{d}	71.81±1.76^{d}
FB2	7.24±0.34^{d}	13.39±0.54^{c}	1.90±0.15^{d}	5.17±0.64^{b}	1.70±0.11^{e}	72.50±1.22^{e}
FB3	6.85±0.34^{b}	13.20±0.67^{b}	1.77±0.09^{c}	6.39±1.01^{d}	1.54±0.12^{b}	72.02±1.69^{d}
FC	10.55±0.19^{g}	12.87±0.40^{a}	1.24±0.07^{a}	4.53±0.44^{a}	1.36±0.06^{a}	71.54±0.69^{c}

Values are presented as mean ± Standard Deviation recorded from triplicate readings.

At 95% confidence level, means ± standard deviation with similar / different superscripts along the same column are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).

Key: as in Table 4.18, Page 101.

4.8.4 Phytochemical content of starter-fermented blend

Table 4.21 shows the effect of fermentation time on sample F4 fermented with *L. pentosus*(1.5 x 10^8 CFU/mL) (sample FA1). The result reveals the phytic acid and saponin contents increased by 12 hours and decreased with increase in fermentation time; polyphenol content decreased up till 36 hours of fermentation time and increased slightly by 48 hours of fermentation time while tannin and oxalate contents decreased within the first 36 hours and increased thereafter except tannin which decreased at 48 hours of fermentation time.

Phytic acid content ranges from 2.01 mg/g obtained by 24 hours of fermentation to 1.51 mg/g obtained by 36 hours of fermentation. Tannin content ranges between 15.7 mg/g obtained by 36 hours of fermentation and 9.28 mg/g obtained by 12 hours of fermentation time. Polyphenol content ranges from 1.89 mg/g obtained by 48 hours of fermentation time to 0.31 mg/g obtained by 36 hours of fermentation time. Oxalate content ranges between 1.01 mg/g obtained by 48 hours of fermentation and 0.55 mg/g obtained by 12 hours of fermentation. Saponnin content ranges from 0.04 mg/g obtained by 12 hours of fermentation time and 0.01 mg/g obtained by 36 hours of fermentation time.

Table 4.21 shows result of analysis of phytochemical content of sample F4 fermented with 3.0 x 10^8 CFU/mL *L. pentosus*(sample FA2).From the figure, the phytic acid, polyphenol and saponin contents decreased within the first 12 and 24 hours and increased as fermentation time increased while by 12 hours and 24 hours flavonoid content increased and decreased respectively

The highest and lowest phytic acid content was obtained by 48 hours and by 12 hours of fermentation time respectively. Polyphenol content ranges from 2.08 mg/g obtained by 48 hours of fermentation time and 0.33 mg/g obtained by 24 hours of fermentation time. Saponnin content ranges from 0.11 mg/g obtained by 48 hours of fermentation time and 0.004 mg/g obtained by 12 hours of fermentation time. Flavonoid content ranged between 0.067 mg/g obtained by 24 hours and 0.18 mg/g obtained by 48 hours.

Phytochemical contents of blend fermented with 2.0 mL of *L. pentosus* (sample FA3) as shown in table 4.21 reveals the phytic acid and alkaloid contents

increased by 12 hours of fermentation time, decreased by 24 hours and increased as fermentation time increased; tannin and flavonoid contents reduced within the first 24 hours and 36 hours and increased as fermentation time increased; oxalate content increased as fermentation time progressed while saponin content reduced and increased by 12 hours as well as 24 hours respectively and decreased as fermentation time increased.

Phytic acid content was recorded highest by 12 hours of fermentation time and lowest by 36 hours. Tannin content was noted to be highest by 48 hours of fermentation time and lowest recorded by 24 hours. Oxalate content ranged between 1.09 mg/g obtained by 48 hours of fermentation time and 0.72 mg/g obtained in its unfermented form. The alkaloid content ranges from 0.56 mg/g obtained by 24 hours of fermentation time to 0.002 mg/g obtained by 0 hour of fermentation time. The saponnin content ranges from 0.023 mg/g obtained at 24 hours of fermentation time and 0.009 mg/g obtained by 36 hours of fermentation time. Flavonoid content ranges from 0.24 mg/g obtained by 48 hours of fermentation time and 0.07 mg/g obtained by 12 hours.

The result of analysis of phytochemical composition of formulated blend fermented with 1.5 x 10^8 CFU/mL*L. plantarum* (sample FB1) shown in table 4.21 reveals the phytic acid, oxalate and polyphenol contents increased by 12 hours of fermentation time, decreased up till 36 hours and increased by 48 hours; tannin content decreased up till 24 hours, increased by 36 hours and reduced by 48 hours while alkaloid content increased as fermentation time progressed.

Phytic content ranges from 2.99 mg/g obtained by 12 hours of fermentation time and 1.51 mg/g obtained by 36 hours of fermentation time. Tannin content ranges from 10.0 mg/g obtained by 36 hours of fermentation time to 5.90 mg/g obtained by 48 hours. Highest polyphenol and oxalate content was obtained by 48 hours while their lowest was obtained by 36 hours. Alkaloid content ranges from 0.04 mg/g obtained by 36 hours of and 0.002 mg/g obtained by 0 hours of fermentation time.

The result of the analysis of phytochemical content of blend fermented with 3.0 x 10^8 CFU/mL *L. plantarum*(sample FB2) shown in table 4.21 revealed alkaloid, polyphenol,

oxalate and tannin contents increased by 12 hours, reduced by 24 hours and increased as fermentation time progressed while phytic acid content decreased for the first 36 hours and increased by 48 hours of fermentation time.

Phytic acid content ranges from 3.16 mg/g obtained by 12 hours and 1.49 mg/g obtained by 36 hours. Tannin content ranges from 12.17 mg/g obtained by 48 hours and 0.04 mg/g obtained by 24 hours. Polyphenol content ranges between 1.64 mg/g obtained by 48 hours and 0.49 mg/g obtained by 24 hours. Oxalate content ranges from 0.81 mg/g obtained by 48 hours and 0.64mg/g obtained by 36 hours. Alkaloid content ranges from 0.63 mg/g obtained by 12 hours and 0.002 mg/g obtained by 0 hours of fermentation time.

The result of the analysis of phytochemical content of the blend fermented with 6.0 x 10^8 CFU/mL *L. plantarum*(sample FB3) as shown in table 4.21 shows the polyphenol contents decreased by 36 hours of fermentation time and increased as fermentation time increased; oxalate, tannin and flavonoid contents decreased by 12 hours and increased as fermentation time progressed while alkaloid content increased as fermentation time increased.

The highest and lowest tannin content was recorded by 48 hours and 12 hours of fermentation time respectively. Polyphenol content ranges from 1.49 mg/g to 1.37 mg/g obtained by 48 hours and 24 hours of fermentation time respectively. Oxalate content was recorded to be highest by 48 hours and lowest obtained by 12 hours of fermentation time. Alkaloid content was highest by 12 hours and lowest in its unfermented form. Flavonoid content ranges from 0.19 mg/g and 0.06 mg/g obtained by 48 hours and 12 hours respectively.

The result of the analysis of phytochemical content of blend fermented with combined *L. plantarum* (1.5 x 10^8 CFU/mL) and *L. pentosus* (6.0 x 10^8 CFU/mL) (sample FC) is shown in table 4.21. It was observed that the polyphenol and phytic acid content decreased within 24 hours, increased till 36 hours and decreased by 48 hours while alkaloid and saponin contents increased till 24 hours, decreased by 36 hours and increased by 48 hours. The

Oxalate and saponin contents were highest by 48 hours while tannin and polyphenol contents were highest before fermentation commenced. The lowest oxalate and tannin

contents were observed by 24 hours while the alkaloid, saponin and flavonoid contents were observed to be highest before commencement of fermentation. Alkaloid content was highest by 24 hour and phytic acid content was highest by 0 hours of fermentation time and lowest by 48 hours of fermentation time.

Table 4.21: Effect of using varying inoculum sizes on phytochemical content of starter-fermented blend

Sample code	FT (Hour)	Phytochemical Composition (mg/g)						
		Oxalate	Tannin	Alkaloid	Polyphenol	Saponnin	Flavonoid	Phytic acid
FA1								
	0	0.72^{c}	9.91^{b}	0.002^{a}	0.97^{d}	0.02^{a}	0.08^{a}	1.81^{b}
	12	0.53^{a}	9.91^{a}	0.14^{e}	0.66^{c}	0.04^{a}	0.14^{a}	3.49^{d}
	24	0.63^{b}	9.91^{c}	0.02^{b}	0.62^{b}	0.03^{a}	0.10^{a}	2.01^{c}
	36	0.73^{c}	16.27^{e}	0.67^{c}	0.31^{a}	0.01^{a}	0.14^{a}	1.51^{a}
	48	1.01^{d}	12.47^{d}	0.10^{d}	1.89^{e}	0.01^{a}	0.14^{a}	1.57^{a}
FA2	0	0.72^{b}	9.91^{c}	0.002^{a}	0.97^{d}	0.016^{c}	0.08^{b}	1.81^{b}
	12	0.44^{a}	11.35^{c}	0.180^{e}	0.69^{c}	0.004^{a}	0.14^{c}	1.31^{a}
	24	1.27^{e}	9.64^{b}	0.019^{b}	0.33^{a}	0.008^{b}	0.06^{a}	1.83^{b}
	36	0.82^{c}	8.34^{a}	0.393^{d}	0.49^{b}	0.009^{b}	0.14^{d}	1.83^{b}
	48	1.01d	15.30e	0.025c	2.08^{e}	0.107d	0.15^{e}	1.93^{b}
FA3	0	0.72^{a}	9.91^{c}	0.002^{a}	0.97^{b}	0.02^{d}	0.08^{b}	1.81^{b}
	12	0.74^{a}	8.27^{b}	0.32^{ab}	0.86^{a}	0.01^{c}	0.08^{a}	3.49^{d}
	24	0.82^{b}	8.04^{a}	0.56^{b}	0.98^{b}	0.023^{e}	0.09^{c}	1.35^{a}
	36	0.99^{c}	13.07^{d}	0.05^{a}	0.86^{a}	0.001^{a}	0.14^{d}	1.33^{a}
	48	1.09^{d}	13.39^{e}	0.19^{a}	1.66^{c}	0.009^{b}	0.24^{e}	2.16^{c}
FB1	0	0.72^{b}	9.91^{d}	0.002^{a}	0.97^{c}	0.02^{b}	0.08^{b}	1.81^{b}
	12	0.82^{c}	9.64^{c}	0.03^{c}	1.35^{d}	0.01^{a}	0.08^{a}	2.99^{d}
	24	0.74^{b}	8.72^{b}	0.03^{c}	0.63^{b}	0.06^{c}	0.11^{d}	1.95^{c}
	36	0.57^{a}	10.00^{d}	0.04^{b}	0.55^{a}	0.01^{a}	0.09^{c}	1.51^{a}
	48	1.18^{d}	5.90^{a}	0.01^{d}	2.09^{e}	0.01^{a}	0.16^{e}	1.88^{bc}
FB2	0	0.72^{b}	9.91^{c}	0.002^{a}	0.97^{c}	0.02^{a}	0.08^{a}	1.81^{b}
	12	0.73^{b}	10.69^{d}	0.63^{e}	1.18^{d}	0.004^{a}	0.05^{a}	3.16^{c}
	24	0.72^{b}	8.04^{a}	0.144^{b}	0.49^{a}	0.74^{c}	0.06^{a}	1.84^{b}
	36	0.64^{a}	8.45^{b}	0.148^{c}	0.72^{b}	0.01^{a}	0.19^{b}	1.49^{a}
	48	0.81^{c}	12.17^{e}	0.24^{d}	1.64^{e}	0.01^{b}	0.09^{a}	1.83^{b}
FB3	0	0.72^{c}	9.91^{c}	0.002^{a}	0.97^{b}	0.02^{c}	0.08^{b}	1.81^{c}
	12	0.55^{a}	7.98^{a}	0.19^{a}	1.05^{c}	0.12^{b}	0.56^{a}	2.17^{e}
	24	0.65^{b}	8.12^{b}	0.06^{a}	0.37^{a}	0.13^{e}	0.14^{c}	1.68^{b}
	36	1.00^{d}	10.42^{d}	0.11^{a}	0.97^{b}	0.01^{a}	0.16^{d}	1.51^{a}
	48	1.18^{e}	11.62^{e}	0.18^{a}	1.50^{d}	0.03^{d}	0.19^{e}	2.12^{d}
FC	0	0.72^{a}	9.91^{e}	0.002^{a}	0.97^{e}	0.02^{a}	0.08^{a}	1.81^{a}
	12	0.64^{a}	4.24^{b}	0.017^{a}	0.40^{a}	0.04^{c}	0.83^{e}	0.83^{a}
	24	1.09^{a}	2.92^{a}	0.079^{a}	0.45^{b}	0.05^{d}	0.79^{b}	0.83^{a}
	36	0.76^{a}	4.90^{d}	0.018^{a}	0.48^{d}	0.03^{b}	0.82^{d}	2.48^{a}
	48	0.82^{a}	4.283^{c}	0.021^{a}	0.47^{c}	0.06^{e}	0.81^{e}	0.62^{a}

Values are presented as mean ± recorded from triplicate readings.

At 95% confidence level, means ± standard deviation with similar / different superscripts along the same column are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).

Key: as in Table 4.18, Page 101.

Table 4.22 shows effect of fermentation and use of varying inoculum loads of starters individually and in combination on phytochemical composition of sample F4. The phytic acid, tannin, alkaloid, polyphenol contents were observed to be lower in blend fermented with the starters in combination and highest in the samples fermented with the starters singly. The lowest oxalate, saponnin and flavonoid contents were observed in the blends fermented with the starters singly.

The highest phytic acid content recorded with *L. pentosus* (1.5 x 10^8 CFU/mL) fermented blend (sample FA1) was not significantly different ($P \leq 0.05$) from other starter fermented blends except the combined *L. plantarum* (1.5 x 10^8 CFU/mL) and *L. pentosus* (6.0 x 10^8 CFU/mL) fermented sample F4 (sample FC) which had the lowest (1.31 mg/g). Oxalate content was noted highest in the blend fermented using 6.0 x 10^8 CFU/mL *L. pentosus* (sample FA3) and not significantly different ($P \leq 0.05$) from blends fermented using *L. pentosus* (3.0 x 10^8 CFU/mL) (sample FA2), *L. plantarum* (1.5 x 10^8 CFU/mL) (sample FB1), *L. plantarum* (6.0 x 10^8 CFU/mL) (sample FB3) and sample FC. The lowest oxalate content (0.72 mg/g) obtained in the blend fermented with 1.5 x 10^8 CFU/mL *L. plantarum* (sample FB2) was not significantly different from that obtained in the blend fermented with 1.5 x 10^8 CFU/mL *L. pentosus* (sample FA1). Tannin content was significantly different ($P \leq 0.05$) from each other with highest recorded in sample FA1 and lowest in sample FC. Alkaloid content was highest in sample FA3 but not significantly ($P \leq 0.05$) from that obtained in sample FB2 and lowest obtained in sample FC. The polyphenol content was significantly different ($P \leq 0.05$) from each other with its highest content obtained in 2.0 mL *L. plantarum* fermented blend (sample FB3) and lowest recorded in sample FC. The saponnin content was highest in sample FB2 and lowest in sample FA1 which is not significantly different ($P \leq 0.05$) from its value obtained in sample FB1. Flavonoid content was highest in sample FC and lowest in sample FB2. The flavonoid contents in samples FA1, FA2, FA3 and FB1 were not significantly different ($P \leq 0.05$) from each other.

Samples FA3 and FB1 were observed to have the best proximate and phytochemical composition based on results of analysis obtained in this study hence, were subjected to other physico-chemical analysis.

Table4.22: Effect of varying inoculum sizes on the phytochemical composition of starter-fermented blend

Sample Code	Phytochemical content (mg/g)						
	Phytic acid	Oxalate	Tannin	Alkaloid	Polyphenol	Saponnin	Flavonoid
FA1	2.08±0.20^{b}	0.73±0.04^{a}	11.68±0.68^{g}	0.07±0.01^{ab}	0.89±0.15^{b}	0.02±0.01^{ab}	0.12±0.02^{b}
FA2	1.74±0.06^{b}	0.85±0.08^{b}	10.91±0.64^{f}	0.05±0.02^{ab}	0.91±0.17^{c}	0.03±0.01^{bc}	0.11±0.01^{ab}
FA3	2.03±0.21^{b}	0.87±0.04^{b}	10.54±0.61^{e}	0.24±0.07^{c}	1.07±0.08^{f}	0.01±0.00^{a}	0.13±0.02^{b}
FB1	2.03±0.14^{b}	0.81±0.05^{b}	8.83±0.41^{b}	0.02±0.00^{a}	1.12±0.15^{g}	0.02±0.02^{ab}	0.10±0.01^{ab}
FB2	2.03±0.16^{b}	0.72±0.01^{a}	9.85±0.40^{d}	0.23±0.06^{c}	1.00±0.11^{e}	0.17±0.08^{d}	0.09±0.01^{a}
FB3	1.86±0.07^{b}	0.82±0.06^{b}	9.61±0.37^{c}	0.11±0.03^{b}	1.97±0.10^{d}	0.04±0.01^{c}	0.13±0.01^{b}
FC	1.31±0.37^{a}	0.81±0.07^{b}	5.25±0.65^{a}	0.03±0.01^{a}	0.55±0.06^{a}	0.04±0.00^{c}	0.67±0.08^{c}

At 95% confidence level, means ± standard deviation with similar / different superscripts along the same column are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).
Key: as in Table 4.18, Page 101.

4.8.5 Viscosity, bulk density and metabolisable energy contents of starter-fermented blend

As shown in table 4.23, viscosity and bulk density decrease and metabolisable energy content increase was observed to be higher in starter fermented blends [6.0 x 10^8 CFU/mL *L. pentosus* fermented blend (sample FA3) and 1.5 x 10^8 CFU/mL *L. plantarum* fermented blend (sample FB1)] compared to unfermented sample F4. Viscosity value was recorded highest in unfermented sample F4 while the lowest was obtained in sample FB1. Highest and lowest bulk density value was obtained in unfermented sample F4 and sample FB1 respectively. The highest bulk density was recorded for the control while the lowest was recorded for sample FB1. The highest metabolisable energy content was recorded for sample FB1 and the lowest was recorded for unfermented sample F4. The bulk density and metabolisable energy content of both starter fermented sample F4 were not significantly ($P \leq 0.05$) different among each other but significantly different ($P \leq 0.05$) from the unfermented sample F4.

Table4.23:Physico-chemical properties of the starter-fermentedblends

Physico-chemical Property	F4	FB1	FA3
Viscosity(cP)	3.50^{c}	1.15^{a}	2.31^{b}
Bulk density(g/ml)	6.48^{b}	5.32^{a}	5.98^{a}
Metabolisable energy(Kcal/100g)	387.61^{a}	436.71^{b}	436.54^{b}

At 95% confidence level, means with similar / different superscripts along the same row are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).
Key: F4= 70% Millet, 22% Cowpea, 5% Groundnut, 3% *Moringa oleifera,* FB1= 1.5 x 10^{8} CFU/mL *L. plantarum* fermented F4, FA3= F4. 6.0 x 10^{8} CFU/mL *L. pentosus* fermented F4

4.8.6 Mineral content of starter-fermented blend

The mineral content of starter fermented sample F4 is shown in table 4.24. Unfermented sample F4 had significantly lower ($P \leq 0.05$) mineral content relative to starter fermented samples (FA3 and FB1). Magnesium, calcium, manganese, sodium, potassium, copper and zinc were highest in 1.5 x 10^8 CFU/mL *L. plantarum* fermented blend (sample FB1) while iron and phosphorus were recorded highest in blend fermented with 6.0 x 10^8 CFU/mL *L. pentosus* (sample FA3) and unfermented sample F4 (control).

4.8.7 Vitamin content of starter-fermented blends

The result of analysis of the vitamin content of the starter-fermented blends is shown in table 4.25. Higher vitamin contents were observed for the starter fermented samples except vitamin C content which was higher in the control compared to 1.5 x 10^8 CFU/mL *L. plantarum* fermented blend (sample FB1). Highest contents of Vitamin B3, Vitamin B6, Vitamin A, Vitamin B1, Vitamin B2, Vitamin D, Vitamin E, Vitamin B9 and Vitamin K were recorded in 6.0 x 10^8 CFU/mL fermented *Lpentosus* blend (sample FA3) while Vitamin B5 was recorded highest in 1.5 x 10^8 CFU/mL *L. plantarum* fermented blend (sample FB1). There were significant differences ($P \leq 0.05$) in vitamin contents among samples.

4.8.8 Amino acid content of starter-fermented blends

The result of analysis of the amino acid content of the starter-fermented sample F4 is presented in table 4.26. The result shows the non-essential amino acid content (glycine, alanine, serine, proline, aspartate, arginine, tyrosine, cystine, glutamate) and essential amino acid content valine, threonine, methionine, phenylalanine, histidine, tryptophan) were higher in 1.5 x 10^8 CFU/mL *L. plantarum* fermented sample F4 (sample FB1) and 6.0 x 10^8 CFU/mL *L. pentosus* fermented sample F4 (sample FA3) compared to unfermented sample F4 (control) while isoleucine, leucine and lysine contents were higher in unfermented sample F4 relative to both starter-fermented sample F4 (control).

MINERAL (mg/L)	F4	FB1	FA3
Calcium	0.54±0.34[a]	1.14±0.12[c]	1.05±0.37[b]
Magnesium	11.48±0.21[a]	13.18±0.21[c]	12.86±0.24[b]
Potassium	68.5±0.34[a]	131.75±0.16[c]	110.25±0.16[b]
Sodium	7.96±0.01[a]	21.15±0.01[c]	19.4±0.01[b]
Manganese	0.28±2.58[a]	0.42±6.93[a]	0.39±6.30[a]
Iron	1.00±0.01[a]	1.17±0.01[b]	1.28±0.01[c]
Copper	0.11±0.01[a]	0.18±0.01[b]	0.17±0.01[b]
Zinc	0.85±0.01[a]	0.89±0.01[b]	0.83±0.01[a]
Phosphorus	11.335±0.02[c]	9.13±0.01[a]	10.42±0.03[b]

Table4.24:Mineral contents of starter-fermented blend

At 95% confidence level, means ± standard deviation with similar / different superscripts along the same row are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).
Key: F4= 70% Millet, 22% Cowpea, 5% Groundnut, 3% *Moringa oleifera,* FB1= 1.5 x 10^8 CFU/mL *L. plantarum* fermented F4, FA3= F4. 6.0 x 10^8 CFU/mL *L. pentosus* fermented F4

Table4.25:Vitamin composition of starter-fermeted blends (mg/100g)

Vitamins	F4	FB1	FA3
Vitamin B3	2.27±0.01[a]	2.50±0.01[b]	2.69±0.01[c]
Vitamin B6	0.30±0.01[a]	0.34±0.01[a]	0.39±0.01[b]
Vitamin C	4.40±0.01[a]	4.38±0.01[a]	4.76±0.01[b]
Vitamin A (µg/)	0.23±0.01[a]	0.24±0.01[a]	0.26±0.01[a]
Vitamin B1	0.58±0.01[a]	0.70±0.01[b]	0.82±0.01[c]
Vitamin B2	0.30±0.01[a]	0.42±0.01[b]	0.48±0.01[c]
Vitamin D (µg/)	2.7E-05±0.01[a]	4.4E-05±0.01[a]	4.7E-05±0.01[a]
Vitamin E	1.58±0.01[a]	1.79±0.01[b]	1.87±0.01[c]
Vitamin B9	6.4E-06±0.01[a]	9E-06±0.01[a]	1.1E-05±0.01[a]
Vitamin K (µg/)	0.001±0.01[a]	0.001±0.01[a]	0.002±0.01[a]
Vitamin B5	0.30±0.01[a]	0.38±0.01[b]	0.31±0.01[a]

At 95% confidence level, means ± standard deviation with similar / different superscripts along the same row are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).

Key: F4= 70% Millet, 22% Cowpea, 5% Groundnut, 3% *Moringa oleifera,* FB1= 1.5 x 10^8 CFU/mL *L. plantarum* fermented F4, FA3= 6.0 x 10^8 CFU/mL *L. pentosus* fermented F4.

Non-essential amino acid (cysteine) content was higher in unfermented sample F4 relative to 6.0 x 10^8 CFU/mL*L. pentosus* fermented blend F4 (sample FA3) only.

The highest non-essential amino acids [aspartate, arginine, tyrosine, cystine, glutamate], highest essential amino acids [phenylalanine and histidine] and lowest essential amino acid content [isoleucinewere recorded in sample FB1. Non-essential amino acids [glycine, alanine, serine, proline] and essential amino acids [valine, threonine, methionine, phenylalanine, histidine and tryptophan] where recorded lowest in the control compared to sample FB1 and sample FA3. Essential amino acid [isoleucine, lysine and leucine] were recorded highest in the control compared to sample FB1 and sample FA3. Lowest non-essential amino acid [cysteine] and highest essential amino acid [lysineand leucine] was recorded in sample FA3. Non-essential amino acid [glycine), alanine, serine, proline and essential amino acid [valine, threonine, methionine) and tryptophan were recorded highest in sample FA3. Essential amino acid (threonine, valine, isoleucine, lysine, leucine, phenylalanine, histidine, methionine and tryptophan) contents were significantly different ($P \leq 0.05$) among the samples while of all the non-essential amino acids, only aspartate content was not significantly different ($P \leq 0.05$).

Sample FB1 (1.5 x 10^8 CFU/mL*L. plantarum* fermented sample F4)had the best bulk density, metabolisable energy content, mineral content, vitamin composition and amino acid composition hence was evaluated for its sensory qualities and chosen to be fed to the Wistar rats in the animal experiment.

4.8.9 Sensory quality of starter-fermented formulated blend

Result of analysis of organoleptic properties of starter-fermented blend (F4) as assessed by the judges is presented in table 4.27. The result revealed higher mean value rating of texture, aroma, appearance, taste and overall acceptance for the 1.5 x 10^8 CFU/mL*L. plantarum* fermented blend (sample FB1) while low value rating of texture, aroma, appearance, taste and overall acceptance was recorded for the control. Mean values of organoleptic properties except the appearance among all samples was significantly different ($P \leq 0.05$).

Table4.26:Amino acid content of starter-fermented blends (mg/100g)

Amino Acid	F4	FB1	FA3
Glycine	2.85±0.01[a]	2.89±0.01[b]	3.05±0.01[c]
Alanine	2.52±0.01[a]	2.56±0.01[b]	2.86±0.01[c]
Serine	2.79±0.01[a]	2.85±0.01[b]	2.92±0.01[c]
Proline	1.54±0.01[a]	1.58±0.01[a]	1.72±0.01[b]
Aspartate	7.12±0.01[a]	7.18±0.01[b]	7.15±0.01[ab]
Arginine	4.63±0.01[a]	5.53±0.01[c]	5.41±0.01[b]
Tyrosine	3.83±0.01[a]	3.98±0.01[c]	3.88±0.01[b]
Cystine	1.29±0.01[b]	1.30±0.01[b]	1.24±0.01[a]
Glutamate	13.09±0.01[a]	13.21±0.01[c]	13.14±0.01[b]
Valine	3.79±0.01[a]	3.86±0.01[b]	4.17±0.01[c]
Threonine	1.41±0.01[a]	1.44±0.01[a]	2.19±0.01[b]
Isoleucine	4.03±0.01[c]	3.65±0.01[a]	3.71±0.01[b]
Leucine	5.97±0.01[c]	5.22±0.01[b]	4.88±0.01[a]
Lysine	3.85±0.01[b]	3.83±0.01[b]	3.78±0.01[a]
Methionine	1.39±0.01[a]	1.40±0.01[a]	1.52±0.01[b]
Phenylalanine	5.39±0.01[a]	5.52±0.01[c]	5.44±0.01[b]
Histidine	0.98±0.01[a]	1.19±0.01[b]	1.17±0.01[b]
Tryptophan	0.85±0.01[a]	0.91±0.01[b]	1.47±0.01[c]

At 95% confidence level, means ± standard deviation with similar / different superscripts along the same row are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).
Key: F4= 70% Millet, 22% Cowpea, 5% Groundnut, 3% *Moringa oleifera,* FB1= 1.5 x 10^8 CFU/mL *L. plantarum* fermented F4, FA3= 6.0 x 10^8 CFU/mL *L. pentosus* fermented F4.

Table4.27: Sensory evaluation of starter-fermented blend

PARAMETERS	F4	FB1
Texture	3.50 ± 0.5^{a}	6.50 ± 0.5^{b}
Aroma	5.50 ± 0.5^{a}	7.00 ± 0.0^{b}
Taste	3.00 ± 0.5^{a}	7.00 ± 0.0^{b}
Appearance	5.50 ± 0.5^{a}	6.00 ± 0.5^{a}
Overall Acceptability	4.50 ± 0.5^{a}	7.50 ± 0.5^{b}

At 95% confidence level, means ± standard deviation based on forty jugdes, with similar / different superscripts along the same row are not significantly different / significantly different from one another. Duncans Multiple Range Test (DMRT).
Key: F4= 70% Millet, 22% Cowpea, 5% Groundnut, 3% *Moringa oleifera,* FB1= 1.5 x 10^{8} CFU/mL *L. plantarum* fermented F4.

4.9 Biological evaluation for Nutritional study

4.9.1 Heamatological analysis

Table 4.28 reveals the animals' hematology in the group fed with the conventional feed (group CF). The Heamoglobin (HB), Red Blood Cell (RBC), platelet, Mean Cell Hemoglobin (MCH), monocyte, eosinophil counts were recorded highest within week 3 and week 4 while white blood cell, lymphocyte counts were recorded lowest within week 1 and week 2. Packed Cell Volume (PCV), HB, RBC, White Blood Cell (WBC), platelet counts ranged from 34.25% - 50.50%, 11.63 $g100mm^{-1}$ – 16.28 $g100mm^{-1}$, 5.74×10^6 mm^{-1} – 8.13×10^6 mm^{-1}, 3550×10^3 mm^{-1} – 6837×10^3 mm^{-1}, 69,000 – 188,500 respectively.Mean Cell Hemoglobin Concentration (MCHC), MCH, Mean Cell Volume (MCV), lymphocyte, neutrophil, monocyte and eosinophil counts ranged between 31.86% and 34.03%, 19.26 Pg and 20.33 Pg, 59.28 μm^{-3} and 63.83 μm^{-3}, 64.25% and 73.00%, 23.50% and 33.25%, 1.50% and 2.75%, 0.75% and 2.00% respectively. These observations fall within the range of reference value reported for healthy Wistar rats and were significantly different ($P \leq 0.05$) at different time of treatment except MCH as well as lymphocyte counts.

Complete blood count analysis in animals feed with fermented blend (group FB) as shown in table 4.29 reveals the highest and lowest PCV, RBC, HB and platelet counts were recorded at week 1 and week 2 respectively. WBC, eosinophil, neutrophil and lymphocyte counts were recorded highest at week 4. PCV, HB, RBC, platelet, MCHC, MCH, MCV, lymphocyte, neutrophil, monocyte and eosinophil counts ranged between 30.75% - 49.75%, 10.03 $g100mm^{-1}$ – 16.18 $g100mm^{-1}$, 4.95×10^6 mm^{-1} – 8.04×10^6 mm^{-1}, 2100×10^3 mm^{-1} – 6787.50×10^3 mm^{-1}, 99750 – 16900, 32.45% - 33.24%, 19.34Pg – 20.85Pg, 58.43 μm^{-3} – 62.96 μm^{-3}, 60.75% - 72.25%, 25.25% - 35%, 0.50% - 2.00%, 0.00% - 2.50% respectively and falls within the range of reference value reported for healthy Wistar rats (University of Pennsylvania school of Veternary Medicine, 2012). The effect of treatment with fermented food over weeks 2, 3 and 4 on hematological variables was significantly different ($P \leq 0.05$) in PCV, HB, platelet, RBC, neutrophil as well aslymphocyte counts only.

Table 4.30shows the analysis of hematological parameters in animals fed with starter-fermented blend and 1 x 10^6 CFU/mL probiotic (group FBP) showing a decrease in PCV, HB, RBC, WBC and lymphocyte counts with increase in treatment time while MCHC, MCH, MCV, and monocyte counts increased within the first three weeks of treatment. PCV, HB, RBC, WBC, platelet, MCHC, MCH, MCV, lymphocyte, neutrophil, monocyte and eosinophil counts ranged between 49.00% and 31.75%, 16.25 g100mm^{-1} and 10.63 g100mm^{-1}, 7.97 × 10^6 mm^{-1} and 5.19 × 10^6 mm^{-1}, 8262.5 × 10^3 mm^{-1} and 3987.5 × 10^3 mm^{-1}, 182,000 and 72,750, 33.94% and 33.14% 21.06 Pg and 19.29 Pg, 62.07 µm^{-3} and 28.42 µm^{-3}, 72.00% and 61.75%, 34.50% and 24.00%, 2.50% and 1.50%, 2.50% and 1.75% respectively. These observations were within the standard blood count of healthy Wistar rats and significantly different ($P \leq 0.05$) over the treatment time exceptfor WBC, MCHC, monocyte and eosinophil counts.

In the group of animals fed with the commercially sold complementary food (group Control), complete blood count analysis revealed their PCV and WBC decreased with increase in treatment time; HB, RBC, MCHC, MCH, MCV and lymphocyte counts were recorded lowest at week 3 of treatment time (Table 4.31). PCV, HB, RBC, WBC, platelet, MCHC, MCH, lymphocyte, neutrophil, monocyte and eosinophil counts ranged from 45.00% - 36.33%, 15.10 g100mm^{-1} – 10.63 g100mm^{-1}, 7.54 × 10^6 mm^{-1}– 5.31 × 10^6 mm^{-1}, 8333.33 × 10^3 mm^{-1} – 3133.33 × 10^3 mm^{-1}, 216, 750 – 54,666.67, 33.55% -25.09%, 20.86 Pg - 15.01 Pg, 62.79 µm^{-3} – 44.77 µm^{-3}, 71.75% - 49%, 30.67% – 24.50%, 1.75% - 1.33%, 2.33% - 1.50% respectively. All hematological variables were within range of reference value reported for healthy Wistar rats. PCV, HB, RBC, WBC and platelet count only were significantly different ($P \leq 0.05$) at different treatment times.

Table4.28: Hematological variables in Wistar rats fed with conventional feed (CF) for nutritional study

Parameters	Sampling Time (Week)			
	1	2	3	4
Packed cell volume (%)	50.50±6.20^{b}	42.75±3.15^{ab}	49.25±3.75^{b}	34.25±3.45^{a}
Hemoglobin Conc. (g 100 mm^{-1})	16.05±1.89^{b}	13.90±1.08^{ab}	16.28±1.16^{b}	11.63±1.09^{a}
Red blood cell (×10^{6}mm^{-1})	7.86±0.82^{b}	7.22±0.56^{ab}	8.13±0.61^{b}	5.74±0.57^{a}
White blood cell (×10^{3}mm^{-1})	3550±139.94^{a}	6837.5±1014.35^{b}	5162.5±679.58^{ab}	4612.5±368.20^{a}
Platelet	156000±359.96^{b}	69000±6096.45^{a}	71500±21285.75^{a}	188500±22765.10^{b}
MCHC (%)	31.86±0.26^{a}	32.489±0.15^{ab}	33.08±0.22^{bc}	34.03±0.52^{c}
MCH (Pg)	20.33±0.35^{a}	19.26±0.29^{a}	20.06±0.43^{a}	20.30±0.35^{a}
MCV (µm^{-3})	63.83±1.54^{b}	59.28±0.93^{a}	60.66±1.61^{ab}	59.67±0.22^{a}
Lymphocyte (%)	73.00±3.11^{a}	70.0±1.58^{a}	71.75±3.97^{a}	64.25±2.87^{a}
Neutrophils (%)	23.75±3.12^{a}	27.5±1.56^{ab}	23.5±3.86^{a}	33.25±2.72^{b}
Monocytes(%)	1.50±0.29^{a}	1.75±0.48^{ab}	2.75±0.25^{b}	1.75±0.25^{ab}
Eosinophils(%)	1.75±0.48^{ab}	0.75±0.48^{a}	2.00±0.00^{b}	0.75±0.25^{a}

Values are presented as mean ± standard deviation from four Wistar rats in each group
Values in same rownot having same superscript significantly differ at 95% confidence level(DMRT)
Standard blood count of rats within the weaning age of 6 - 8 weeks. Hemoglobin (10 – 20), Packed Cell Volume (33 – 50), White Blood Cells (5000 – 5500), Red Blood Cells (5 - 10), and Platelets (80 000– 115 000).
Key: MCHC= Mean Corpuscular Hemoglobin. MCH= Mean Corpuscular Hemoglobin. MCV= Mean Corpuscular Volume.

Table4.29: Hematological variables in Wistar rats fed with fermented blend (FB) for nutritional study

Parameters	Sampling Time (Week) 1	2	3	4
Packed cell volume (%)	49.75±3.07^{b}	30.75±5.76^{a}	35.25±1.89^{a}	33.75±5.81^{a}
Hemoglobin Conc. (g 100 mm^{-1})	16.18±0.88^{b}	10.03±2.21^{a}	11.70±0.53^{a}	11.13±1.85^{a}
Red blood cell (×10^{6}mm^{-1})	8.04±0.39^{b}	4.95±1.27^{a}	5.66±0.43^{a}	5.79±1.00^{a}
White blood cell (×10^{3}mm^{-1})	6300±1559.38^{a}	6787.5±699.88^{a}	5862.5±1073.81^{a}	2100±10520.14^{a}
Platelet	169000±12369.32^{c}	99750±32024.97^{a}	129500±6614.38^{ab}	143000±9949.87^{bc}
MCHC (%)	32.56±0.29^{a}	32.45±1.52^{a}	33.24±0.30^{a}	33.09±0.30^{a}
MCH (Pg)	20.11±0.14^{a}	20.39±1.10^{a}	20.85±0.69^{a}	19.34±0.28^{a}
MCV (µm^{-3})	61.78±0.92^{a}	62.96±4.38^{a}	62.68±1.53^{a}	58.43±0.35^{a}
Lymphocyte (%)	72.25±2.21^{b}	60.75±4.17^{a}	62.50±2.63^{a}	62.50±3.97^{a}
Neutrophils (%)	25.25±2.59^{a}	35.00±4.24^{b}	31.75±2.097^{ab}	33.50±3.12^{ab}
Monocytes(%)	0.50±0.29^{a}	1.75±0.47^{a}	1.75±0.48^{a}	2.00±0.41^{a}
Eosinophils(%)	1.00±0.71^{a}	2.50±0.48^{a}	0.00±0.65^{a}	1.50±0.50^{a}

Values are presented as mean ± standard deviation from four Wistar rats in each group
Values in same row not having same superscript significantly differ at 95% confidence level (DMRT).
Standard blood count of rats within the weaning age of 6-8 weeks. Hemoglobin (10 – 20), Packed Cell Volume (33 – 50), White Blood Cells (5000 – 5500), Red Blood Cells (5 - 10), and Platelets (80 000– 115 000).
Key: MCHC= Mean Corpuscular Hemoglobin. MCH= Mean Corpuscular Hemoglobin. MCV= Mean Corpuscular Volume.

Table4.30: Hematological variables in Wistar rats fed with fermented blend plus probiotics (1×10^6 CFU/mL) (FBP) fornutritional study

	Sampling Time (Week)			
Parameters	**1**	**2**	**3**	**4**
Packed cell volume (%)	49.00±2.04^{c}	38.25±1.25^{b}	35±0.82^{ab}	31.75±2.98^{a}
Hemoglobin Conc. (g 100 mm^{-1})	16.25±0.78^{c}	12.95±0.42^{b}	11.88±0.24^{ab}	10.63±1.09^{a}
Red blood cell ($\times10^6mm^{-1}$)	7.97±0.17^{c}	6.71±0.18^{b}	5.65±0.19^{a}	5.19±0.54^{a}
White blood cell ($\times10^3mm^{-1}$)	8262.5±2557.94^{a}	7450±563.84^{a}	5800±961.34^{a}	3987.5±51.54^{a}
Platelet	182000±20054.09^{b}	72750±10842.62^{a}	158250±12963.89^{b}	167500±8568.35^{b}
MCHC (%)	33.141±0.33^{a}	33.86±0.17^{a}	33.94±0.45^{a}	33.37±0.53^{a}
MCH (Pg)	20.36±0.69^{ab}	19.29±0.15^{b}	21.06±0.29^{b}	20.46±0.29^{ab}
MCV (μm^{-3})	61.41±1.55^{b}	28.42±0.35^{a}	62.07±1.14^{b}	61.37±1.24^{b}
Lymphocyte (%)	72.00±1.08^{b}	64.25±2.09^{a}	62±2.68^{a}	61.75±2.66^{a}
Neutrophils (%)	24.00±1.47^{a}	31.75±2.29^{b}	34±2.12^{b}	34.5±3.57^{b}
Monocytes(%)	1.50±0.29^{a}	2.25±0.48^{a}	2.5±0.29^{a}	1.50±0.50^{a}
Eosinophils(%)	2.50±0.50^{a}	1.75±0.63^{a}	1.75±0.75^{a}	2.25±0.75^{a}

Values are presented as mean ± standard deviation from four Wistar rats in each group
Values in same row not having same superscript significantly differ at 95% confidence level (DMRT).
Standard blood count of rats within the weaning age of 6-8 weeks. Hemoglobin (10 – 20), Packed cell volume (33 – 50), white blood cells (5000 – 5500), Red blood cells (5 - 10), and platelets (80 000– 115 000).
Key: MCHC= Mean Corpuscular Hemoglobin. MCH= Mean Corpuscular Hemoglobin. MCV= Mean Corpuscular Volume.

Table4.31: Hematological variables in Wistar rats fed with commercially sold complementary food (Control) for nutritional study

Parameters	Sampling Time (Week)			
	1	2	3	4
Packed cell volume (%)	45.0±2.27^{b}	43±4.93^{b}	42.33±2.33^{ab}	36.33±1.86^{a}
Hemoglobin Conc. (g 100 mm^{-1})	15.1±0.80^{b}	14.17±1.72^{b}	10.63±0.72^{ab}	12.07±0.57^{a}
Red blood cell (×10^{6}mm^{-1})	7.54±0.45^{b}	7.15±0.95^{b}	5.31±0.35^{ab}	5.79±0.35^{a}
White blood cell (×10^{3}mm^{-1})	4587.5±643.68^{a}	8333.33±961.48^{b}	5700±785.81^{a}	3133.33±133.33^{a}
Platelet	216750±9809.65^{b}	54666.67±13383.24^{a}	133250±20840.67^{ab}	145000±5686.24^{ab}
MCHC (%)	33.55±0.22^{a}	32.89±0.27^{a}	25.09±0.14^{a}	33.22±0.16^{a}
MCH (Pg)	20.06±0.27^{a}	19.89±0.25^{a}	15.01±0.37^{a}	20.86±0.27^{a}
MCV (µm^{-3})	59.80±0.67^{a}	60.49±1.25^{a}	44.77±1.25^{a}	62.79±0.58^{a}
Lymphocyte (%)	71.75±2.09^{a}	69.67±3.84^{a}	49±4.07^{a}	65.67±1.86^{a}
Neutrophils (%)	24.50±2.22^{a}	28.0±3.00^{a}	24.5±5.97^{a}	30.67±2.19^{a}
Monocytes(%)	1.75±0.25^{a}	1.33±0.33^{a}	1.50±0.50^{a}	1.67±0.33^{a}
Eosinophils(%)	2.00±0.71^{a}	2.33±0.67^{a}	1.50±00.87^{a}	2.00±0.00^{a}

Values are presented as mean ± standard deviation from four Wistar rats in each group
Values in same row not having same superscript significantly differ at 95% confidence level (DMRT).
Standard blood count of rats within the weaning age of 6-8 weeks. Hemoglobin (10 – 20), Packed cell volume (33 – 50), white blood cells (5000 – 5500), Red blood cells (5 - 10), and platelets (80 000– 115 000).
Key: MCHC= Mean Corpuscular Hemoglobin. MCH= Mean Corpuscular Hemoglobin. MCV= Mean Corpuscular Volume.

At week 1 animals fed with starter-fermented blend (group FB) and animals fed with starter fermented blend and 1 x 10^6 CFU/mL probiotic (group FBP) had the highest HB, WBC, MCH and eosinophil counts of 16.25 g100mm^{-1}, 8,262.5 × 10^3mm^{-1}, 20.36 Pg and 2.50% respectively. Animals in group FB only had the highest RBC, neutrophil counts. The lowest PCV, HB, RBC, MCH, MCV and lymphocyte counts were recorded in the animals of group Control. Hematological variables in all groups were not significantly different ($P \leq 0.05$) (except MCHC) and were within the standard blood count of healthy Wistar rats as shown in table 4.32.

As shown in table 4.33, the lowest hematological counts of PCV, HB, WBC, MCHC lymphocyte and the highest counts of platelet, MCH, MCV, neutrophiland eosinophil at week 2 was recorded in animals fed with fermented blend (group FB). These observations were significantly different ($P \leq 0.05$) in RBC, HB, PCV, platelet, MCV, and lymphocyte count and all fall within the range of standard blood count for healthy Wistar rats.

From table 4.34, animals fed with fermented blend and probiotic (group FBP) at week 3 had the highest platelet, MCHC, MCH and neutrophil counts; the animals fed with the commercially sold complementary food (group Control) had the lowest HB, RBC, MCHC, MCH, MCV, lymphocyte and monocyte counts. In all groups, hematological variables are within the range of standard blood count reported for healthy Wistar rats and were significantly different ($P \leq 0.05$) for PCV, HB, RBC as well as platelet counts.

By the fourth week, as presented in table 4.35, the highest hematological counts of PCV, HB, MCH, MCV and lymphocytewere recorded in animals fed with the commercially sold

Table4.32:Effect of feeding on hematological variables in Wistar rats after one week

	Formulated blends			
Parameters	**CF**	**FB**	**FBP**	**Control**
Packed cell volume (%)	50.50±6.20[a]	49.75±3.07[a]	49.00±2.04[a]	45.00±2.27[a]
Hemoglobin Conc. (g 100 mm^{-1})	16.05±1.89[a]	16.18±0.88[a]	16.25±0.78[a]	15.10±0.80[a]
Red blood cell ($\times 10^6 mm^{-1}$)	7.86±0.82[a]	8.04±0.39[a]	7.97±0.17[a]	7.54±0.45[a]
White blood cell ($\times 10^3 mm^{-1}$)	3550±139.94[a]	6300±1559.38[a]	8262.50±2557.94[a]	4587.50±643.68[a]
Platelet	156000±359.96[a]	169000±12369.32[a]	182000±20054.09[a]	216750±9809.65[a]
MCHC (%)	31.86±0.26[a]	32.56±0.29[ab]	33.14±0.33[bc]	33.55±0.22[c]
MCH (Pg)	20.33±0.35[a]	20.11±0.14[a]	20.36±0.69[a]	20.06±0.27[a]
MCV (μm^{-3})	63.83±1.54[a]	61.78±0.92[a]	61.41±1.55[a]	59.80±0.67[a]
Lymphocyte (%)	73.00±3.11[a]	72.25±2.21[a]	72.00±1.08[a]	71.75±2.09[a]
Neutrophils (%)	23.75±3.12[a]	25.25±2.59[a]	24.00±1.47[a]	24.50±2.22[a]
Monocytes(%)	1.50±0.29[a]	0.50±0.29[a]	1.50±0.29[a]	1.75±0.25[a]
Eosinophils(%)	1.75±0.48[a]	1.00±0.71[a]	2.50±0.50[a]	2.00±0.71[a]

Values are presented as mean ± standard deviation from four Wistar rats in each group
Values in same row not having same superscript significantly differ at 95% confidence level (DMRT).
Standard blood count of rats within the weaning age of 6-8 weeks. Packed cell volume (33 – 50), Hemoglobin (10 – 20), red blood cells (5 - 10), white blood cells (5000 – 5500) and platelets (80 000– 115 000).
Key: MCHC= Mean Corpuscular Hemoglobin. MCH= Mean Corpuscular Hemoglobin. MCV= Mean Corpuscular Volume. CF= Conventional Feed. FB= Fermented Blend. FBP=Fermented Blend + Probiotics (3.1×10^6 CFU/mL). Control= Commercial weaning Feed.

Table4.33:Effect of feeding on hematological variables in Wistar rats after two weeks

	Formulated blends			
Parameters	**CF**	**FB**	**FBP**	**Control**
Packed cell volume (%)	42.75±3.15^{b}	30.75±5.76^{a}	38.25±1.25^{ab}	43.00±4.93^{b}
Hemoglobin Conc. (g 100 mm^{-1})	13.90±1.08^{b}	10.03±2.21^{a}	12.95±0.42^{ab}	14.17±1.72^{b}
Red blood cell (×10^{6}mm^{-1})	7.22±0.56^{b}	4.95±1.27^{a}	6.71±0.18^{ab}	7.15±0.95^{b}
White blood cell (×10^{3}mm^{-1})	6837.5±1014.35^{a}	6787.5±699.88^{a}	7450±563.84^{a}	8333.33±961.48^{a}
Platelet	69000±6096.45^{ab}	99750±32024.97^{b}	72750±10842.62^{ab}	54666.67±13383.24^{a}
MCHC (%)	32.489±0.148^{a}	32.45±1.52^{a}	33.86±0.17^{a}	32.89±0.27^{a}
MCH (Pg)	19.26±0.29^{a}	20.39±1.10^{a}	19.29±0.15^{a}	19.89±0.25^{a}
MCV (µm^{-3})	59.28±0.93^{ab}	62.96±4.38^{b}	28.42±0.35^{a}	60.49±1.25^{ab}
Lymphocyte (%)	70.00±1.58^{b}	60.75±4.17^{a}	64.25±2.09^{ab}	69.67±3.84^{b}
Neutrophils (%)	27.50±1.56^{a}	35.00±4.24^{a}	31.75±2.29^{a}	28.00±3.0^{a}
Monocytes(%)	1.75±0.48^{a}	1.75±0.47^{a}	2.25±0.48^{a}	1.33±0.33^{a}
Eosinophils(%)	0.75±0.48^{a}	2.50±0.48^{a}	1.75±0.63^{a}	2.33±0.67^{a}

Values are presented as mean ± standard deviation from four Wistar rats in each group

Values in same row not having same superscript significantly differ at 95% confidence level (DMRT).

Standard blood count of rats within the weaning age of 6-8 weeks. Packed cell volume (33 – 50), Hemoglobin (10 – 20), red blood cells (5 - 10), white blood cells (5000 – 5500) and platelets (80 000– 115 000).

Key: MCHC= Mean Corpuscular Hemoglobin. MCH= Mean Corpuscular Hemoglobin. MCV= Mean Corpuscular Volume. CF= Conventional Feed. FB= Fermented Blend. FBP=Fermented Blend + Probiotics (3.1× 10^{6} CFU/mL). Control= Commercial weaning Feed.

Table4.34:Effect of feeding on hematological variables in Wistar rats after three weeks

	Formulated blends			
Parameters	**CF**	**FB**	**FBP**	**Controll**
Packed cell volume (%)	49.25±3.75^{b}	35.25±1.89^{a}	35.00±0.82^{a}	42.33±2.33^{ab}
Hemoglobin Conc. (g100m^{-1})	16.28±1.16^{b}	11.70±0.53^{a}	11.88±0.24^{a}	10.63±0.72^{ab}
Red blood cell (×10^{6}mm^{-1})	8.13±0.61^{b}	5.66±0.43^{a}	5.65±0.19^{a}	5.31±0.35^{ab}
White blood cell (×10^{3}mm^{-1})	5162.50±679.58^{a}	5862.5±1073.81^{a}	5800±961.34^{a}	5700±785.81^{a}
Platelet	71500±21285.75^{a}	129500±6614.38^{b}	158250±12963.89^{b}	133250±20840.67^{b}
MCHC (%)	33.08±0.22^{a}	33.24±0.30^{a}	33.94±0.45^{a}	25.09±0.14^{a}
MCH (Pg)	20.06±0.43^{a}	20.85±0.69^{a}	21.06±0.29^{a}	15.01±0.37^{a}
MCV (µm^{-3})	60.66±1.61^{a}	62.68±1.53^{a}	62.07±1.14^{a}	44.77±1.25^{a}
Lymphocyte (%)	71.75±3.97^{a}	62.50±2.63^{a}	62.00±2.68^{a}	49.00±4.07^{a}
Neutrophils (%)	23.50±3.86^{a}	31.75±2.097^{a}	34.00±2.12^{a}	24.50±5.97^{a}
Monocytes(%)	2.75±0.25^{a}	1.75±0.48^{a}	2.50±0.29^{a}	1.50±0.5^{a}
Eosinophils(%)	2.00±0^{a}	0.00±0.65^{a}	1.75±0.75^{a}	1.50±00.87^{a}

Values are presented as mean ± standard deviation from four Wistar rats in each group
Values in same row not having same superscript significantly differ at 95% confidence level (DMRT).
Standard blood count of rats within the weaning age of 6-8 weeks. Packed cell volume (33 – 50), Hemoglobin (10 – 20), red blood cells (5 - 10), white blood cells (5000 – 5500) and platelets (80 000– 115 000).
Key: MCHC= Mean Corpuscular Hemoglobin. MCH= Mean Corpuscular Hemoglobin. MCV= Mean Corpuscular Volume. CF= Conventional Feed. FB= Fermented Blend. FBP=Fermented Blend + Probiotics (3.1× 10^{6} CFU/mL). Control= Commercial weaning Feed.

complementary food (group CWF). The lowest hematological counts of WBC, platelet, MCHC, MCH, MCV, were recorded in the animals fed with starter-fermented blend (group FB). These values were within the range of reference value reported (University of Pennsylvania school of Veternary Medicine, 2012) for healthy Wistar rats and significantly different ($P \leq 0.05$) for MCH and MCV counts.

Parameters	Formulated blends			
	CF	FB	FBP	Control
Packed cell volume (%)	34.25±3.45[a]	33.75±5.81[a]	31.75±2.98[a]	36.33±1.86[a]
Hemoglobin Conc. ($g100m^{-1}$)	11.63±1.09[a]	11.13±1.85[a]	10.63±1.09[a]	12.07±0.57[a]
Red blood cell ($\times10^{6}mm^{-1}$)	5.74±0.57[a]	5.79±1.00[a]	5.19±0.54[a]	5.79±0.35[a]
White blood cell ($\times10^{3}mm^{-1}$)	4612.5±368.20[a]	2100±10520.14[a]	3987.5±51.54[a]	3133.33±133.33[a]
Platelet	188500±22765.1[a]	143000±9949.87[a]	167500±8568.35[a]	145000±5686.24[a]
MCHC (%)	34.03±0.52[a]	33.09±0.30[a]	33.37±0.53[a]	33.22±0.16[a]
MCH (Pg)	20.30±0.35[ab]	19.34±0.28[a]	20.46±0.29[b]	20.86±0.27[b]
MCV (μm^{-3})	59.67±0.22[ab]	58.43±0.35[a]	61.37±1.24[bc]	62.79±0.58[c]
Lymphocyte (%)	64.25±2.87[a]	62.50±3.97[a]	61.75±2.66[a]	65.67±1.86[a]
Neutrophils (%)	33.25±2.72[a]	33.50±3.12[a]	34.50±3.57[a]	30.67±2.19[a]
Monocytes(%)	1.75±0.25[a]	2.00±0.41[a]	1.50±0.50[a]	1.67±0.33[a]
Eosinophils(%)	0.75±0.25[a]	1.50±0.50[a]	2.25±0.75[a]	2.00±0.00[a]

Table4.35:Effect of feeding on hematological variables in Wistar rats after four weeks

Values are presented as mean ± standard deviation from four Wistar rats in each group
Values in same row not having same superscript significantly differ at 95% confidence level (DMRT).
Standard blood count of rats within the weaning age of 6-8 weeks. Packed cell volume (33 – 50), Hemoglobin (10 – 20), red blood cells (5 - 10), white blood cells (5000 – 5500) and platelets (80 000– 115 000).
Key: As Table 4.34, page 132.

4.9.2 Liver function test

Table 4.36 shows the result of the analysis of liver function test of animals in all groups over the period of study. Animals fed with conventional food (group CF) had the highest Aspartate aminotransferase (AST), Alanine phosphatase (ALP) and Alanine aminotransferase (ALT) concentrations recorded at week 3 and their lowest between week 1 and week 2 with values ranging from 45.00 – 41.25 (AST), 34.00 – 29.50 (ALT) and 115.50 – 81.25 (ALP). Over the periods of sampling, the analysed AST, ALT and ALP concentration valuesdiffered significantly ($P \leq 0.05$) within the group.

For animals fedstarter-fermented food (group FB), analysis of liver function parameters showed AST, ALT and ALP had highest concentrations between week 2 and week 3 while the least AST and ALT was recorded at week 4 and ALP at week 1. Liver enzymes concentrations ranged between 45.75 IU/L and 41.00 IU/L (AST); 33.75 IU/L and 31.00 IU/L (ALT); 119.50 IU/L and 81.50 IU/L (ALP). AST concentration over the weeks under review was not significantly different ($P \leq 0.05$) within the group.

Analysis of liver function revealed the lowest and highest AST, ALT and ALP concentrations were obtained at week 1 and week 4 respectively in animals fed with fermented food plus probiotic (group FBP) with values ranging from 41.00 IU/L – 44.75 IU/L1 (AST), 33.75 IU/L – 29.50 IU/L (ALT), and 119.00 IU/L – 85.00 IU/L (ALP). Statistical analysis showed only AST concentration was not significantly different ($P \leq 0.05$) over the sampling time within the group

Animals fed with the commercially sold complementary food (group Control) after analysis of their liver function showed the lowest and highest AST, ALT and ALP were recorded at week 3 and week 4 respectively. With values ranging between 30.50 IU/L and 46.33 IU/L (AST), 21.50 IU/L and 35.00 IU/L (ALT), 75.25 IU/L and 121.67 IU/L (ALP). Statistical analysis revealed only ALP concentration was significantly different ($P \leq 0.05$) over the sampling periods.

Table4.36: Liver Function analysis in Wistar rats fed with diets for 28 days

Parameters (IU/L)/ Groups	Sampling Time (Week)			
	1	2	3	4
CF				
AST	42.25±0.63[a]	41.25±2.25[a]	45.50±0.29[a]	44.00±2.74[a]
ALT	30.50±0.29[ab]	29.50±1.85[a]	34.00±0.41[b]	33.00±0.41[ab]
ALP	81.25±1.66[a]	105.00±4.71[b]	115.50±5.01[b]	112.50±0.75[b]
FB				
AST	44.50±1.56[a]	44.25±1.68[a]	45.75±1.93[a]	40.00±2.48[a]
ALT	31.75±0.95[a]	32.00±0.96[a]	33.75±1.60[a]	31.00±6.61[a]
ALP	81.50±2.22[a]	119.50±2.23[b]	115.75±3.95[b]	110.00±5.29[b]
FBP				
AST	41.00±1.47[a]	42.50±1.94[a]	42.75±0.63[a]	44.75±1.38[a]
ALT	29.50±1.56[a]	30.25±1.55[ab]	31.50±0.65[ab]	33.75±1.03[b]
ALP	85.50±3.43[a]	112.75±8.03[b]	113.75±3.95[b]	119.00±1.29[b]
Control				
AST	43.25±1.11[a]	42.33±0.67[a]	30.50±2.84[a]	46.33±0.33[a]
ALT	31.25±0.75[a]	30.00±1.16[a]	21.50±2.02[a]	35.00±0.58[a]
ALP	78.00±2.04[a]	114.00±4.73[b]	75.25±6.83[b]	121.67±0.88[b]

Values are presented as mean ± standard deviation obtained from four Wistar rats in individual group
Values in same row not having same superscript significantly differ at 95% confidence level (DMRT).
Key: as in Table 4.34, page 132. ALP= Alkaline Phosphatase,ALT= Alanine Transaminase,AST= Aspartate Transaminase.

The results of the liver function tests of rats treated with different experimental diet in 4 weeks is presented in table 4.37. The result shows AST concentration was highest in fermented food fed animals (group FB) while it was lowest in the animals fed with fermented food plus probiotic (group FBP) in the first week. The concentration of ALT in plasma of animals of group FB was highest and lowestin plasma of the animals fed with fermented food plus probiotic (group FBP). ALP concentration of the animals fed with group FBP was highest and lowest in the animals fed with the commercially sold complementaryfood (group Control). There was no significant difference ($P \leq 0.05$) in the AST, ALT and ALP concentration in the plasma of animals in all the groups within the first week of experiment.

As revealed in the table after two weeks of experimentation, the AST concentration in animals fed with fermented food (group FB) was highest while it was least in the animals of group CF. Alanine aminotransferase (ALT) concentration recorded in the animals fed with fermented food (group FB) was highest and lowest in the animals fed with conventional food (group CF). ALP concentration ranges from 119.50 IU/L recorded in the animals of group FB to 105.00 IU/L recorded in group CF. Statistical analysis revealed there was no significant difference ($P \leq 0.05$) in the AST, ALT and ALP concentration of animals in group CFin week 2.

From this table as well, AST concentration value after three weeks ranged from 45.75 IU/L in group FB and 30.50 IU/L in group Control. Alanine aminotransferase (ALT) in plasma of group CF was highest and lowest in group CWF. The concentration of ALP of animals fed with fermented food (group FB) was highest and lowest in animals fed with the branded complementary blend (group Control). The concentration of ALT among the experimental groups varied significantly ($P \leq 0.05$).

Result from the table also shows the activities of the AST range between 46.33 IU/L recorded in group Controland 40.00 IU/L recorded in group FBat week four. The concentration of ALT was highest in group Control and lowest in group FB. Highest ALP concentration was recorded in group Control and lowest in group FB. Statistical analysis showed no significant increase or reduction ($P \leq 0.05$)

Table4.37:Nutritional study of the liver function analysis in Wistar rats among fed-groups

		Liver Function Analysis (IU/L)		
Experimental Group	**Treatment time (Week)**	**AST**	**ALT**	**ALP**
CF	1	42.25±0.63[a]	30.50±0.29[a]	81.25±1.66[a]
FB		44.50±1.56[a]	31.75±0.95[a]	81.50±2.22[a]
FBP		41.00±1.47[a]	29.50±1.56[a]	85.50±3.43[a]
Control		43.25±1.11[a]	31.25±0.75[a]	78.00±2.04[a]
CF	2	41.25±2.25[a]	29.50±1.85[a]	105.00±4.71[a]
FB		44.25±1.68[a]	32.00±0.96[a]	119.50±2.32[a]
FBP		42.50±1.94[a]	30.25±1.55[a]	112.75±8.03[a]
Control		42.33±0.67[a]	30.00±1.16[a]	114.00±4.73[a]
CF	3	45.50±0.29[a]	34.00±0.41[b]	115.50±5.01[a]
FB		45.75±1.93[a]	33.75±1.60[b]	115.75±6.61[a]
FBP		42.75±0.63[a]	31.50±0.65[ab]	113.75±3.95[a]
Control		30.50±2.84[a]	21.50±2.02[a]	75.25±6.83[a]
CF	4	44.00±2.74[a]	33.00±1.69[a]	112.50±5.075[a]
FB		40.00±2.48[a]	31.00±1.16[a]	110.00±5.29[a]
FBP		44.75±1.38[a]	33.75±1.03[a]	119.00±1.29[a]
Control		46.33±0.33[a]	35.00±0.58[a]	121.67±0.88[a]

Values are presented as mean ± standard deviation from four Wistar rats in individual group
Values in same column not having same superscript significantly differ at 95% confidence level (DMRT)
Key: as in Table 4.34, page 132. ALT= Alanine Transaminase, AST= Aspartate Transaminase, ALP= Alkaline Phosphatase.

in AST, ALT as well as ALP concentrations in the serum of animals of all the groups.

4.9.3 Physiological observations for nutritional study

Table 4.38 shows the average feed intake, mean food conversion ratio (FCR), mean protein efficiency ratio (PER), final weight, initial weight, as well asaverage weight gain of the experimental animal dietary groups for 28 days.

In all groups and at different sampling time except days 14 and 21, highest mean feed intake, mean protein efficiency ratio, weight gain and least mean feed conversion ratio were obtained in fermented blend and probiotic fed animals (group FBP). At all sampling time also, the lowest average weight gain and mean feed intake was obtained in animals fed with the commercially sold complementary food (group Control).

The initial weight of animals ranged between 156.25 g and 110.67 g among the groups. At all sampling time, the least average weight gain was observed in animals fed with the commercially sold complementary blend (group Control) while the highest was recorded in group FBP. The average weight gain ranged from -9.00 g to 41.50 g. Among all the groups at all sampling time, average weight gain was significantly different ($P \leq 0.05$) from each other.

Least mean feed intake was obtained in group Control animals while it's highestwas recorded in groups FB and group FBP at day 28. Feed intake mean value in groups increased as treatment time increased and differ significantly ($P \leq 0.05$) among groups at all sampling periods.

Mean feed conversion ratio was obtained least in group Control on day 21. It was recorded highest in group CF on day 14. The mean value of feed conversion ratio differ significantly ($P \leq 0.05$) among groups at all sampling periods.

The mean protein efficiency ratio (PER) was recorded highest in group FBP while it was recorded lowest in group Control at all sampling time. It recorded highest 2.06 in group FFP and lowest in group Control. The mean values of protein efficiency ratio differ significantly ($P \leq 0.05$) among groups atall sampling periods.

Table4.38: Effect of fermented blend on physiology of Wistar rats

Experimental Diet	Sampling time (Day)	Initial weight (g)	Final weight (g)	Average weight gain (g)	Mean feed intake (g)	Mean FCR	Mean PER
CF	7	156.25^{c}	163.50^{b}	7.25^{a}	70.00^{b}	10.00^{b}	0.63^{a}
FB		153.75^{b}	172.75^{c}	19.00^{b}	77.00^{c}	4.05^{a}	0.94^{b}
FBP		147.75^{a}	170.00^{c}	22.25^{b}	78.40^{c}	3.69^{a}	1.05^{c}
Control		154.25^{b}	159.75^{a}	5.50^{a}	63.00^{a}	11.33^{b}	0.93^{b}
CF	14	140.75^{a}	150.75^{b}	10.00^{b}	154.00^{b}	15.40^{d}	0.90^{bc}
FB		143.50^{b}	160.25^{c}	16.75^{c}	161.00^{c}	9.61^{c}	0.83^{b}
FBP		146.50^{c}	167.50^{d}	21.00^{d}	162.40^{c}	7.73^{b}	1.04^{c}
Control		146.67^{c}	143.67^{a}	-3.00^{a}	126.00^{a}	-42.00^{a}	-0.50^{a}
CF	21	150.25^{d}	144.50^{b}	-5.75^{b}	237.30^{b}	-41.30^{b}	0.52^{b}
FB		143.50^{b}	162.75^{d}	19.25^{c}	247.80^{c}	12.87^{cd}	0.95^{c}
FBP		130.00^{a}	152.75^{c}	22.75^{d}	249.90^{c}	10.99^{c}	1.13^{cd}
Control		146.67^{c}	137.67^{a}	-9.00^{a}	210.00^{a}	-636.40^{a}	-0.06^{a}
CF	28	133.00^{c}	143.25^{b}	10.25^{b}	322.00^{b}	10.00^{c}	0.92^{c}
FB		135.00^{d}	151.5^{c}	16.25^{c}	336.00^{c}	4.05^{b}	0.81^{b}
FBP		119.25^{b}	160.75^{d}	41.50^{d}	336.00^{c}	3.69^{a}	2.06^{d}
Control		110.67^{a}	107.67^{a}	-3.00^{a}	308.00^{a}	11.33^{d}	-0.50^{a}

Values are presented as mean ± standard deviation from four Wistar rats in individual group
Values in same column for each sampling time not having same superscript significantly differ at 95% confidence level (DMRT)
Key: As in Table 4.34, page 132. PER= Protein efficiency ratio, FCR= Feed conversion ratio.

4.10 Biological evaluation of Wistar rats induced with diarrhoea

Animals in groups induced with diarrhoea were observed to pass out loose watery stool and total number of diarrhoeal feaces and water content of stools was observed to decrease in diarrhoea induced animals fed with the fermented food and probiotic (group IFBP), fermented food (group IFB) and Loperamide HCl-Tm (group ICFL) in comparison with animals fed with conventional food and induced with diarrhoea (group ICF/positive control) after 24 hours (Table 4.39). The loss of body weight in animals of group IFBP, group IFB and group ICFL was lower compared to that which was observed in group ICF/positive control.Animals in groups induced with diarrhoea had reduced activity, raised fur and evident weight loss. In addition, it was observed that 24 hours post diarrhoea induction showed no visible symptom of diarrhoea except in 3 animals fed with conventional food and induced with diarrhoea (group ICF/positive control).

4.10.1 Heamatological studies of animals six hours and twenty four hours post diarrhoeal induction

It was observed that six hours post diarrhoea induction, group IFB had the highest value of PCV, HB, MCHC, lymphocyte, monocyte and lowest value of WBC, platelet and neutrophil (21.50%) counts. For animals fed with conventional food and induced with diarrhoea (group ICF/positive control), lowest hematological variables of PCV, HB, RBC, MCHC, lymphocytes and highest WBC, MCH, MCV and neutrophil counts were recorded. Six hours after inducing diarrhoea, hematological variables, in all groups were observed to be within the range of standard blood count for Wistar rats except those of animals fed with conventional food and induced with diarrhoea (group ICF/positive control) and differ significantly ($P \leq 0.05$) in all groups apart from WBC, platelet, monocyte as well as eosinophil counts as shown in table 4.40a -.4.40b.

On thesetables, heamatological analysis 24 after inducing diarrhoea shows group IFB had the lowest PCV, HB, RBC, MCH, MCV, neutrophiland eosinophil counts. The highest platelet, neutrophil and eosinophil counts were obtained in IFBP. These observations differ significantly ($P \leq 0.05$) within all groups apart from platelet, MCV as well as monocyte counts. PCV and HB counts in group UCF/negative control and group ICF/positive control

respectively; platelet count in group IFB and group IFBP were not within the range of reference value of blood count in Wistar rats.

As revealed in table 4.40a - 4.40b, analysis of hematological parameters of animals fed with conventional food (group UCF/negative control) shows lesser value were recorded in the lymphocyte and monocyte counts 24 hour after inducing diarrhoea. All parameters consider except WBC, neutrophil and monocyte counts only differ significantly ($P \leq 0.05$).

In group ICF/positive control animals, analysed heamatological variables 24 after diarrhoea induction increased except MCH, MCV, lymphocyte and eosinophil counts relative to observations at 6 hours after diarrhoea induction. Significant increase ($P \leq 0.05$) was observed in HB, PCV, RBC as well as MCHC counts only as shown in table 4.40a- 4.40b.

Table 4.40a - 4.40b revealed 24 hours after inducing diarrhoea, MCHC, MCH, lymphocyte, monocyte and eosinophil counts were higher in group IFB compared to observations of these heamatological parameters 6 hours after inducing diarrhoea. Significant decrease ($P \leq 0.05$) was recorded in MCHC, MCH as well as lymphocyte counts only; significant increase ($P \leq 0.05$) was recorded in monocyte as well as eosinophil counts only.

Analysis of hematological parameters in animals fed with fermented food, probiotic (group IFPB) showed higher values 24 hours after inducing diarrhoea compared to observations 6 hours after inducing diarrhoea except for MCHC count. Statistical analysis revealed lymphocyte count decreased while WBC, platelet and neutrophil counts increased significantly ($P \leq 0.05$) with time as presented in table 4.40a -4.40b.

Table4.39: Physical features ofdiarrhoea-induced Wistar rats

Experimental Group	Number of animals	Average weight before inducing diarrhoea	Average weight after inducing diarrhoea	Presence of diarrhoea after 6 Hours	Diarrhoeal stool after 6 Hours	Diarrhoeal stool after 24 Hours	Raised Fur	Reduced activity
UCF	5	69.00	66.00	0	0/5	0/5	0	0
IFB	5	82.00	76.00	++	5/5	0/5	++	++
IFBP	5	85.00	78.00	++	5/5	0/5	++	++
ICF	5	72.00	60.00	++	5/5	3/5	+	+
ICFL	5	73.00	69.00	++	4/5	0/5	++	++

Key: UCF: Conventional Feed, IFB: Fermented Feed (Induced With Diarrhoea), IFBP: Fermented Feed + Probiotic Cell Suspension (1×10^6 CFU/mL) (Induced With Diarrhoea), CFD: Conventional Feed (Induced With Diarrhoea), ICFL: Conventional Feed + Loperamide HCl-Tm (Induced With Diarrhoea).++ = Copious, + = Mild, 0 = Absent

In animals fed with conventional food, induced with diarrhoea and treated with Loperamide HCl-Tm (group ICFL), analyzed hematological variables were reduced 6 hours after inducing diarrhoea except for lymphocyte and eosinophil counts relative to values obtained 24 hours after inducing diarrhoea. Statistical analysis revealed lymphocyte count decreased while WBC, platelet and neutrophil counts increased significantly ($P \leq 0.05$) as shown in table 4.40a - 4.40b.

Table4.40a: Hematological features of Wistar rats for anti-diarrhoea study

Diets	PCV		HB		RBC		WBC		Platelets	
	6 hr	24 hr	6 hr	24 hr	6 hr	24 hr	6 hr	24 hr	6 hr	24 hr
UCF	46.50±1.32^{a}	63.00±0.41^{a} *	15.30±0.32^{a}	21.30±0.12^{a} *	7.93±0.28^{a}	9.90±0.02^{a} *	2600±414.33^{a}	3550±142.89^{b}	72750±5793.32^{a}	128500±18337.12^{a} *
IFB	29.00±10.40^{b}	62.50±1.04^{a} *	9.48±3.51^{b}	21.25±0.13^{a} *	4.53±1.92^{b}	9.92±0.02^{a} *	3375±430.84^{a}	4425±208.67^{a}	91000±4358.89^{a}	115500±14121.50^{a}
IFBP	48.00±0.71^{a}	52.25±3.28^{b} *	16.35±0.46^{a}	17.10±0.99^{b} *	7.95±0.29^{a}	8.53±0.37^{b} *	2700±511.53^{a}	4450±400.52^{ab}	60250±18213.43^{a}	163500±22812.64^{a} *
ICF	43.00±0.00^{a}	52.50±2.10^{b}	14.23±0.10^{a}	18.73±0.58^{b}	7.34±0.04^{a}	9.34±0.34^{a}	2912.5±311.16^{a}	3410±1032.48^{b} *	92000±7516.65^{a}	166500±13883.44^{a} *
ICFL	47.50±2.10^{a}	52.75±3.33^{b}	15.73±0.59^{a}	17.78±0.87^{b}	7.96±0.31^{a}	8.62±0.47^{b}	2962.5±407.93^{a}	3537.5±227.65^{b} *	74750±9507.67^{a}	148250±17927.52^{a} *
STANDARD	33 – 50		10 – 20		5 – 10		5,000 - 5,500		80,000 – 115,000	

Values are presented as mean ± standard deviation from four Wistar rats in individual group
Values in same columnnot having same superscript significantly differ at 95% confidence level (DMRT)
Values in same row with asterisk significantly differ at 95% confidence level using T Test
Key: UCF: Conventional Feed, IFB: Fermented Feed (Induced With Diarrhoea), IFBP: Fermented Feed + Probiotic Cell Suspension (1×10^{6} CFU/mL) (Induced With Diarrhoea), CFD: Conventional Feed (Induced With Diarrhoea), ICFL: Conventional Feed + Loperamide HCl-Tm (Induced With Diarrhoea). PCV: Packed cell volume. HB: RBC: Red blood cell. Hemoglobin. WBC: White blood cell.

Table4.40b: Hematological features of Wistar rats for anti-diarrhoea study

Diets	MCHC		MCH		MCV		Lymphocytes		Neutrophils		Monocytes		Eosinophils	
	6 hr	24 hr	6 hr	24 hr	6 hr	24 hr	6 hr	24 hr	6 hr	24 hr	6 hr	24 hr	6 hr	24 hr
UCF	32.93±0.33^{b}	33.81±0.12^{a} *	19.34±0.31^{b}	21.51±0.09^{a} *	58.72±0.54^{b}	63.62±0.37^{a} *	74.25±0.75^{a}	53.00±1.23^{bc} *	24.25±0.48^{b}	43.75±0.09^{b}	1.50±0.29^{a}	1.25±0.25^{a}	0.00±0.00^{a}	2.00±0.41^{ab} *
IFB	32.00±0.65^{b}	34.02±0.38^{a} *	23.86±2.32^{a}	21.42±0.12^{a}	75.07±8.72^{a}	63.00±1.01^{a}	60.50±7.27^{b}	51.75±0.75^{bc}	33.75±5.57^{a}	45.00±1.47^{b}	1.50±0.29^{a}	1.75±0.25^{a}	1.75±0.63^{a}	1.50±0.65^{ab}
IFBP	34.08±1.06^{a}	32.76±0.32^{b} *	20.66±0.95^{b}	0.00±0.32^{b} *	60.58±1.45^{b}	61.08±1.28^{a} *	75.00±0.41^{a}	64.00±2.92^{c} *	21.5±0.87^{b}	33.75±2.75^{b} *	1.75±0.25^{a}	1.50±0.29^{a}	1.75±0.75^{a}	0.75±0.25^{a}
ICF	33.08±0.24^{a}	31.49±0.39^{a}	19.39±0.14^{b}	20.07±0.22^{ab}	58.61±0.28^{b}	63.78±1.44^{a}	71.00±0.58^{a}	50.00±2.12^{ab} *	24.75±1.11^{b}	49.00±2.35^{a} *	1.25±0.25^{a}	1.50±0.20^{a}	1.50±0.50^{a}	2.75±0.25^{b}
ICFL	33.13±0.23^{a}	33.82±1.14^{ab}	19.78±0.28^{ab}	20.67±0.51^{b}	59.69±0.99^{b}	61.17±0.77^{a}	74.00±0.71^{a}	58.25±3.82^{a} *	22.5±0.96^{b}	38.25±1.42^{c} *	1.50±0.65^{a}	1.75±0.48^{a}	1.50±0.65^{a}	1.25±0.48^{b}

Values are presented as mean ± standard deviation from four Wistar rats in individual group
Values in same column not having same superscript significantly differ at 95% confidence level (DMRT)
Values in same row with asterisk significantly differ at 95% confidence level using T Test
Key: UCF: Conventional Feed, IFB: Fermented Feed (Induced With Diarrhoea), IFBP: Fermented Feed + Probiotic Cell Suspension (1×10^{6} CFU/mL) (Induced With Diarrhoea), CFD: Conventional Feed (Induced With Diarrhoea), ICFL: Conventional Feed + Loperamide HCl-Tm (Induced With Diarrhoea). MCH= Mean Corpuscular Hemoglobin. MCHC= Mean Corpuscular H`emoglobin. MCV= Mean Corpuscular Volume

4.10.2 Estimation of protein concentration in the whole colon of rats induced with diarrhoea, treated with experimental diets

Protein level as presented in figure 4.7 revealed an increase six hour post diarrhoea inductionin animals of group ICFL (treated with Loperamide HCl-Tm) compared with uninduced animals fed with conventional food (group UCF/negative control). It was highest (4.03 mg/mL) in animals of group ICFL (fed with conventional food,induced and treated with Loperamide HCl-Tm) and lowest (2.05 mg/mL) in animals fed with conventional food (group UCF/negative control).An increased protein level was recorded 24 hours after induction in animals of group IFB (fed with fermented food) relative to what was recorded of animals in group ICF. Twenty four hours post diarrhoea induction, protein level was recorded highest (5.69 mg/mL) in animals of group IFB (fed with fermented food) and lowest (1.43 mg/mL) in animals of group IFBP (fed with fermented food plus probiotic). Statistical analysis shows protein concentration was significantly higher ($P \leq 0.05$) in animals fed with conventional food and induced with diarrhoea (group ICF/positive control) when compared with animals fed with conventional food (group UCF/negative control) at the six and twenty four hours mark**.**

4.10.3 Estimation of glutathione (GSH) level in the whole colon of rats induced with diarrhoea and treated with experimental Diet

Figure 4.8 shows6 hours post diarrhoea induction estimation of GSH level decreased and increased in group ICF/positive control and IFBP respectively compared to its level in group UCF/negative control. The result also shows GSH level was recorded highest in animals of group IFBP and lowest in group ICF/positive control. Difference observed in GSH level in group ICF/positive control) and IFBP animals was significant ($P \leq 0.05$) six hours after inducing diarrhoea. By 24 hours, GSH level increased ingroups IFB and group IFBP compared to its level in animals fed with conventional food (group UCF/negative control) and animals in other groups. GSH level was recorded highest in group IFBP and lowest in group ICF/positive control. Statistical analysis revealed significant increase ($P \leq 0.05$) in GSH level of animals in group IFBP 24 hours after inducing diarrhoea.

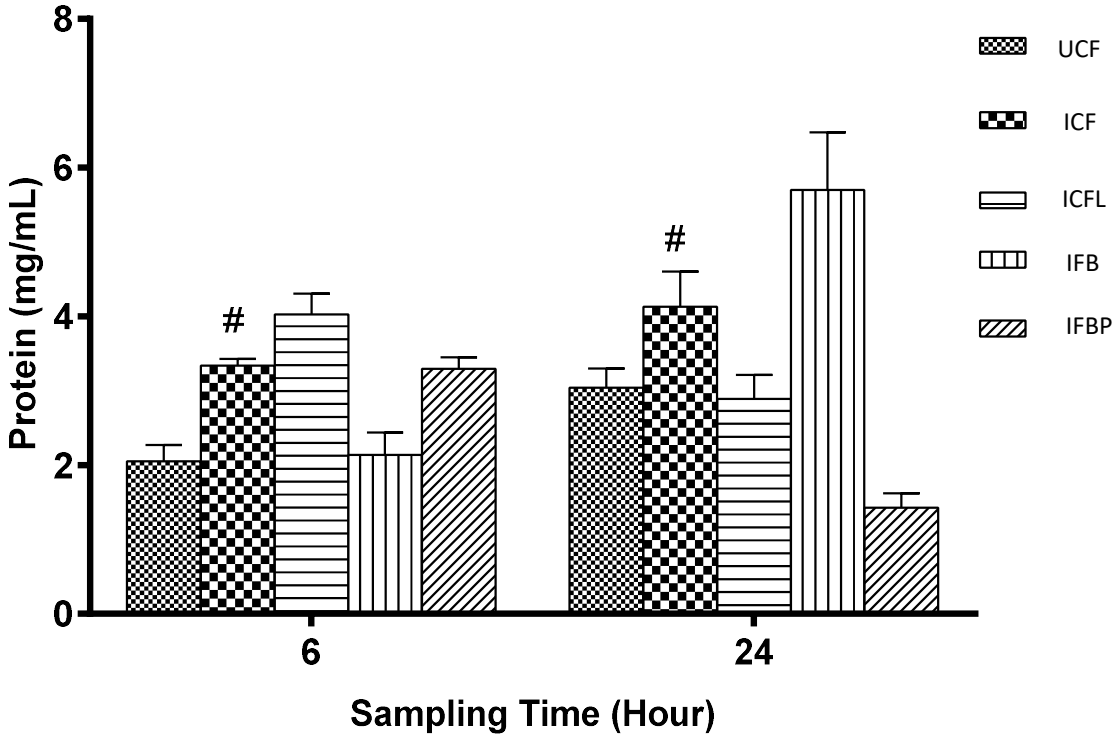


Figure 4.7: Histogram showing effect of experimental diets on protein concentration level in Wistar rats

The level of significance is indicated as # $P \leq 0.05$ compared with the UCF group.

Key: UCF: Conventional Feed, IFB: Fermented Feed (Induced With Diarrhoea), IFBP: Fermented Feed + Probiotic Cell Suspension (1×10^6 CFU/mL) (Induced With Diarrhoea), CFD: Conventional Feed (Induced With Diarrhoea), ICFL: Conventional Feed + Loperamide HCl-Tm (Induced With Diarrhoea).

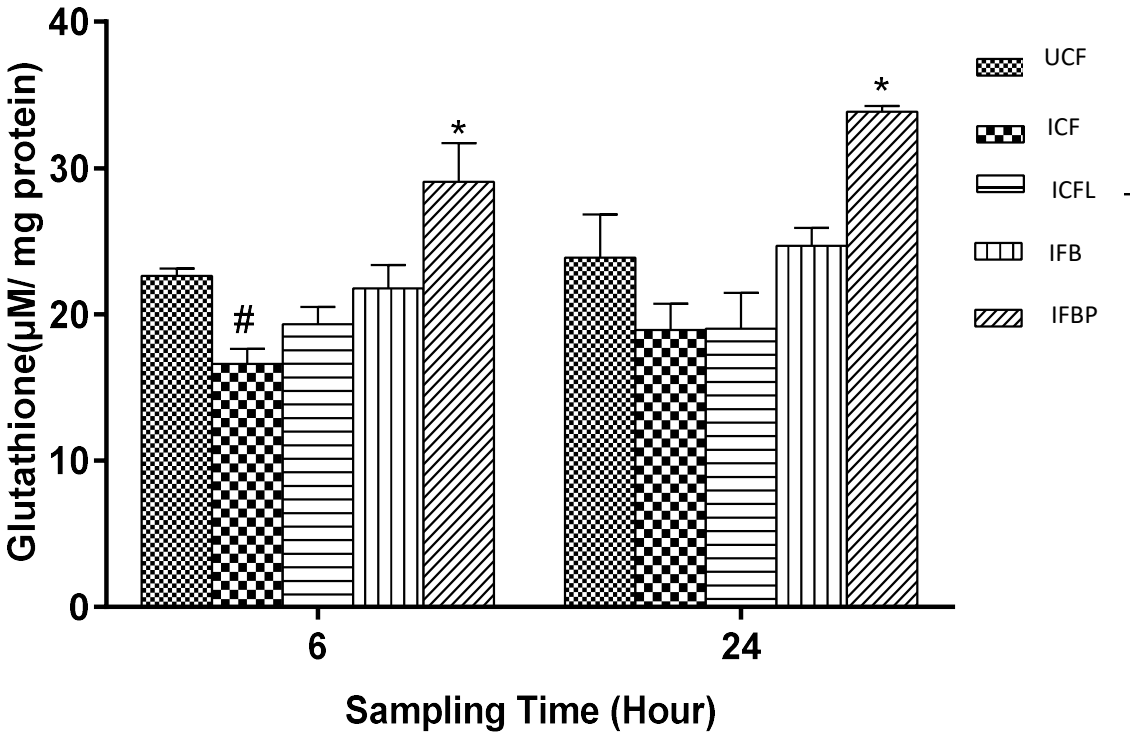


Figure 4.8: Histogram showing effect of experimental diets on Glutathione (GSH) level in Wistar rats

The level of significance is indicated as *& # P ≤ 0.05 compared with theUCF group.

Key: UCF: Conventional Feed, IFB: Fermented Feed (Induced With Diarrhoea), IFBP: Fermented Feed + Probiotic Cell Suspension (1×10^6 CFU/mL) (Induced With Diarrhoea), CFD: Conventional Feed (Induced With Diarrhoea), ICFL: Conventional Feed + Loperamide HCl-Tm (Induced With Diarrhoea).

4.10.4 Estimation of catalase (CAT) level in the whole colon of rats induced with diarrhoea and treated with experimental diet

In figure 4.9, catalase level estimation showed a decrease in animals of all groups except those in group IFBP compared with animals of the negative control/group UCF 6 hours post diarrhoea induction. CAT level ranged between 26.18 µM/mg protein observed in group ICF/positive control and 43.76 µM/mg protein observed in animals of group IFBP six hour after diarrhoea induction. There was a significant decrease ($P \leq 0.05$) in CAT level obtained in group ICF/positive control while a significant increase ($P \leq 0.05$) was recorded in groups IFB and IFBP six hours after inducing diarrhoea. CAT level 24 hours post diarrhoea induction was revealed to significantly increase ($P \leq 0.05$) in animals of groups IFB and IFBP compared with animals of the negative control/group UCF and positive control group UCF. The CAT level was highest in animals of group IFBP and lowestin group UCF/positive control twenty-four hours post diarrhoea induction.

4.10.5 Estimation of nitrite (NO) level in the whole colon of rats induced with diarrhoea and treated with experimental diet

As presented in figure 4.10 an estimation of nitrite level 6 hours after inducing diarrhoea shows an increase in animals of groups IFB and IFBP and a decrease in animals of group ICFL compared with animals of the negative control/group UCF. Nitrite level six hours after inducing diarrhoea was recorded highest in animals of group IFB and lowest in animals of group ICFL. Statistical analysis shows non-significant difference ($P \leq 0.05$) among all the groups. However, a significant increase ($P \leq 0.05$) in NO level in animals of groups IFB and IFBP relative to animals of negative control/group UCF 24 hours after inducing diarrhoa. At this timing, nitrite level ranged from 8.35 µM/mg protein in group FFD to 3.01 µM/mg protein in group UCF/negative control.

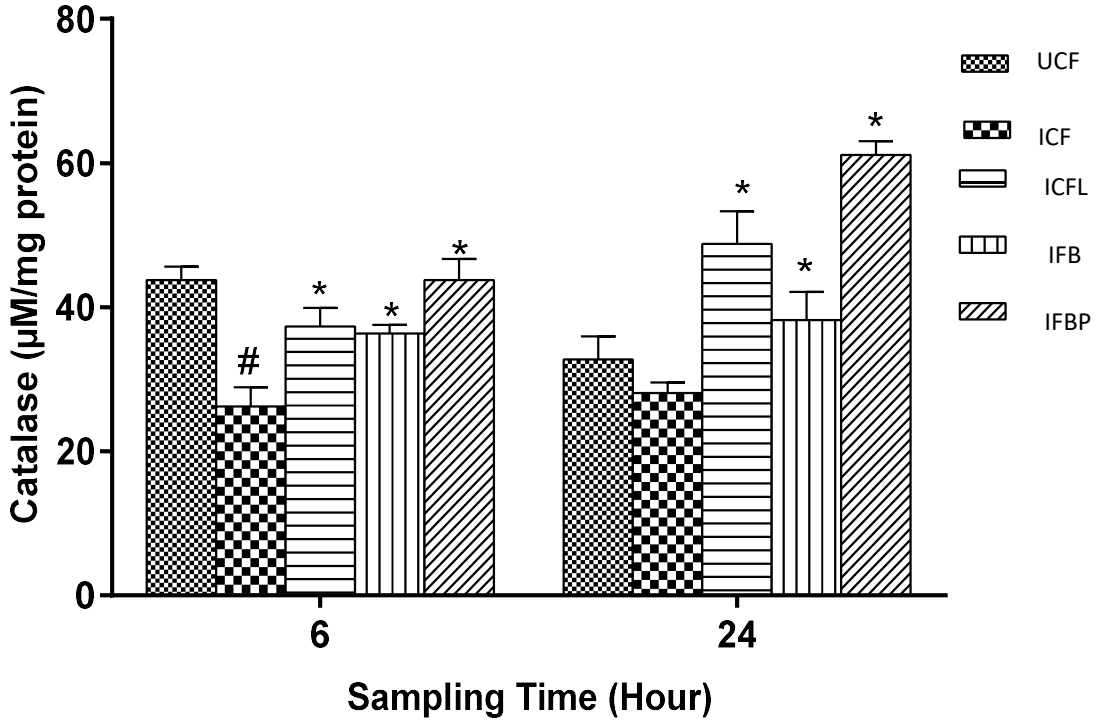


Figure 4.9: Histogram showing effect of experimental diets on Catalse (CAT) level in Wistar rats

The level of significance is indicated as * & # $P \leq 0.05$ compared with the UCF group.

Key: UCF: Conventional Feed, IFB: Fermented Feed (Induced With Diarrhoea), IFBP: Fermented Feed + Probiotic Cell Suspension (1×10^6 CFU/mL) (Induced With Diarrhoea), CFD: Conventional Feed (Induced With Diarrhoea), ICFL: Conventional Feed + Loperamide HCl-Tm (Induced With Diarrhoea).

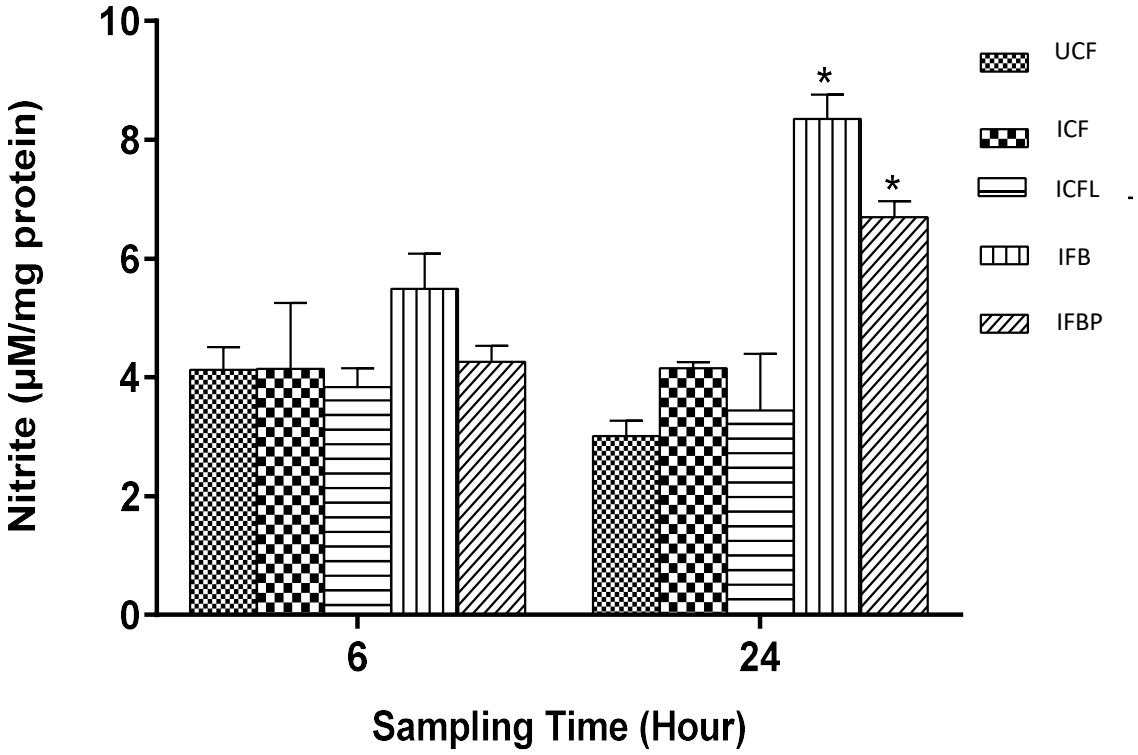


Figure 4.10: Histogram showing effect of experimental diets on Nitrite (NO) level in Wistar rats.

The level of significance is indicated as * P≤0.05 compared with the UCF group.

Key: UCF: Conventional Feed, IFB: Fermented Feed (Induced With Diarrhoea), IFBP: Fermented Feed + Probiotic Cell Suspension (1×10^6 CFU/mL) (Induced With Diarrhoea), CFD: Conventional Feed (Induced With Diarrhoea), ICFL: Conventional Feed + Loperamide HCl-Tm (Induced With Diarrhoea).

4.10.6 Histological studies of colon tissues of male Wistar rats 6 and 24 hours post diarrhoea induction

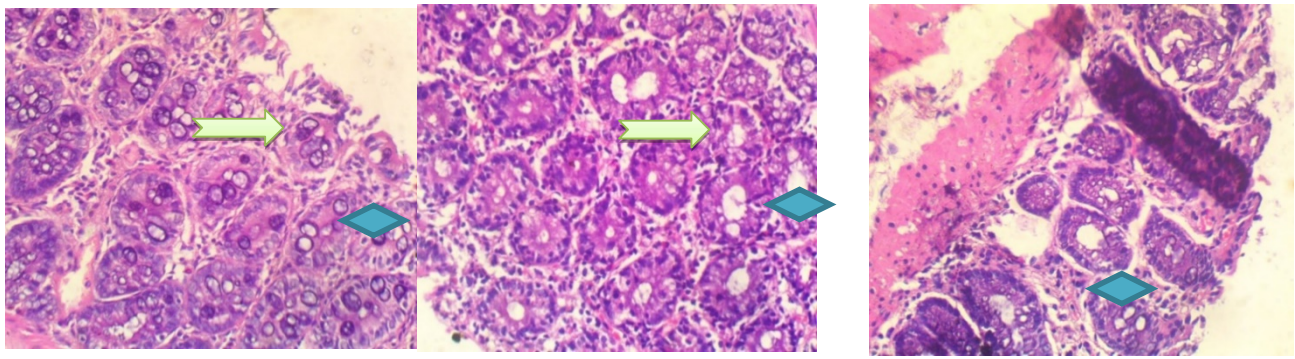
The histology of colon tissue of animals in rats fed conventional feed without diarrhoea(group UCF)after six hours was observed to have villi of normal height covered with fairly intact covering columnar epithelium. Moderate amounts of pericryptal resident inflammatory cells and crypts which appear normal are observed. Goblet cells are almost absent andtheir tunics appear normal. Generally, there is no remarkable vascular change (Plate 4.1a).

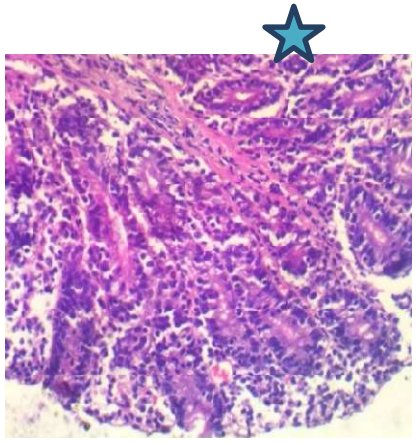
Plate 4.1b shows the histology of colon tissue of rats fed conventional feed six hours after inducing diarrhoea(group ICF). The villi height is observed to be low and reduced. The covering columnar epithelium is discontinuous and disrupted at a few foci.There are scanty amounts of pericryptal resident inflammatory cells and the crypts appear disrupted at a few foci.Moderate goblet cell hyperplasia at intact foci are observed. There is mild expansion of the tunic submucosa. Generally there were no remarkable vascular change.
Histological examination of colon tissue of rats fed conventional feed and treated with Loperamide HCl-Tm(group ICFL) six hours after diarrhoea induction is shown in plate 4.1c. Thevilli height were revealedlow and reduced. The covering columnar epithelium is intact and moderate amounts of pericryptal resident inflammatory cells were observed. The crypts appear elongated and show moderate goblet cell hyperplasia at intact foci. There is moderate congestion of blood vessels and other tunics appear normal.

Plate 4.1d shows the histology of colon tissue of rats fed fermented food six hours after inducing diarrhoea (group IFB). Compared to observations in group ICF, the villi height is normal, enormous and covered with intact covering columnar epithelium. There are moderate amounts of pericryptal resident inflammatory cells. There are foci of degenerate crypts as well as regenerating cryptal glands. There is marked goblet cell hyperplasia as other tunics appear normal. No remarkable vascular change was observed.
Histological examination of colon tissue of rats fed fermented food plus probiotic and induced with diarrhoea (group IFBP)reveals the enormous villi height is normal and

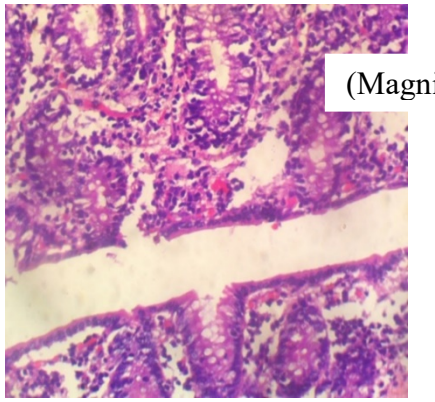
enormous, and covered with intact covering columnar epithelium. There are moderate amounts of pericryptal resident inflammatory cells. There are foci of sloughing off of cells of the cryptal glands. There is marked goblet cell hyperplasia as other tunics appear normal. No remarkable vascular change was observed(Plate 4.1e).

a b c





e



Necrosis of cryptal gland
Globlet cell hyperplasia
Pericryptal inflammatory cells
Degenerated cryptal gland
Disrupted covering epithelium
Pericryptal epithelieum

(Magnification: x 400)

d

Plate 4.1: Histology of colon of Wistar rats of experimental group treated with (a.) conventional feed before inducing diarrhoea. (b.) conventional feed 6 hours after inducing diarrhoea. (c.) fermented feed 6 hours after inducing diarrhoea. (d.) fermented feed plus probiotic 6 hours after inducing diarrhoea. (e.) conventional food plus Loperamide HCl-Tm 6 hours after inducing diarrhoea.

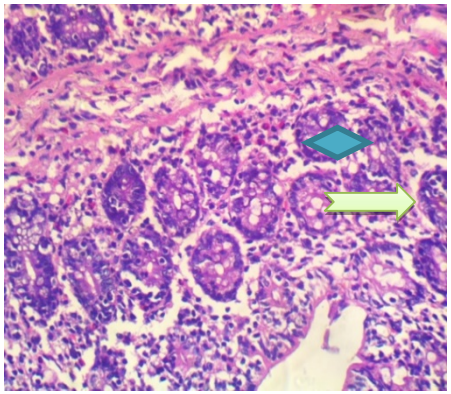
The histology of colon tissue of rats of the UCF group after 24 hours shows the villi height is low and reduced. The covering columnar epithelium is discontinuous and disrupted at a few foci. Scant amounts of pericryptal resident inflammatory cells were observed and the crypts appear normal and show moderate goblet cell hyperplasia. A mild expansion of the tunic submucosa was observed but no remarkable vascular change (Plate 4.2a).

An examination of the histology of the colon tissue of rats of group ICF 24 hours after inducing diarrhoea as shown in plate 4.2b reveals the villi height is low and reduced. The covering columnar epithelium is markedly discontinuous and disrupted. There are moderate amounts of pericryptal resident inflammatory cells. The crypts appear normal and show marked goblet cell hyperplasia. Other tunics appear normal. No remarkable vascular change was observed generally.

In rats of the ICFL group, histological examination shows the villi height is normal and enormous, and covered with intact covering columnar epithelium. There are moderate amounts of pericryptal resident inflammatory cells. The crypts appear normal showing moderate goblet cell hyperplasia. Other tunics appear normal and mild congestion of mucosal blood vessels was observed (Plate 4.2c).

The histological examination of colon tissue of rats of IFB group 24 hour after inducing diarrhoea as presented in plate 4.2d shows the villi height is normal, enormous and covered with intact covering columnar epithelium. There are scant amounts of pericryptal resident inflammatory cells. The crypts appear normal and show marked goblet cell hyperplasia. Other tunics appear normal and no remarkable vascular change was observed.

The histology of colon tissue of rats of group IFBP 24 hours post diarrhoea induction as shown in plate 4.2e reveals the villi height is normal, enormous and covered with intact covering columnar epithelium. A scant amount of pericryptal resident inflammatory cells was observed. The crypts appear normal and show a few foci of Goblet cell (mild goblet cell hyperplasia). Other tunics appear normal and no remarkable vascular change was observed.



a

b

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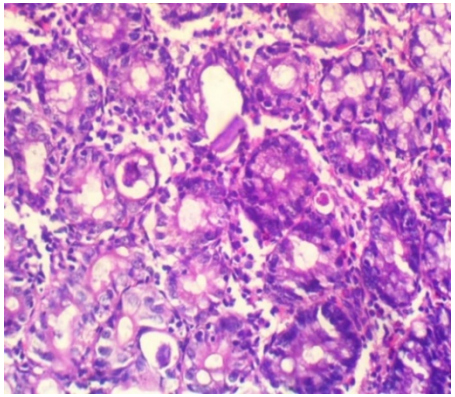
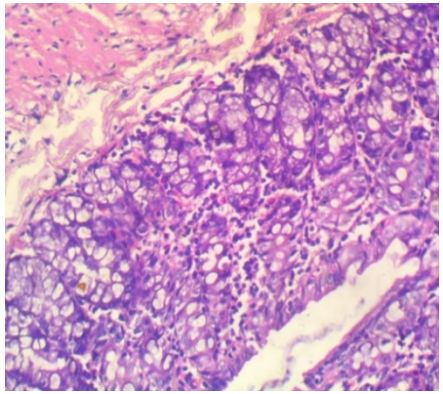




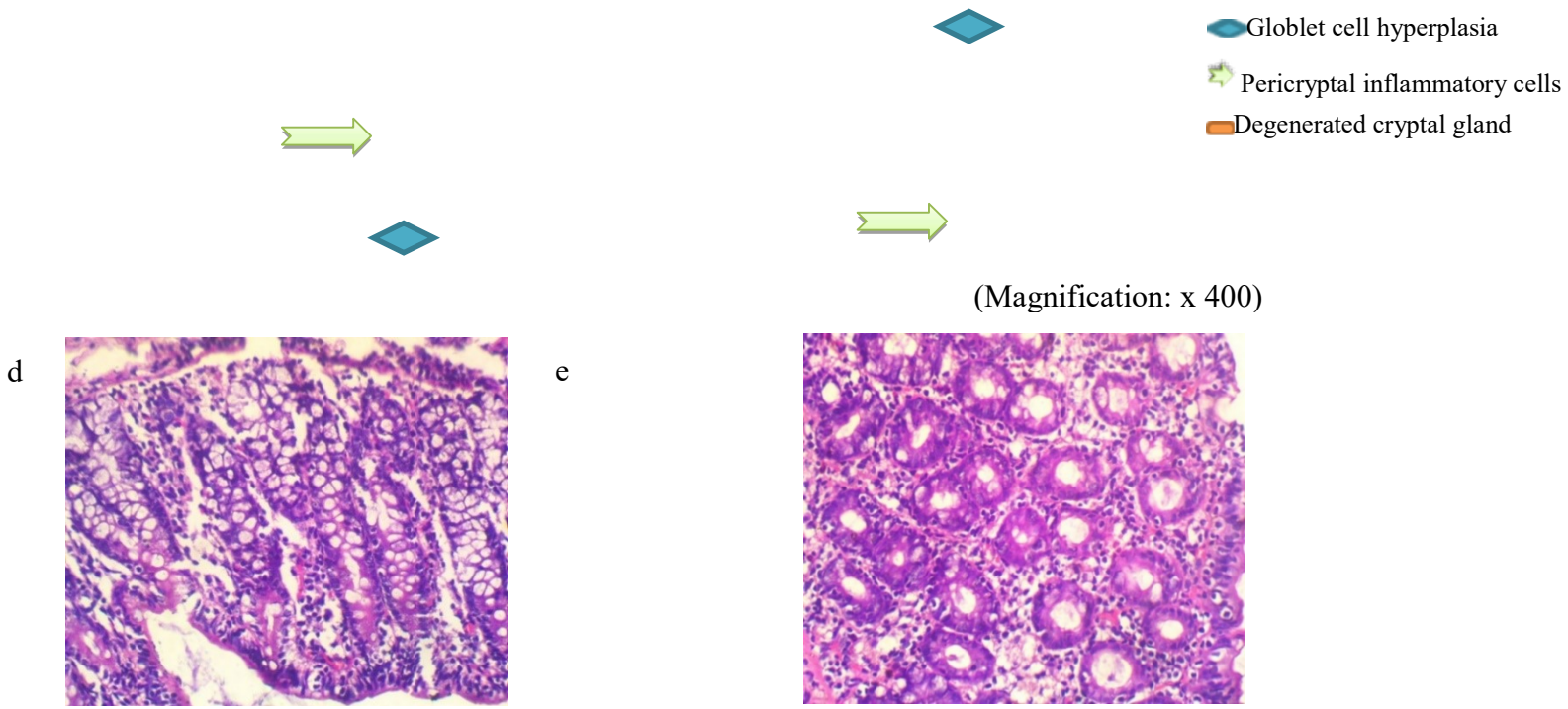


Plate 4.2: Histology of colon of Wistar rats of experimental group treated with (a.) conventional feed before inducing diarrhoea (b.) conventional feed 24 hours after inducing diarrhoea. (c.) fermented feed 24 hours after inducing diarrhoea. (d.) fermented feed plus probiotic 24 hours after inducing diarrhoea. (e.) conventional food plus loperamide 24 hours after inducing diarrhoea.

CHAPTER FIVE

DISCUSSION

5.1 Discussion

Changes observed in pH as well as total titratable acidity (TTA) of spontaneously fermented formulated slurry reported is similar withrecorded effects of fermentation on cereals based complementary blends(Giri *et al*., 2018).

The pH of the spontaneously fermented blends was observed to have decreased over 72 hours relative to the unfermented samples. Decrease in pH of the spontaneously fermenting blends over the period of fermentation could be attributedto the presence and activity of lactic acid bacteria and other microoganisms resulting in the breakdown of carbohydrates into alcohols, sugars, as well as organic acids.

An accelerated acidification was noticed during controlled fermentation compared with the spontaneous fermentation in this study as significant reduction in pH was detected with increase in fermentation time after inoculation of the starters. The pH of the starter fermented blend was observed to have reduced over 48 hours of fermentation compared with unfermented blend. The observed decrease could be linked this time around to the complete dominance and activity of lactic acid bacteria in the fermentation set up. This agrees with findings of Onuoha *et al*. (2017) who reported same observation during controlled fermentation of pearl millet used for flour production. The lowered pH as observed in the fermented samples is a factor known not to support life activities of spoilage and pathogenic microorganisms including spore forming ones (Giri *et al*., 2018). Also, the low pH values in the range of 3.5 - 4.5 as recorded in the fermented food formulation has been reported to contribute to pH decrease in the gastro-intestinal tract

enhancing stability of microbes hence contributing to the favorable effects of probiotics consumed beyond nutritional gains (Dangang *et al*., 2018).

The total titratable acidity increased as fermentation time progressed in the spontaneously fermented complementary blends relative to the unfermented samples after 72 hours of fermentation. This noted increase could be an effect of lactic acid bacteria in the fermenting medium. Similar to this finding is that of Inyang *et al*., (2019) who reported increased total titratable acidity during the fermentation of sorghum-cowpea flour. An increased total titratable acidity in starter-fermented blend was observed as fermentation time increased after 48 hours of fermentation time. The dominating LAB in the fermenting medium could be a reason for this observation which has been reported to aid in the upgrade of sensory qualities as well as microbiological safety of fermented foods (Ojokoh *et al*., 2015). Fortification of blends with *M. oleifera* was observed to bring about increasing pH and TTA. This shows that these blends will be suitable for microbial stability in the gastro-intestinal tract. Wakil and Alao (2013) observed similar trend as the quantity of *M. oleifera* supplementation increased in fermented cereal-legume blends.

The microbiological analysis of the spontaneously fermentated formulated samples revealed a consortium of microorganisms consisting of lactic acid bacteria, enterics, aerobes, moulds with predominance of *L. plantarum*. The observed predominance of *Lactobacillus plantarum* may be as a result of produced lactic acid with low pH which inhibits growth of other microorganisms during carbohydrate fermentation. Adesulu and Awojobi (2014) gave comparable report during fermentation of cerealand also reported that cereals are a rich hub of lactic acid bacteria. Other studies have also shown cereal fermentation aids in altering the pH of fermented slurry such that it does not favour growth of other bacteria. Antimicrobial compounds produced by lactic acid bacteria during the process of fermentation is another factor linked to this observed predominance of LAB, an assertion which compares with findings of (Ojokoh *et al*., 2015).

MacConkey agar selective for the isolation of enterobacteriacea and other Gram negative bacteria had evident growth at 0 hour only in all the blends, an indication that the blendswere well pretreated and processed hygienically. Lowered acidity of the fermenting blends may have become intolerable for the survival of these enterics.This findings is comparable withfindings of Adegbehingbe *et al*. (2017) which reports growth of enterobacteriacea only at the early hours of fermentation experiment. The elimination of Gram negative enteropathogens is a quality coveted after in fermentation of complementary foods because the transmission of pathogens en-route the handling processes to the infant can be prevented by the activities of other fermenting organisms such asLAB. This enhances the safety quality of the food.

The phenotypic identification of the isolates on MRS agar based on morphology, biochemical characteristics and sugar fermentation testsindicating: Gram positive rods, catalase negative as well as non-motile indicates that the isolates were *Lactobacillus* spp. This observation is similar to the finding of Wakil and Olorode (2018) who isolated similar species from fermenting acha.

There were notable improved changes in the proximate content in terms of the crude protein, crude fibre, ash and carbohydrate after fermentation suggesting that fermentation time has positive effect on blends' proximate composition. Best considerable results were recorded after 48 hours of fermentation, which is in agreement with that of Inyang *et al*. (2019) who reported highest proximate composition of co-fermented sorghum-cowpea flour after 48 hours of fermentation time.

Moisture content reduction in the fermented formulated blends was observed in this study. This report compared with that of Wakil and Ola (2018) who reported same trend in fermented maize-tigernut blend. A reduced moisture content of complementary food contributes to its safety and keeps its nutritional quality suggesting they could be kept for long without deterioration as spoilage causing microorganisms find it difficult to thrive in low moist environment (Inyang *et al*., 2019). This is avital consideration for local feeding

methods practiced by busy Nigerianmothers for instancewho prepare dried infants' formula in large quantities and preserve in containers to avoid processing every now and then to have spare time as well as adequate energy for remaining domestic activities.

Increased crude protein content observed as fermentation time increased in this study, could result from mobilization of stored nitrogen to synthesize high quality protein through increased bacteria activity present during fermentation.This is important to infant who depend on gruels of cereal to meet a balanced energy and protein needs. Dangang *et al*. (2018) reported a comparable observation in *Lactococcus lactis* species fermented mungbean, Irish potato, papaya and red kidney bean. This noted increase could also be a result of the synergy between *M. oleifera*,cowpea and groundnut reported to be highly nutritious, that make up the formulated blends (Shiriki *et al*., 2015).

Reduction incontent of crude fat of complementary blends compares with the findings of Msheliza *et al*. (2018), who found a reduction in crude fat content of roasted and fermented sorghum and soybean blend.Thefat content reduction during fermentation may be due to increased activity oflipolytic enzymes produced by microorganismsthat hydrolyzes fat contents to form glycerol and fatty acid. A higher proportion of fat content is said to be advantageous as it contains essential fatty acids which increase the energy content of food and promote good health. But reduced unsaturated oils vulnerable to becoming rancid or having off flavour in fermented samples would enhance the keeping quality as well as general acceptability of complementary food (Ikujenlola, 2014).

Decreasing crude fibre content observed withincrease in fermentation time of naturally fermented and most of the starter fermented blends is comparable to that of Inyang *et al*. (2019) after co-fermentation of sorghum-cowpea flours. This reduction may have occurred because of enzymatic activities on the content of fibre of the composite blend. Low crude fibre content is anticipatedafter fermentation aslikely implications of consuming high fibre food includes of irritation of the gut mucosa, increased bulk as well as lower calorie density, reduced digestibility, reduced vitamin as well as mineral availability. This fosters

a lowering of the required daily nutritional supply and adverse effects on intestinal mucosa in infants. Inceased crude fibre as recorded in some of the blends may be due to incorporation of dried *M. oleifera* leaves. This observed increase is similar tothat reported by Wakil and Alao (2013) during fermentation of *M. oleifera* fortified millet based complementary food.Shiriki *et al*. (2015) also reported a reduced crude fibre content in food blends supplemented with leaves of *M. oleifera*.

The ash content levelof a fermented food substrate communicates the level of minerals present in it. The ash content level of the formulated samples was noted to increasewith progress in fermentation time. This observation has similarity with findings of Msheliza *et al*. (2018) after fermenting a cereal based food. Also,it could be as a result of supplementing the blends with *M. oleifera* as observed by Shiriki *et al*. (2015), who recorded increase in ash content level of formulations fortified with this leaf resulting in a higher level of mineral elements in the diets. Minerals are linked with the ash content and are required for growth and development(Dangang *et al*., 2018). They are important in infant diets to supply nutritional requirement of infants for micronutrients,particularlyzinc, iron, calcium, phosphorus as well as vitamins (A, B, C group) required in the rapid phase of growth (the weaning age) and regulation of various body functions.

Carbohydrate content known as nitrogen free extract in the blend decreased as fermentation progressed. This is largely due to carbohydrate being a key substrate usually utilized as energy and nutrient source by fermenting microorganism. Findingpresented by Inyang *et al*. (2019) shows similar trend after fermenting sorghum-cowpea flours.

The results of the phytochemical content of the starter-fermented blend relative to the control are not similar tothe observation of Ojokoh *et al*. (2015) who presented reduced phytochemical contents of a fermented food after fermentation. These phytochemicals locked up richly in dried leaves of *M. oleifera*(Aljohani and Abduljawad, 2018) could have been released into the fermenting medium as fermentation time progressed hence effecting the observed increases of the analysed phytochemicals. However observed

decrease in phytochemical level during spontaneous fermentation of the blends compares with reports of (Ojha*etal*., 2017).

Phytate is naturally occurring in cereals and legumes and when its presence is above certain level could be worrisome. As observed in some samples, the phytic acid content reducedas fermentation time progressed. This observation agrees with finding of Ojha *et al*. (2017) who noted a decrease in content of phytic acid during fermentation of sorghum flour. Hydrolysis of phytic acid by pearl millets' indigenous phytase as well as fermenting microorganisms have been observed to be a reason for the loss of phytic acid in the course of fermentation. Insoluble complexes formed with trace elements like iron, zinc as well as copper by phytic acid reduces these minerals bioavailability resultantly depleting haemoglobin production and impairing metabolic processes (James *et al*., 2015).

The polyphenol content of some formulated blends reduced as fermentation time progressed. A similar reduction in polyphenols was reported to have occurred due to lactic acid fermentation of maize and tiger-nut (Wakil and Ola, 2018). However, in some formulated samples the polyphenols increased as fermentation time progressed a finding which agrees with observation of Khetarpaul and Chauhan (1989) who reported same trend in pearl millet flour fermentation as a result ofmicrobial hydrolysis of tannins which is condensed thus releasing phenols of lower molecular weight. Noted increased polyphenol quantity may be due toactivities of microbial enzymes which consequentlyyield more easilyaccessible form of plant chemicals such as tannin,alkaloid, flavoniod etc. The non-complex phenolic conversion as well as depolymerisation of high molecular weight phenolic compounds is boosted by presence of lactic acid bacteria during fermentation as reported by (Nazarni *etal*., 2016).

The observed decrease in tannin content of some of the formulated blends as fermentation time increases could be due to the break down of tannic acid into smaller component due to microbial activity and tannin acyl hydrolases (Onuoha *et al*., 2017). Reduced tannin during fermentation connotes: increased bioavailability of proteins as tannin complexes

are hydrolysed, palatability and reduced damage to intestinal tract (James*et al*., 2015). Recently, ahigh level ofcuriosity in antioxidant activity of tannins as well as their prospective health profits, particularly in the prevention of cardiovascular as well as cancer disease in adults has been observed(Inyang *et al*., 2019).

Increased flavonoid content was noted as fermentation time progressed in agreement with report of Nazarni *et al*. (2016)who recordedsimilar increase in flavonoid content in fermented tigarun flower compared to its content in fresh tigarun flower. This could have occurred due to activities of microbial enzymes like amylase, glucosidase, cellulose, invertase, esterase, lipase or tannase produced during fermentation which hydrolyse glucosides, and plant cell walls or break starch down. These enzymes play a disintegration role of the plant cell wall matrix henceaiding release of flavonoid (Hur *et al*., 2014). The β- glucosidase of microbial origin as observed in *L.plantarum* documented to have strong glucosidase activity could hydrolyse the phenolics and flavonoid (Nazarni *et al*., 2016).

Oxalate content was observed to have reduced after fermentation. Similar report was made byOjha *et al*. (2017) that oxalate content level was significantly lowered in the course of malting as well as fermentation of sorghum flour. Oxalate present in the body combines with Ca^{2+}, Fe^{2+} for instance producing their salts which are insolubleand could block kidney tubules engenderingformation of kidney stones (James *et al*., 2015). Therefore,the drop in oxalate contentssuggestsa rise in mineral bioavailability as well aslessening of renal dysfunction.

Reduced alkaloid and saponin content was observed with progress in fermentation time,an occurrence which could have arisen from leaching of thisphytochemicals into the fermenting water and activities of microorganisms during fermentation. This is in agreement with finding of Onuoha *et al*. (2017) whostudied the effect of fermentation on the physico-chemical, phytochemicals as well as proximate composition of pearl millet used for flour production.

According to Nazarni *et al*. (2016), the presence of phytochemicals could confer a broad spectrum for pathogen inhibition however low it is compared with standard antibacterial drug.

The obtained proximate and phytochemical content of the blend fermented with varying inoculum sizes of *L. pentosus* and *L. plantarum*varied. This may be linked to the uniqness of these strains of LAB.From the results obtained as well as those of other researchers, optimum inoculum density for attaining preferred products in fermentation varies extensively depending on the growth rate of the isolate used as well as the substrate they are meant to colonize and utilize (Kumar *et al*., 2011). Inoculum size below optimum will affect the time needed for cells to proliferate, colonize and utilize substrate and produce desired products (Ramachandran *et al*., 2004). At the other extreme, higher inoculum density above optimum has been generally witnessed to affect desired outcomes undesirably (Ibrahim *et al*., 2012). This can be connected to the fact that too much microbial biomass is formed as a result of higher inoculum thus leading to depletion of nutrient in shorter time without adequate metabolite production (Kumar *et al*., 2011). Hence, a suitable inoculum size is needed if the highest desired result must be achieved (Ibrahim *et al*., 2012).

The LABcell free culture supernatant showed substantial antagonistic action against selected pathogenic bacteria in this study as *Staphylococcus aureus* was notably susceptible to metabolites produced by majority of the LAB isolated from the fermenting blends. This observation is similar to that of Giri *et al*. (2018), who reported the growth of pathogens including *Listeria monocytogens*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* from various sources was inhibited by LAB isolates. This may be due to production of secondary metabolites like organic acids (acetic and lactic) and bacteriocins, antimicrobial peptides and toxic oxygen metabolites. Lactic acid bacteria in previous studies have been noted to produce peptides having antimicrobial properties against food borne pathogens thereby being beneficial for food preservation (Garcia *et al*., 2016).

Antimicrobial compound production capacity is oneof many significant qualities required for display of a probiotic effect in the gastro intestinal tract of a host.

Having intrinsic resistance to antibiotics is a natural property of LAB (Manini *et al*., 2016). The LAB isolated from the fermenting formulated blends were resistant to amoxyllin, clavulanate, gentamycin, ceflazidime, cefurozine, ceftriazone, erythromycin, cloxillin and ofloxicine. Components of cell wall liketeichoic acid, polysaccharide, and peptidoglycans which makes it impermeable, seems to be the main mechanism of resistance to this antibiotics. This finding compares with that of Pundir *et al*. (2013) who suggested administering LAB isolates to patients already administeredbroad spectrum antibioticsmay be of substantial help in quick recovery due to swiftestablishment of required microbial flora. However, report of this current study is dissimilar to the report of Giri *et al*. (2018) who in their experiment observed all LAB isolates were only resistant tovancomycin and susceptible to other antibiotics.

The result of this research showed that LAB isolated from fermented formulations were capable of tolerating 2 - 10% concentration of NaCl, a finding which agrees with that of Qing *et al*. (2015) who observed growth of *Lactobacillus plantarum* in NaCl concentration of 0 -10% from fermented soybean paste and Thakkar *et al*. (2015) whose lactic acid bacteria isolates tolerated 8 - 12% NaCl concentration. At these high concentrations, bacteria cells would experience cell pressure loss which in turn disturb the physiology, enzymes activity as well ascell metabolism. The LAB isolates'ability to withstand a stress conditions (1 - 6.5% salt concentration)is due to their enablement to efficiently store osmo- as well as cryoprotective solutes such as carnitine and betaine making them preferred as good probiotic. Moreover tolerance to high salt concentrations initiates metabolic activity which yields acid that inhibits growth of undesirable microorganisms (Thakkar *et al*., 2015).

Resistance to a low pH (high acidic condition) is a key criterion for probiotic selection. The pH of a stomach after meal ranges between pH 1 and pH 4, a stress situation

probiotics have to pass through (Hawaz, 2014). This high acid condition has been reported to stimulate the residency of beneficial bacteria in the stomach (Puphan *et al*., 2015). The results as noted in this study showed the LAB isolated from fermenting blends could tolerate extreme pH of 2 – 3. This is in agreement with the report of Wakil and Olorode (2018), who observed lactic acid bacteria survived acidic condition at pH 2 as well as pH 3 after incubation for 4 hours. The ability of LAB isolates to grow in high acid condition may possibly be linked to their acid tolerance response which is dependent: on their cell wall components such as peptidoglycan, teichoic acid and colonic acid; culture medium type and conditions (Kalhoro *et al*., 2019).

The appropriate physiological concentrations of human bile ranges between 0.1%and 0.5% and staying time is proposed to be 4 hours (Mathara *et al*., 2008). In this research the lactic acid bacteria isolates grew at different bile concentrations used with observable decrease in viable count of cells. This might be linked to the ability of certain strains of LAB to release bile salt hydrolase enzymes which act as catalyst for hydrolysis of bile salt into amino acid residues with reduced solubility hence resisting the bile salt (Mishra and Prasad, 2005). These results agrees with Kalhoro *et al*. (2019) who reported similar survival of LAB isolates in 0.5% - 1% bile concentration after three hours of incubation. Wakil and Olorode (2018) also reported tolerance of lactic acid bacteria strains in bile (0.05 - 1.0%).

The period of entrance of LAB through the mouth up to their release time into the stomach where the gastric juice is found is usually within 90 minutes (Chou and Weimer, 1999). A sizeable number of LAB isolated from the formulated blends survived in the presence of simulated gastric juice up till 180 minutes. Wakil and Olorode (2018) also reported lactic acid bacteria isolates from fermenting blends survivedcircumstances similar to the gastrointestinal environment which may be due to a factor like the composition of cell wall component which promote their ability to tolerate low pH.

The next challenge an effective probiotic should overcome having survived the upper gastrointestinal transit is adherence to the small intestine. Cell surface hydrophobicity is considered anessentialfeature in adhesion together with proliferation of microorganism in the intestinal epithelial cells (Giri *et al*., 2018). Previous studies suggested the capacity of beneficial micro-organisms to adhere, support colonization of the gut and instituting a barrier which inhibits enteropathogens from establishing an infection (Syal and Vohra, 2014). This observation suggests the complexity of the cell surface mosaic ensuing from hydrophobic as well as hydrophilic appendages together with other macromolecular components may be responsible for the differences in hydrophobicity (Giri *et al*., 2018).

Safety is also asort after criteria in the FAO/WHO (2002) guiding principle on consideration and selection of probiotics to be utilized as feed supplement or food.

All LAB isolatedin this study showed no gelatinase and DNAase activity suggesting they are not virulent. This observation is comparable to observations of Thakar *et al*. (2015) who suggested microorganisms should not produce this enzyme so they can be function as probiotic in feed supplement as well as food. Gelatinase activity has been reported to derange the mucoid lining while extracellular DNAase through the degradation of the neutrophil extracellular traps is reported to provide growth advantage for disease causing microrganisms by increasing nucleotides pool due to hydrolysis of DNA which aids pathogens spread by liquefying pus and aiding the evasion of the innate immune response (Hasegawa *et al*., 2010).

Exopolysaccharide (EPS) occur as a capsule or slime lightly tied to the surface of a cell playing a protection role against osmotic stress, antibiotics, toxic compounds, desiccation and bacteriophages in locations they are found (Syal and Vohra, 2014). EPS manufacturing bacteria are known to have a solid capability to tolerate technological stress and survive transition through the gastrointestinal tract (Patel *et al*., 2012). They reduce pathogenic biofilm development, modulate adhesion to epithelial cells among other qualities. Exopolysaccharides commonly produced by LAB are known for their ability to impact thickening and gelling properties thereby improving the viscosity and texture of food substances. These qualities make them better bacteria compared to their non-

producing counterparts (Patel *et al*., 2012). Findings of this research agrees with that of Wakil and Olorode (2018) who reported EPS production by LAB strains obtained from fermenting acha blends in their study.

Absence of haemolysis is very important characteristic and prerequisite for selecting any probiotic bacteria. Non-haemolytic characteristic of LAB as observed in this study agrees with findings of non-haemolysis in lactic acid bacteria isolates reported by Kalhoro *et al*. (2019). Inability of these LAB to be haemolytic connotes they are not capable of destroying animal red blood cell hence are not capable to contribute toward increasing a chance of infection.

The inability of the selected lactic acid bacteria to release lecithinase or phospholipases known to be enzymes connotes they do not have the capacity to destroy tissues of animal by splitting the phospholipid lecithin, a complex usually known as emulsifying agents existing in serum, tissues, as well as egg yolk and play a role in pathogenicity. This report is similar to that of McChung and Taobe (1947) who in their study isolated bacteria that were negative for lecithinase production.

Ability of lactic acid bacteria to produce enzymes (proteolytic, amylolytic as well as lipolytic) which are needed to breakdown key nutrient in food substances shows they could play a part in making nutrients available to microorganisms which in turn improve and impact specific required quality on these food substances. Furthermore, the capability to produce hydrolytic enzymes assists an organism to compete favorably in its habitat with other microorganisms. Isolated lactic bacteria in this study showed proteolytic, lipolytic and amylolytic properties suggesting they could be suitable starters. Similar finding was reported by Ojha *et al*.(2017).

Minerals are indispensable nutrients which are requiredtoexpedite accurate functioning of certain organsin the body. Increased content of the calcium, iron, zinc, manganese, magnesium, potassium, and phosphorus in this study could be connected to hydrolysis of specific phytochemicals through microbial enzymes in the fermentation medium. This

observation agrees with that of Wakil and Ola (2018) who reported that fermentation was more effective in increasing bioavailability of calcium, phosphorus, iron, etc. in maize-tigernut formulated blend. James *et al*. (2015) reported that fermentation increased minerals bioavailability in cereals mixed with legumes via microbialphytase hydrolytic action leading to the release of more calcium, magnesium, iron, and zinc etc. Reduced content of zinc and phosphorus as observed in in this studyas well could be linked to their absorption by the fermenting microorganism. This observation agrees with the finding of Dangang *et al*.(2018) who reported a reduction in level of minerals in their fermentation experiment as a result of their use up by fermenting organisms.

Apart from the obvious effect of fermentation on the mineral content of the formulated blends,the supplementation effect of dried leaves of *M. oleifera* powder identified as a highly nutritious plant may have contributed to the noted increase in minerals content. This further confirms the exceptional nutritive quality of *M. oleifera* leaves as reported by other researchers (Aljohani and Abduljawad, 2018).

Result of analysis of vitamin content of formulated blends revealed increased vitamin content values in fermented samples compared to samples that were unfermented. This report is comparable with report of Msheliza *et al*. (2018) having observed that thiamine, nicotinic acid, riboflavin were higher after fermentation relative to the unfermented roasted sorghum and soybean blend. Wakil and Ola (2018) also reported starter fermentation increased the vitamins content in maize-tigernut blend. LAB isolates involved in the fermentation process may have synthesized these vitamins during the course of their metabolic activities. The rich vitamin content of *M. oleifera* leaves and legumes may also have added to the overall improved vitamin contents of the fermented blend. This assertion is similar to report of Aljohani and Abduljawad (2018) after fortifying a food blend with *M. oleifera* leaves.

Some amino acids which are not synthesized by the body but providedin a food mix have uniqueroles to play in ensuringaccurate body functions and growth. Thesemust be

supplied in precise quantity viaconsumed diets to achieve a desired metabolic result. Result of the amino acid analysis revealed increase in obtained values in fermented samples relative to samples that were not fermented. Fermentation was noted to have increased the amino acid contents of the blend significantly and this report is comparable to that which Nwabueze *et al*. (2018) reported during fermentation and sprouting of blended millet and bambara nut significantly increased the analyzed essential amino acid content through hydrolytic reactions of proteolytic enzymes released by the lactic acid bacteria in their fermentation set up. Inyang *et al*. (2019) also reported maximum amount of essential amino acids was obtained after co-fermenting sorghum-cowpea blend. This observation can be linked to the effect of formulation ratio which had rich legumes and dried leaves of *M. oleifera* added to the blend. Reduction in the concentration of some amino acids of the blend during fermentation may have occurred due to their use by the fermenting microorganism for growth and production of metabolites. This agrees with findings of Sengev *et al*. (2016) who observed reduced amino acid content of sorghum-based complementary foods.Values recorded of tryptophan a limiting essential amino acid and others essential amino acid obtained of the starter-fermented complementary blend in this research are within the range indicated in the dietary reference intake (Institute of medicine, 2002)

Viscosity which is a measure of resistance to flow of fluidy food is one of the key determinants of food acceptability to mothers and children. Food is observed to have a visco-elastic form and complementary food which has high viscosity is typically unacceptable to growing infants as it causes suffocation as well as a measure of difficulty during feeding. Report of this research shows viscosity of the formulated blend decreased upon fermentation an observation similar to report of Msheliza *et al*. (2018) having fermented sorghum and soyabean. This may be as a result of theaction of amylase which produces components which are more soluble and the other actions of microorganisms in the process of fermentation. Also, reduction in protein and carbohydrate interaction in the course of treatment may yield a change in viscosity (Ojha *et al*., 2017).

The weight,particles of flour blends can bear if allowed to directly rest on one another is known as bulk density. Inmost samples, measured bulk density was observed to decrease as fermentation time progressed in agreement with the report of Msheliza *et al*. (2018) who observed a reduction in the bulk density of sorghum and soybean flour after fermentation. In cereals, starch break-down throughfermentation process reduce itsstarch content and resultantly reduces it bulk density making it suiableuse as food for infants (Ojha *et al*.,2017). High bulk density poses a nutritional threat because it can limit the nutrient intake per feed below the recommended level and its general acceptability (Lohia and Udipi, 2015).

The metabolisable energy was observed to increase with increase in fermentation time in starter fermented blends in agreement with the observation of Dangang *et al*. (2018) who fermented formulated mungbean, Irish potato, red kidney bean as well as papaya with *Lactococcus lactis*. This increase could be a result of the content of fat which happens to be on the high side in the formulated samples.Fat on its own comprises about twice the value of food energy of carbohydrate and protein (Osborne and Voogt, 1978). A notable balanced proximate composition could also have resulted in an increased calorific value. The energy content recorded in this study can be compared to resultpresented by Ojokoh *et al*. (2015) on the energy content of fermented pearl millet and acha flour blends.

Enhanced sensory qualities recorded with increase in fermentation timecompares with that of Wakil and Ola(2018) on starter-fermented maize-tigernut blend. Inyang *et al*. (2019) also reported fermentation enhanced the sensory attributes of gruels making it more preferred by jugdes who developed no side effect in any form after consuming the preparation.

The weight gained by Wistar rats as shown in nutritional evaluation revealed the probiotic-fermented blend had good nutritional effect compared to conventional food and commercially sold complementary food. This observation could be as a result of the ratio formulation of the nutritionally rich constituent such as cereal, legumes and *M. oleifera*

which make up the formulated blend which was further enriched through the process of lactic acid fermentation. This observation is corroborated by the results of the protein efficiency ratio observed in Wistar ratsfed the probiotic-fermented blend and the probiotic LAB which is significantly higher compared to that of Wistar ratsfed the conventional food and commercially sold complementary food.

Haematological result obtained in this study showed the Wistar ratsfed the probiotic-fermented food and the probiotic LAB were healthy as their haematological variables noted to be within the normal range of reference value of standard blood count were at par with that obtained in animals fed commercially sold complementary food. This may be as a result of the rich nutritional composition of the probiotic-fermented food. This report compares with that of Wakil and Ola (2018) who reported formulated maize-tigernut blend had outstanding nutritional qualities revealed in the haematology of Wistar rats compared to nutrend.

The liver is known to play key role in body metabolism but its cellular integrity can be compromised when there is a disease condition. Therefore, its test of function is performed to assess its pathological condition (Oyagbemi and Odetola, 2010). In assessment of an occurrence of liver damage by the probiotic fermented food, the probiotic-fermentedfood and the probiotic bacteria, prominent tests like AST, ALT and ALP levels were evaluated. Transaminase enzymes are greatly involved in the release of energy, rearrangement of protein-building blocks, bone growth and determination of the integrity of plasma membrane such that an alteration in their levels beyond permitted levels suggests a damage to cells, tissues and important organs of the body. In case of liver membrane damage or necrosis, these enzymes are released and circulated into the living system. This can be measured in the serum as markers of hepatic damage (Nordqvist, 2017).

In this study, serum level of AST, ALT and ALP inWistar ratsfed the probiotic-fermented food and the probiotic LABwithin permitted range (as no recorded value was 10-20 times

the control) were not significantly altered suggesting more release of energy positively linked to improve feed intake together with general activity recorded in these animals. This is in agreement with report of Banwo *et al*. (2020) who observed normal transaminase enzyme and glucose level linked to improved feed intake in experimental rats. Non significant alterations of the levels of these enzymes also suggest that no damage was done to the hepatic cells implying the fermented food and the probiotic bacteria administered was non-toxic rather they seem to confer protection and maintain the hepatic cells functional integrity in the animals.

Obtained heamtological results of diarrhoea induced Wistar rats fed the probiotic-fermented food, the probiotic LAB and those treated with Loperamide HCl-Tm was stabilized physiologically compared to that of Wistar rats fed convetional feed and induced with diarrhoea which had a significantly reduced PCV, RBC and HB together with a non-significantly increased WBC, all not within the reference range of standard blood count set by the University of Pennsylvania school of Veternary Medicine, 2012. These observations should be as a result of electrolyte imbalances and dehydration which alters the normalequilibrium of electrolyte and water and electrolytes i.e. potassium, sodium and chloride in the animal system.

The reduced diarrhoeal activity (pass out of loose watery stool, reduced activity, raised fur) recorded in Wistar rats fed the probiotic-fermented food, the probiotic LAB and those treated with Loperamide HCl-Tm as compared to those fed conventional food suggests the food hasantidiarrhoea properties. This reflection is similar to the report of Holowacz *et al*. (2016) who documented a decreased diarrhoeal incidence in rats treated with probiotic mixture. The perceived antidiarrhoeal effect of the probiotic-fermented food and the probiotic bacteria could be linked to its rich nutritional composition and inherent antioxidants such as alkaloids, flavonoids and tannin which were confirmed by the analysis carried out in this research. Antioxidants metabolites (released by the probiotic bacteria) with capacity to resist reactive oxygen species which have been reported by Wang *et al*. (2017) might have ameliorated the diarrhoegenic situation.

Comparatively, the heamatological variables in Wistar rats fed probiotic-fermented foodand that obtained for Wistar rats uninduced and fed with conventional foodafter six and twenty four hours were normal and within the reference range of standard blood count set by the University of Pennsylvania school of Veternary Medicine, 2012. This implies the fermented food protected the Wistar rats induced with diarrhoea to an extent and helped with treatment and recovery within twenty four hours. Holowacz and others (2016) documented a decreased diarrhoeal incidence in rats treated with probiotic mixture

Worthy of note is the similarity between the observations 6 and 24 hours post diarrhoea induction timing on obtained haematological parameters obtained of Wistar rats fed the probiotic-fermented food and the probiotic LAB and those treated with Loperamide HCl-Tm. In the animals of both groups, haematological variables such as WBC, platelet, lymphocyte and neutrophils only were noted to positively change significantly. This seem to suggest a similarity in the mechanism of antidiarrhoegenic action of Loperamide HCl-Tm a known standard drug for treating diarrhoea and the fermented food plus probiotic.

The biochemical assays carried out in this study revealed that the experimental diet stimulated production of varying levels of antioxidant. Studies have revealed oxidative stress plays majorpart in pathogenesis of gastrointestinal diseases like diarrhoea and gastritis in humans.Both preclinical and clinical investigations have shown raised oxidative stress level during periods of gastrointestinal disease, which often leads to a sudden onset of symptoms of these diseases (Renuka *et al*., 2015).

Glutathione (GSH) is a water-soluble tripeptide made up of the glutamine, amino acids, glycine and cysteine. GSHis a major endogenous anti-oxidant manufactured by the cells which functions majorly as a detoxifier of various peroxide as well as electrophilic compounds through catalysis by glutathione peroxidases as well as glutathione S-transferases (GST). It couldplay a non-enzymatic antioxidant rolethroughuninterrupted interaction of –SH group with reactive oxygen species(Anderson and Seilhamer, 1997). It participates in free radicals as well as reactive oxygen species neutralization. As reflected

in the result of this study 6 and 24 hours post induction, GSH levels in Wistar rats fed with conventional feed and induced with diarrhoea were significantly lower compared to uninduced Wistar rats also fed conventional feed. This indicate an increased oxidative burden in Wistar rats fed induced and fed conventional feed which should be a consequence of diarrhoea incidence. The observed increase in GSH level in induced Wistar rats fed probiotic-fermented food, [significantly different ($P \leq 0.05$)], probiotic-fermented food plus probiotic LAB andLoperamide HCl-Tmmight be an adaptive response to the diarrhoegenic insult.

Catalase (CAT) is an enzymatic antioxidant which is a component of the body antioxidant defense systems that play key role in detoxifying free radicals manufacturedwhen there is oxidative stress. This enzymatic antioxidant is a homoprotein, contained in the peroxisomes or the microperoxisomes which catalyzes decomposition of H_2O_2 to H_2O and O_2 and as a result shielding the cell from oxidative impairment by H_2O_2 as well as OH (Naganuma *et al*., 1990). According to the result of this study, catalase activitydeclined significantly ($P \leq 0.05$) in induced Wistar rats fed conventional food compared to those induced and fed the probiotic-fermented food, the probiotic-fermented food plus probiotic LAB. Decrease in Catalse level may have occurred because of saturation of the enzyme superoxide dismutase (SOD)[that help reduce oxidative tension] with superoxide radicals or decreased expression of SOD which makes the colonic mucosa susceptible to damage by ROS. As CAT decomposes H_2O_2 generated by SOD, reduced CAT activity may be due to decrease in substrate (H_2O_2) level. Significantly elevated activity of CAT observed in induced Wistar rats fed the probiotic-fermented food, the probiotic-fermented food plus probiotic LAB and Loperamide HCl-Tm six and twenty-four hours after, may be in response to diarrhoegenic effect of castor oil and an adaptive response to counter the effect of the increased oxidative stress caused by the physiological disorder.Chakaraborty *et al*. (2009) reported inducement of antioxidant enzyme on toxic insult and reported increased activity of CAT which decomposes H_2O_2 generated by SOD would lead to a decrease in oxidative stress.

This result can be corroborated by the observation of estimated GHS level in the animals of the groups mentioned respectively and are comparable with results of Renuka *et al*. (2015) who presented remarkable rise in CAT and GSH level after inducing colon injury using N, N-dimethylhydrazine dihydrochloride (DMH) and treating with extract of Ocimum gratissimum.

Nitrite (NO) is known to abate the oxidation chemistry hence protecting against cell death facilitated by ROSlike H_2O_2, alkylhydroperoxides as well as xanthine oxidase (Çiftci *et al*., 2015). The weakening of metal or peroxide oxidative chemistry and lipid peroxidation as wellseems to be the key chemical mechanisms through which NO possibly will limit oxidative hurt to cells of mammals. NO can control cellular and physiological processes to limit oxidative damage therebyrestraining processes such as leukocyte adhesion is in addition to afore mentioned chemical and biochemical properties(Çiftci *et al*., 2015).

In this study, the raised level of nitrite in induced Wistar rats fed the probiotic-fermented food, the probiotic-fermented food plus probiotic LAB after 6 and 24 hours suggests nitrite an antioxidant agent was adequately high enough to counteract the oxidizing effect of reactive oxygen species in these Wistar rats. This implies therefore, there was low oxidative stress in these Wistar rats. The lowest nitrite levels observed in induced Wistar rats fed with conventional food and those treated with Loperamide HCl-Tm suggests the measure of this antioxidant might not be adequately high enough to counteract oxidizing effect of ROSimplying slower recovery process in these Wistar rats. In a similar work Çiftci *et al*. (2015) reported serum nitrite level was not adequately high to compensate for the high serum reactive oxygen species levels in patients diagnosed with non-alcoholic fatty liver disease which he suggested implied increased oxidative stress in these patients.

Noted protection against ROS as well as generated free radicals in induced Wistarrats may have been mediated through activities of these anti-oxidant enzymes enhanced by natural antioxidants present in the composite ingredients (Aljohani and Abduljawad, 2018).

Thereforeobservations of this researchpropose that administration of probiotic-fermented food as well asprobiotic-fermented food plus probiotic bacteria shows a comparable measure of protective effect against castor oil induced diarrhoea with Loperamide HCl-Tm. This suggests that fermented food and fermented food plus probiotic bacteria have an antidiarrhoeal effect by modulation of oxidative stress.

The villi generally increases the internal surface area of the intestinal walls for water and nutrient absorption (Sharma e*t al*., 2015). A loss or damage of villi due to inflammation or microbial infection results in reduced absorption in the gastro intestinal tract hence, resulting in continuous diarrhoea as observed in induced Wistar rats fed conventional food which showed reduced number of villi with low height as compared to enormous villi which had normal height observed in uniduced Wistar rats fed conventional food and induced Wistar rat fed probiotic-fermented food as well as probiotic-fermented food plus probiotic bacteria. Goblets cells, on the other hand are responsible for the secretion of mucus in the digestive tract and subsequently giving it its characteristic mucosal membrane structure Johnansson *et al*. (2013). In all induced Wistar rats, goblet cell hyperplasiawhich refers to the enlargement of the intestine due to increase in the number of goblet cells resulting from uncontrolled cell-division leading to increased mucus production as evident in the animals droppings also showing signs of presence of diarrhoea. Loperamide HCl-Tm treated Wistar rats showed moderate congestion of blood vessels suggesting possible side effect when used in diarrhoea treatment, while Wistar rats fed probiotic fermented food and probiotic fermented food plus probiotic mix showed normal crypt hence supporting the effectiveness of probiotic fermented food and probiotic mix.These observations are in agreement with reports of Banwo *et al*. (2020) who reported the antidiarrhoeal effect of plant materials used in treatment of castor oil induced diarrhoea in Wistar rats.A comparable result was also obtained by Renuka *et al*. (2015) in injured colon of rats induced with N, N-dimethylhydrazine dihydrochloride. Twenty four hours after inducing diarrhoea, there was absence of prominent histological changes in all groups. This observation corroborates reports that an episode of diarrhoea condition should ends within 24 to 48 hours (WHO, 2014).

CHAPTER SIX

SUMMARY, CONCLUSION, AND RECOMENDATION

6.1 Summary

An improved nutritional effect of fortified already supplemented millet cowpea and groundnut complementary food with dried powdered leaves of *M. oleifera* has been highlighted in this study.

6.2 Conclusion

Forty-eight (48) hour spontaneously fermented F4 had improved nutritional composition.

Lactobacillus plantarum-MCB4, *Lactobacillus plantarum*-MCB18, *Lactobacillus pentosus*-MCB47 isolated from fermenting formulations demonstrated good starter and probiotic qualities *in vitro*.

Forty-eight hours*L. plantarum*-MCB18(1.5×10^8 CFU/mL) fermented F4 formulated blend had improved physico-chemical qualities expected of a good complementary diet as illustrated by*in vitro*study.

Consumption of F4 formulated complementary blend fermented using *L. plantarum*-MCB18 as starter and ingestion of *L. plantarum*-MCB18 (dose X 10^6 CFU/mL) as probiotic had preventive effect against malnutrition and diarrhoea as illustrated by *in vivo* study.

6.3 Recommendation

Further work targeted at finding more appropriate formulation ratio and processing methods (which include prospecting for appropriate inoculum sizes of starter cultures) that

can best improve physico-chemical parameters of complementary blends made from cheaper and more readily available resources in the immediate environment should be done.

Prospecting for more lactic acid bacteria with probiotic potentials whose list remains inexhaustible isolated from these complementary blends and other food matrix should be worked upon for application in protecting against diseases and consequently depending less on chemically synthesized drugs.

Contributions to knowledge

This work was carried out in order to formulate a complementary blend with improved nutritional qualities with focus on its anti-diarrhoeal potentials.

1. Development of a new complementary food formulation(70% millet, 22% cowpea, 5% groundnut, 3% *M. oleifera*) with improved nutritional qualities.
2. Isolation and characterization of*Lactobacillusplantarum*-MCB18 with unique inherent probiotic potentials as revealed by *in-vitro* studies.
3. Biological evaluation of healthy male Wistar rats fed *L. plantarum*-MCB18 (1.5×10^8 CFU/mL) fermented complementary blend (F4B1) showed improved health status.
4. Consumption of*L. plantarum*-MCB18 (F4B1) fermented complementary blend and its ingestion (dose × 10^6 CFU/mL) helped stabilize a diarrhoea-like condition induced by castor oil as revealed by *in-vitro* studies.

REFERENCES

Abdulkadir, M. and Danjuma, J.B. 2015. Microbial profile and nutritional quality during the fermentation of cereal based weaning food fortified with soya bean and tiger nut using starter culture. *World Scientific News*. **24**: 103-115.

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

Adegbehingbe, K.T.; Fakoya, S. and Adeleke, B.S. 2017. Effect of Fermentation on Nutrient and Antinutrient Contents of Fermented Whole and Ground African Breadfruit (*Treculia africana*) Seeds.*Microbiology Research Journal International*. **20**(5): 1-11. Article no.MRJI.34444 ISSN: 2456-7043.

Adenuga, W. 2010. Nutritional and sensory profiles of sweet potato based infant weaning food fortified with cowpea and peanut. *Journal of Food Technology*.**8**(5): 223-228.

Adesulu, A.T. and Awojobi, K.O. 2014. Enhancing sustainable development through indigenous fermented food products in Nigeria. *African Journal of Microbiology Research*. **8**(12):1338-1343.

Afagnigni, A.D., Nyegue, M.A., Foe, C.F.N., Ndam, Y.N., Njayou, F.N., Fonkonua, M.C. and Etoa, F.X. 2017. Antidiarrhoeal activity of *Dissotis multiflora* (sm) Triana (Melastomataceae) leaf extract in Wistar rats and sub-acute toxicity evaluation. *Evidence- Based Complementary and Alternative Medicine*. Article ID. 4038371.

Afify, A.M., El-Beltagi, H.S., Abd El-Salam, S.M. and Omran, A.A. 2011. Bioavailability of iron, zinc, phytate and phytase activity during soaking and

germination of white sorghum varieties. *Asian Pacific Journal of Tropical Biomedicine*. **6**(10): 25512, 1-7.

Agaliya, P.J. and Jeeveratnam, K. 2012. Screening of *Lactobacillusplantarum* isolated from fermented idli batter for probiotic properties. *African Journal of Biotechnology*. **11**(65): 12856-12864.

Ahmed, T. and Kanwal, R. 2004.Biochemical characteristics of lactic acid producing bacteria and preparation of camel milk cheese by using starterculture. *Pakistan Veterinary Journal*.**24**: 87-91.

Akudor, G.C., Muazzam, I., Usman-Idris, M., Megwas U.A., Akpan, J.L., Chilaka, K.C., Okoroafor, D.O. and Osunkwo U.A. 2011. Evaluation of the antidiarrheal activity of methanol leaf extract of Bombax buonopozense in rats. *Ibnosina Journal of Medicine and Biomedical Sciences*. **3**(1): 15-20.

Aljohani, N.E. and Abduljawad, S.H. 2018. Efficacy of Moringa oleifera leaf supplementation for enhance growth performance, haematology and serum biochemistry of rabbits. *Food and Nutrition Sciences*.**9**: 1285-1298.

Allen, S.J., Martinez E.G., Gregorio G.V. and Dans L.F. 2010. Probiotics for treating acuteinfectious diarrhoea. *CochraneDatabase System Review*. **11**: CD003048. Archived from the original on 3 December 2010.

Amarowicz, R. and Pegg, R.B. 2008. Legumes as a source of natural antioxidants. *European Journal of Lipid Science and Technology*. **110**(10): 865-878.

American Academy of Pediatrics, 2012. Breastfeeding and the use of human milk. Pediatrics, 129(3), e827-e841. Retrieved April 27, 2012, from http://pediatrics.aapublications.org/content/129/3/e827.full.pdf+html

Anderson, L. and Seilharmer, J. 1997. A comparison of selected mRNA and protein abundance in human liver. *Electrophoresis*. **18**(3-4): 188-191.

Annor, G.A., Ma, Z. and Boye, J.I. 2014. Crops-legumes in food processing: Principle and Application: Second Edition: Principles and Application: Second edition. Vol. 9780470671146, pp 305-337.

A.O.A.C. 2005. Official Methods of Analysis.18thEdition, AssociationofOfficial Analytical Chemists, Washington DC, 18.

A.O.A.C. 2012. Official methods of analyses, 19th Edition. Association of Official Analytical Chemists, Arltngton, 806-842.

Apaliya, M.T., Kwaw, E., Tchabo, W., Sackey, A.S. and Boateng, N.A.S. 2017. The use of lactic acid bacteria as starter culture and its effect on the proximate composition and sensory acceptability of millet beverage. *International Journal of Innovative Food Science and Technology*. **1**: 1-8.

Armar-Kelemesu, M.A. and Wheeler, E.F. 1991. Weaning practices and their outcome: A critical look with special reference to Ghana. *Bulletin of Noguchi Memorial Institute for Medical Research*. **4**: 3-24.

Astashkina, A.P., Khudyakova, L.I. and Kolbysheva. 2014 Microbiological quality control of probiotic products. *Procedia Chemistry*. **10**: 74-79.

Avwioro, G.O. 2010. Histochemistry and tissue pathology principles and techniques. Claverianum press, Nigeria.

Awika, J.M. 2011. Major Cereal Grains Production and Use around the World. In: Advances in Cereal Science: Implications to Food Processing and Health Promotion. **1089**:1-13.

Aworh, O.C. 2008. The role of traditional processing Technologies In National Development: the West African Experience. *International Union of Food Science and Technology*.**1**:1-18.

Awouters, F, Niemegeers, C.J.E., Lenaerts, F.N. and Janseen, P.A. 1978. Delay of castor oil diarrhoeal inrats: a new way to evaluate inhibitors of prostaglandin biosynthesis. *Journal of Pharmacy and Pharmacology*.**30**:41-53.

Balciunas, E.M., Martinez, F.A.C., Todorov, S.D., deMelo, G.,Franco, B.D. and Convert, A. 2013. Novel biotechnology applicationsof bacteriocins: AReview. *Food Control*. **32**:134-142.

Ballard O. and Morrow A. L., 2013. Human milk composition: Nutrients and bioactive factors. *Pediatric Clinics of North America*.**60**(1): 49–74. Retrieved June 21, 2016, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3586783 [top].

Ballogou,V.Y., Soumanou, M.N., Toukourou, F.and Hounhouigan, J.D. 2013. Structure and nutritional composition of fonio (*Digitaria exilis*): A review. *International Research of Biological Science*. **2**(1): 73-79.

Banwo, K., Alao, M.B. and Sanni, A.I. 2020. Antioxidant and antidiarrhoeal activities of methanolic extract of *Parkiabiglobosa*and leaves of *Parquetinanigrescens*. *Journal of Herbs*, *Spices and Medicinal Plant*s. **26**(1): 14–29.

Baquerizo, Nole, K.L., Yim, E. and Keri, J.E. 2014. Probiotics and prebiotics in dermatology. *American Academy of Dermatologist*.**71**: 814-821.

Basem, A. and John, D., 2013. Anesthesia for otolaryngologic surgery. Cambridge University Press. pp. 282–287. ISBN 1107018676.

Bassey, O.A., Lowry, O.H. and Brock, M.J. 1946. A Method for the Rapid Determination of Alkaline Phosphates with Five Cubic Millimetres of Serum. *Journal of Biology and Chemistry*. **164:** 321–325.

Beutler, E., Duron, O. and Kelly, B.M. 1963. Improved method for the determination of blood glutathione. *Journal of Laboratory and Clinical Medicine*. **61**: 882-888.

Bulut, C. 2003. Isolation and molecular characterisation of lactic acid bacteria from cheese. Izmir Institute of Technology Izmir, Turkey Thesis of MSc; 14.

Chafen, J.J., Newberry, S.J., Ried, M.A., Bravata, D.M., Maglione, M., Suttorp, M.J., Sundaram, V., Paige, N.M., Towfigh, A., Hulley, B.J and Shekelle, P.G. 2010. Diagnosing and managing common food allergies: a systematic review. *Journal of American Medical Association*. **303**(18): 1848-56.

Chakaraborty, P., Hossain, S.U. and Murmu, N. 2009. Modulation of cyclophosphamide-induced cellular toxicity by diphenyl methyl selenocyanate*invivo*, an enzymatic study. *Journal of Cancer Molecules*. **4**(6):183-189.

Charteris, W.P., Kelly, P.M., Morelli, L. and Collins, J.K.1998.Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotics Lactobacilli and Bifidobacterium species in the upper human gastrointestinal tract. *Journal of Applied Microbiology*. **84**(5): 759-768.

Cheesbrough, M. 2006. Haematological tests: abnormal haemolglobin in: District Laboratory practice in tropical countries. Part 2, 2nd ed. Cambridge Universal Press, New York, pp 283-340.

Chelule, P.K., Mokoena, M.P and Gqaleni, N. 2010. Adavantages of traditional lactic acid bacteria fermentation of food in Africa. Current Research Technology and Education in Tropics in Applied Microbiology and Microbial Biotechnology. A Merdez Vilas (Ed) p. 1160-1167.

Chou, L.S. and Welmer, B. 1999. Isolation and characterization of acid- and bile-tolerant isolates from strains of *Lactobacillusacidophilus*. *Journal of Dairy Science*. **82**(1): 23-31.

Choung, M.B. Choi, Y. An, Chu, Y. and Cho, Y. 2003. Anthocyanin profile of Korean cultivated Kidney bean (*Phaseolus vulgaris* L.).*Journal of Agriculture and Food Chemistry*. **51**: 7040-7043.

Çiftci, A., Yilmaz, B., Köklü, S., Yüksel, O., Özsoy, M., Erden, G., Öztürk, G., Akbal, E., Ergül, B., Koçak, E. and Cesur, S. 2015. Serum levels of nitrate, nitrite and advanced oxidation protein products (AOPP) in patients with nonalcoholic fatty liver disease. *Acta Gastro-Enterologica Belgica*. Volume LXXVIII.

Daliri, E.B. and Lee, B.H. 2015. New perspective on probiotics in health and disease. *Food Science and Human Wellness*.**4**: 56-65.

Dangang, B.D.S., Zambou, N.F., Agrawal, R. and Fonteh, A.F. 2018. Effects of Lactococcuslactis sp. On nutritional valueof formulated non-dairy complementary food from Irish potato, mungbean, red kidney bean and papaya. *American Journal of Food and Nutrition*. **6**(1): 1-8.

Danka, P.O., Tsvetkova, D.D., Ivanov, K.V. 2012. Simultaneous identificationand determination of total content of amino acids in food supplements-tablets by gas chromatography. *Asian Journal of Pharmaceutical and Clinical Research*. **5**: 57-58.

David, O.M. and Famurewa, O. 2010. Prophylactic and Bio-therapeutic benefits of ogi: A lactic acid fermented food. *Bulletin of Biological Science***2**(9): 72-77.

De La Cruz, T.E. and Torres, J.M. 2013. Gelatine hydrolysis test protocol. American Society for Microbiology. Microbial Library. p. 1-9.

Dendy, D.A.V. 1995. Sorghum and Millets: Chemistry and Technology. Upton Oxford. United Kingdom. St. Paul MN, USA: American Association of Cereal Chemists.

Denkova, Z and Krastanov, A. 2012. Development of new products: Probiotics and probiotic foods. In: Intech.

Devadas, R.P. Chandersekhar, U. and Bhooma, N. 1984 Nutritional outcome of a rural diet supplemented on children studied from birth to pre-school age. *Industrial Journal Nutrition Diet***21**: 115-123.

Di Carlo, G., Autore, G. andIzzo, A.A. 1993. Inhibition of intestinalmotility and secretory by flavonoids in mice and rats: structure activity relationships. *Journal of Pharmacy and Pharmacology*. **45**:1054-9.

Doherty, S.S. 1981. Inhibition of arachinodic acid release, mechanism by which glucocorticoids inhibit endotoxin-induced diarrhoea. *British Journal of Pharmacology*.**73**:549 – 554.

DuPont, H.L. 2014. "Acute infectious diarrhoea in imunocompetent adults". *The New England Journal of Medicine*. **370**(16): 1532-40.

Ebrahimzadeh, M.A., Nanari, S.F. and Nabari, S.M. 2009. Antioxidant activities of methanol extract of *Sambucus ebulus* L. flower. *Parkistan Journal of Biological Sciences*. **12**(5): 447-450.

Egwim, E., Amanabo, M., Yahaya, A. and Bello, M. 2013. Mycotoxin and Food Safety in Developing Countries, chapter 7 Downloaded from: http://www.intechopen.com/books/ mycotoxin-and-food-safety-indeveloping-Countries. http://dx.doi.org/10.5772/52877.

Eka, E.B., Bene, W., Abbey, and Joyce. O.A. 2010. Nutritional Evaluation of Some Traditional Weaning Foods from Akwa Ibom State, Nigeria. *Nigerian Journal of Biochemistry and Molecular Biology Research Journal*.**25**(1): 65-72.

Eklou, A.S., Ines, M.S. and Francis, N. 2006. Comparative study of drying methods on the seed quality of interspecific NERICA rice varieties and their parents. *African Journal of Biotechnology*.**5**: 1618-1624.

Elmer, G.W. 2001. Probiotics: 'Living Drugs'. *American Society of Health-System Pharmacists*. **58**(12): 1101-1109.

Evans, C.E., Omoaruemike, E.O. and Mohammed, A.V. 2013. Effect of fermentation variables on nutritional and organoleptic scores ofKunu-Zaki produced from rice and achablends. *Advance Journal of Food Science and Technology*. **5**(9): 1117-1119.

Fahey, J.W. 2005. *Moringaoleifera*: A review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Part 1.*Tree for Life Journal*.**1**:5.

Food and Agriculture Organization FAO/WHO. 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Cordoba, Argentina, 1-4 October 2001. ftp:// ftp.fao.org/ es/ esn/ food/ probio/ en.pdf.

Fischer, W., Puls, J., Buhrdorf, R. and Gebert, B. 2001. Systematic mutagenesis of the Helicobacter pylori Cag pathogenicity island: Essential genes for CagA translocation in host cells and induction of interleukins-8. *Molecular Microbiology.***42**(5): 1337-1348.

Food and Agriculture Organization of the United Nations (FAO)/WorldHealth Organisation (WHO), 2002. Guidelines for the evaluation of probiotics in food. Food and AgricultureOrganization of the United Nations and World Health Organization working group report. London. Ontario, Canada. P. 1-11.

Freidman, M. 1996. Nutritional value of proteins from different food resources. A review. *Journal of Agriculture and Food Chemistry*. **44**: 6-29.

Fuglie, L.J. 2000. New Uses of Moringa Studied in Nicaragua. ECHO Development Notes #68.

Galadima, M. and Okolo, O., 2014. Bacterial Agents of Diarrhoea in children aged 0 to 5 years in Minna, Niger State, Nigeria. *International Journal of Current Microbiology and Applied Sciences*. ISSN: 2319-7706. **3**(6): 1048-1054 http://www.ijcmas.com.

Garcia, E.F., Luciano, W.A., Xavier, D.E., da Costa, W.C., de Sousa, O.K. and Franco, O.L. 2016. Identification of lactic acid bacteria in fruit pulp processing byproducts and potential probiotic properties of selected *Lactobacillus* strains. *Frontier In. Microbiology*. **7**:1371. doi: 10.3389/fmicb.2016.01371.

Gassenschmidt, U., Jany, K.D., Tauscher, B. and Neibergall, H. 1995. Isolation and characterization of a flocculating protein from *Moringa oleifera* Lam. *Biochimica Biophysica Acta*.**1243**: 4777-481.

Gerhardt, P., Murray, R.G., Costilow, R.N., Nester, E.W., Wood, W.A., Krieg,N.R. and Phillips, G.B. 1981. Manual of methods for general bacteriology. American Society for Microbiology, Washington, DC 20006.

Gernah, D.I., Ariahu, C.C. and Ingbian, E.K. 2011. Effects of malting and lactic acid fermentation on some chemical and functional properties of maize (*Zea mays*). *American Journal of Food Technology*. **6**(5): 404-412.

Giri, S.S., Sen, S.S., Saha, S., Sukumaran, V. and Park S.C. 2018. Use of a potential probiotic *Lactobacillus plantarum* L7, for the preparation of a rice-based fermented beverage. *Frontiers in Microbiology*. **9**: 473.

Gornall, A.G., Bardawill, C.J. and David, M.M. 1949. Determination of serum proteins by means of the reaction. *Journal of Biology and Chemistry*. **177**(2): 751-766.

Goth, L. 1991. A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chemica Acta*. **15**(2-3): 143-151.

Gupta, H. and Malik, R.K. 2007. Incidence of virulence in bacteriocin – producing enterococcal isolates. *Lait*. **87**:587 – 601.

Gyamfi, E.T., Kwarteng, I.K., Ansah, M.O., Anim, A.K., Ackah, M., Lebene, Kpattah and Nash, O. 2011. *Bentil International Academy of Ecology and Environmental Sciences***1**(3-4): 179-187.

Har, L. W. and Ismail, I. S. 2012. Antioxidant activity, total phenolics and total flavonoids of Syzygiumpolyanthum (Wight) Walp leaves. *International Journal of Medicinal Aromatic Plants*. **2**(2): 219-228.

Hardman, J.G. and Limberd, L.E. 1992. The pharmacological basis of therapeutics in: Goodman and Gilman's (Eds). 10th edition. MacGraw Hill, New York. 914-931.

Hardy, H., Harris, J., Lyon, E., Beal, J. and Foey, A.D. 2013. Probiotics, prebiotics and immunomodulation of gut mucosal defense: Homeostasis and immunopathology. *Nutrients*. **5**(6): 1869-1912.

Harrigan, W.F. and McCance, M.E. 1976. Laboratory methods in food and dairy microbiology. London: Academic Press p. 452-464.

Hasegawa, T., Minami, M., Okamoto, A., Tatsuno, I., Isaka, M. and Ohta,M. 2010. Characterization of a virulence-associated and cell wall located DNase of *Streptococcuspyogens*. *Microbiology*. **186**: 184-190.

Hawaz, E. 2014. Isolation of probiotic lactic acid bacteria from curd and in vitro evaluation of its growth inhibition against pathogenic bacteria. *African Journal of Microbiology Research*. **8**(13): 1419-1425.

Hempel, S., Newberry, S.J., Maher, A.R., Wang, Z., Miles, J.N., Shanman, R., Johnsen, B. and Shekelle, P.G., 2012. Probiotics for the prevention and treatment

of antibiotic-associated diarrhea: a systematic review and meta-analysis. JAMA: *The Journal of the American Medical Association*. **307**(18): 1959–69. doi:10.1001/jama.2012.3507. PMID 22570464.

Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen,S., Calder, P.C and Sanders, M.E. 2014. "Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic". *Nature Reviews. Gastroenterology and Hepatology*. **11**(8): 506–14. doi:10.1038/nrgastro. 2014.66. PMID 24912386.

Holowacz, S., Blondeau, C., Guinobert, I., Guilbot, A., Hidalgo-Lucas, S. and Bisson, J. 2016. Antidiarheal and antinociceptive effects of a probiotic mixture in rats. *Journal of Probiotic Health*. **4**: 3.

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

Hur, S.J., Lee, S.Y., Kim, Y.C., Choi, I. and Kim, G.B. 2014. Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry*.**160**: 346–356.

Ibrahim, D., Puspitaloka, H., Rahim, R. A. and Hong, L. S. 2012. Characterization of Solid State Fermentation Culture Conditions for Growth and Mananase Production by *Aspergillus niger* USM F4 on Rice Husk in Tray System. *British Biotechnology Journal*.**2**(3): 133-145.

Ikujenlola, A.V. 2014. Chemical and functional properties of complementary food blends from malted and unmalted acha (*Digitaria exilis*), soybean (*Glycine max*) and defatted sesame (*Sesamum indicum* L.) flours. *African Journal of Food Science*. **8**(7): 361-367.

Ikujenlola, A.V and Adurotoye, E.A. 2014. Evaluation of quality characteristics of high nutrient dense complementary food from mixtures of malted qualityprotein maize (*Zea mays* L.) and steamed cowpea (*Vigna unguiculata*).*Journal of Food Processing and Technology*. **5**: 291.

Ikujenlola, A.V and Fashakin, J.B. 2005, "The physico-chemical properties of a complementary diet prepared from vegetable proteins".*Journal of Food, Agriculture and Environment*. **3**(3-4): 232-6.

Inceoglu, O., Hoogwout, E. F., Hill, P. and van Elsas, J. D. 2010. Effect of DNA extraction method on the apparent microbial diversity of soil. *Applied Environmental Microbiology*. **76**: 3378-3382.

International Development Research Centre (IDRC), 2016. IDRC - International Development Research Centre. Archived from the original on 9 June 2016.

Inyang, U.E., Umoh, H.I. and Ukwo, S.P. 2019. Effect of Co-fermentation duration on the nutritional composition and anti-nutritional contents of sorghum-cowpea flours and sensory properties of their gruels. *Journal of Environmental Science, Toxicology and Food Technology*. **13**(2): 56-64.

James, S., Oloyede, O.O., Ocheme, O.B., Chinma, C.E. and Agbejule, A.Y. 2015. Proximate antinutrients and sensory properties of 'ogi', a millet based gruel supplemented with treated African oil bean (*Pentaclethramacrophylla* Benth) seed flour. *African Journal of Food Science*. **9**(3): 136-141.

Jan, A., Rafi, M., Mustafa, S., Rasmussen, Z. A., Thobani, S. and Badruddin, S.H. 1997. Evaluation of dwodo (wheat-milk gruel) in children with acute diarrhea. *Journal of Pakistan Medical Association*.**47**(1): 12-16.

Jed, W.F. 2005. *Moringa oleifera*: review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Part1. *Trees for life*.**1**(5).

Jindal, A. 2004. Weaning food. www. healthy baccha.com.

Jirapa, P., Normah, H., Zamaliah, M.M., Asmah, R. and Mohamad, K. 2001. Nutritional quality of germinated cowpea flour (*Vigna unguiculata*) and its application in home prepared powdered weaning foods. *Plant Food Human Nutrition*.**56**(3): 203-216.

Johnansson, M.E.V., Henrik S. and Gunnar, C. H. 2013. The gastrointestinal mucus system in health and disease. HSS public Access Author's Manuscript. PMCID: PMC3758667 NIHMSID: NIHMS495248.

Junior, W.L.G.A., Ferrari, I.S., de Souza, A.Y., Barbosa, A.Y., da costa, M.M., Menezes, D.R. and Dias, F.S. 2015. Principal criteria for selection of lactic acid bacteria for potential use as probiotics in foods. *African Journal of Microbiology Research*. **9**(10): 671-686.

Kailasapathy, K. 2013. Commercial sources of probiotic strains and their validated and potentialhealth benefits. A review. *International Journal ofFermented Foods*. **2**(1): 1-17.

Kalhoro, S.M., Nguyen, L.T. and Anal, A.K. 2019. Evaluation of probiotic potential of the lactic acid bacteria (LAB) isolated from raw buffalo (*Bubalus bubalis*) milk. *Pakistan Veterinary Journal*. 2074-7764.

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

Khetarpaul, N. and Chauhan, B.M. 1989. Effect of fermentation on protein, fat, minerals and thiamine content of pearl millet. *Plant Food Human Nutrition*. **39**:169-177.

Kids Health from Nemours, 2014. Weaning your child. Retrieved June 27, 2016, from http://kidshealth.org/en/parents/weaning.html.

Kim, E.K., Jang, W.H., Ko, J.H., Kang, J.S., Noh, M.J. and Yoo, O.J. 2001. Lipase and its modulator from Pseudomonas sp. strain KFCC10818: Proline-to-glutamine substitution at position 112 induces formation of enzymatically active lipase in the absence of the modulator. *Journal of Bacteriology*. **183**: 5937-5941.

Kjeldahl, J. 1883. "Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern" (New method for the determination of nitrogen in organic substances).*Zeitschrift für analytische Chemie*.**22**(1): 366-383.

Kumar, P., Yadava, R.K., Gollen, B., Kumar, S., Verma, R.K. and Yadav, S. 2011. Nutritional contents and medicinal properties of wheat. *International Journal of Life Science and Medical Research*. **22**:1-10.

Larsen, H.N. and Snieszko, S.F. 1961. Comparison of various methods of determination of haemoglobin in trout blood. *The Progressive Fish-Culturist*.**23**(1):8-17.

Lawrence, R., 1999. Breastfeeding: A Guide for theMedical Profession. 5th edn. Vol. 335. St Louis: Mosby. 338pp. 343–344.

Lee, P.Y., Costumbrado, J., Hsu, C., and Kim, Y.H. (2012). Agarose Gel Electrophoresis for the separation of DNA fregments. *Journal of Visualized Experiments*, (62).

Livingstone, S.A., Feng, J.J. and Malleshi, G.N. 1993. Development and nutritional quality evaluation of weaning foods based on malted popped and roller-dreid wheat and chickpea. *International Journal of Food Science and Technology*.

Lohia, N. and Udipi, S.A. 2015. Use of fermentation and malting for development of ready-to-use complementary food mixes. *International Journal of Food and Nutritional Sciences*. **4**(1). ISSN 2320-7876.

Lucas, E.W. 1979. Food uses of peanut protein. *Journal of American Oil Chemistry Society*.**56**: 425-430.

Mahmoudi, R., Zare, P., Hassanzadeh, P. and Nosratpour, S. 2012. Effect of Teucrium polium Essential Oil on the Physico-chemical and Sensory Properties of Probiotic Yoghurt.*Journal of Food Processing and Preservation*. **38**(3).

Mahnaz, K.I, Che, W.J., Khyrunnisa, B. and Iman, Y. 2012. Screening of antibacterial activity of lactic acid bacteria isolated from fermented vegetables against food pathogens. *Archives Des Science*. **65**(6).

Malleshi, N.G., Daodu, M.A. and Chandrasekhar, A. 1989. Development of weaning food formulations based on malting and roller drying of sorghum and cowpea. *International Journal of Food Science and Technology*.**24**: 511-519.

Manini, F., Casiraghi, M. C., Poutanen, K., Brasca, M., Erba, D., andPlumed-Ferrer, C. 2016. Characterization of lactic acid bacteria isolated from wheat bran sourdough. *LWT-Food Science and Technology*. **66:** 275–283. doi:10.1016/j.lwt.2015.10.045.

Marcó, M.L., Heeney, D., Binda, S., Cifelli, C.J., Cotter, P.D. and Foligne, B. 2017. Health benefits of fermented foods: Microbiota and beyond. *Current Opinion in Biotechnology*. **44:** 94-102.

Marero, L.M., Payumo, E.M., Aguinaldo, A.R., Matsumoto, I. and Homma, S. 1991. The antinutritional factors in weaning foods prepared from germinated legumes and cereals. *Lebensmittelwissenschaft Technol*.**24**: 177-81.

Mastorci, F., Vasalle, C., Chatzianagnostou, K., Marabotti, C., Siddiqui, K., Eba, A.O., Sidi Mhamed, S.A., Bandopadhyay, A., Nazzaro, M.S., Passera, M. and Pingitore, A. 2017. Undernutrition and overnutrition burden for diseases in developing countries: The role of oxidative stress biomarkers to assess disease risk and interventional strategies. *Antioxidants*. **6**: 41.

Mathara, J.M., Schillinger, U., Guigas, C., Franz, C., Kutima, P.M., Mbugua, S.K., Shin, H.K. and Holzapfel, W.H. 2008. Functional characteristics of *Lactobacillus* spp. from fermented milk product in Kenya. *International Journal of Food Microbiology*. **126**:57-64.

McChung L. S, and Toabe R. 1947. The egg yolk plate reaction for the presumptive diagnosis of *Clostridiumsporogenes* and certain species of the gangrene and botulinum groups. *Journal of Bacteriology*. **53**:139-47.

Mesele, A. 2018. A review on food fermentation and the biotechnology of lactic acid bacteria. *World Journal of Food Science and Technology*. **2**(1): 19-24.

Michael, Y. 2008. Infectious Diarrhoea http://www.columbia.edu/itc/hs/medica l/pathophys/id/2008/diarrhoea.

Minka, S.R.and Bruneteau, M. 2000. Partial chemical composition of Bambara pea (*Vigna subterranean* (L) *Verde*). *Food Chemistry*.**68**: 273-376.

Mishra, G., Singh, P., Verma, R., Kumar, S., Srivastav, S., Jha, K., and Khosa, R. 2011. Traditional uses, phytochemistry and pharmacological properties of Moringa oleifera plant: An overview. *Scholars Research Library Der Pharmacia Lettre*.**3**(2): 141-164.

Mishra, V. and Prasad, D. N. 2005. Application of in vitro methods for selection of Lactobacillus casei strains as potential probiotics. *International Journal of Food Microbiology*.**103**(1): 109–115.

Msheliza, E.A., Hussein, J.B., Ilesanmi, J. and Nkama, I. 2018. Effect of fementation and roasting on the physiological properties of weaning food produced from blends of sorghum and soybean. *Journal of Nutrition Food and Science*.**8**(2): 681.

Naganuma, A., Anderson, M.E. and Meister, A. 1990. Cellular glutathione as a determinant of sensitivity to mercuric chloride toxicity: Prevention of toxicity by giving glutathione monoester. *Biochemical Pharmacology*. **40**:693-697.

National Institute of Child Health and Human Development (NICHHD). 2017. (US Department of Health and Human Services) "Breastfeeding and Breast Milk: Condition Information". 19 December 2013. Archived from the original on 27 July 2015. Retrieved 27 July 2015.

Nazarni, R., Purnama, D., Umar, S. and Eni, H. 2016. The effect of fermentation on total phenolic, flavoinoid and tannin content and its relation to antibacterial activity in jaruk tigarun (Crataeve nurvala, Buch HAM). *International Food Research Journal*.**23**(1): 309-315.

Nordqvist, C. 2017. Blood: everything you need to know. Medical News Today. Medilexicon International.

Nwabueze, T.N., James, S., Anuonye, J.C. and Kachiro, K. 2018. Chemical composition of millet-based infant formula supplemented with treated Bambara nut flour. *Arid Journal of Engineering, Technology and Environment*. **14**(4): 37-40.

Nwagu, T. and Amadi, O.C. 2013. Amylolytic, lipolytic and proteolytic activity of Kocuria varians isolated from fermented African oil bean seed (*Pentaclethra macrophylla*). *Scientific Journal of Microbiology*. (3): 65-73.

Odunfa, S.A. 1985 African fermented foods. In Microbiology of Fermented Foods; Wood, B. J. B., Ed.; Elsevier Applied Science Publishers, London. Vol. 2: 155-191.

Oelkel, E.A., Oplinger, E.S., Putnam, D.H., Duurgan, B.R., Doll, J.D., and Undersander, D.J. 1990. Millets. In Alternative Field Crops Manual.of finger millet (*Eleusine coracana*).*Food Chemistry*.**58**(4): 345-350.

Ojha, P., Adhikari, R., Karki, R., Mishra, A., Subedi, U. and Karki T.B. 2017. Malting and fermentation effects on antinutritional components and functional characteristics of sorghum flour. *Food Science and Nutrition*. **6**:47-53.

Ojimelukwe, P.C. 2009. Sourcing and processing of legumes. In: Nigerian agro raw materials development. Volume 1: Some Industrial Crops and Salient Issues (P. A. Onwualu, S. C. Obasi and U. J. Ukpabi editors). Raw Materials Research and Development Council, Abuja.

Ojokoh, A.O., Fayemi, O.E., Ocolo, F.C.K. and Nwokolo F.I. 2015. Effect of fermentation on proimate composition, physico-chemical and microbial characteristics of pearl millet (*Pennisetum glaucum* (L.) R.Br.) and acha*Digitaria exilis* (Kippist) Stapf) flour blends. *Journal of Agricultural Biotechnology and Sustainable Development*. **7**(1): 1-8.

Okaka, J.C. and Porter, N.N. 1979. Physico-chemical and functional properties of cowpea flour processed to reduce beany flavors. *Journal of Food Science*. **44**:1233-1240.

Okoruwa, A.E. 1999. Nutritional value and uses of food legumes in Africa. Paper presented at the legume breeding workshop International Institute of Tropical Agriculture, Ibadan, Nigeria, 27 September- 8 October, 1999.

Olutiola, P. O., Famurewa, O. and Sonntag, H. G. 1991. An introduction to general Microbiology; a practical approach. Hygiene-Institut Dder Universitat Heidelberg, Germany.

Oluwajoba, S.O., Akinyosoye, F.A. and Oyetayo, O.V. 2013. In vitro screening and selection of probiotic lactic acid bacteria isolated from spontaneously fermenting kunu-zaki. *Advances in Microbiology*. **3**:309-316.

Onuoha, E.C., Orukptan, A.A. and Ameh, J.B. 2017. Effect of fermentation (natural and starter) on the physico-chemical, antinutritional and proximate composition of pearl millet used for flour production. *American Journal of Bioscience and Bioengineering*. **5**(1): 12-16.

Ortu, S., Felis, G. E.,Marzotto, M., Deriu, A., Molicotti, P.,Sechi, L. A., Dellaglio, F. and Zanetti, S. 2007. Identification and functionalcharacterization of Lactobacillus strains isolated from milk and Gioddu, a traditional Sardinian fermented milk.*International Dairy Journal*.**17**: 1312-1320.

Ory, R.L. and Conkerton, E.J. 1983. Supplementation of bakery items with high protein peanut flour. *Journal of American Oil Chemistry Society*.**60**: 986-989.

Osborne, D.P. and Voogt P. 1978. Calculation of calorific value. In: The Analysis of nutrients in foods. New York Academic Press.

Ouwehand, A. C., Kirjavainen, P. V., Shortt, C. and Salminen, S. 1999. Probiotics: mechanisms and established effects. *International Dairy Journal*.**9**: 43-52.

Oyagbemi, A.A.and Odetola, A.A.2010. Hepatoprotective effects of ethanolicextract of *Cnidoscolus aconitifolius* on paracetamol-induced hepatic damage in rats. *Pakistan Journal of Biological Sciences*. **13**(4): 164-169.

Oyarekua, M.A. 2011. Evaluation of the nutritional and microbiological status of co-fermented cereals/cowpea 'ogi'. *Agriculture and Biology Journal of North America*. **2**(1): 61-73.

Oyewole, O. A. and Isah, P. 2012. Locally Fermented Foods in Nigeria and their Significance to National Economy: A Review.*Journal of Recent Advances in Agriculture*.**1**(4): 92-102.

Pancholy, S.K. Despande, A.S. and Krall, S. 1978. Amino acids, oil and protein content of some selected peanut cultivars. *Process America Peanut Research & Education Association*.**10**: 30-37.

Paraschiv, D., Vasile, A. and Constantin. 2011. Study of physiological properties of some probiotics in multiple cultures with mesophilic lactic acid bacteria by flora Danica Ch. Hansen commercial starter. *Food Technology*. **35**(2): 56-65.

Patel, A., Lindström, C., Patel A., Prajapati J. and Holst, O. 2012. Probiotic properties of exopolysaccharide producing lactic acid bacteria isolated from vegeTables and traditional Indian fermented foods. *International Journal of Fermented Foods*. **1**(1): 87-101.

Podgorskji, V.S. 2002. Functional food product based on lactic acid bacteria. 1st International congress "Biotechnology- state and prospect of development. p 338.

Prescott, L.M., Harley, J.P. and Klain, D.A. 2002. Microbiology of fermentedfoods. Microbiology 5th edition. McGraw Hill Inc. New York.

Price, M.L 1985. The Moringa Tree. ECHO Technical Note. Educational Concerns for Hunger Organization, N. Ft. Meyers, FL. http://ww.echotech.org/technical/ technotes/moringabiomass.pdf.

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

Puphan, K., Somplang, P., Uriyapongson, S. and Navanukraw C. 2015. Screening of lactic acid bacteria as potential probiotics in beef cattle. *Pakistan Journal of Nutrition*. **14**(8): 474-479.

Qing, L., Xiaoli, L., Mingsheng, D., Jiannzhong, Z. and Ying, W. 2015. Adhesion and probiotic properties of Lactobacillus plantarum isolated from Chinese traditional fermented soybean paste. *Global Advanced Research Journal of Food Science and Technology*. **4**(1):001-009.

Ramachandran, S., Patel, A. K., Nampoothiri, K. M., Francis, F.,Nagy, V., Szackacs, G., Pandey, A. 2004. Coconut oil cake-a potential material for production of a-amylase. *Bioresource Technology*.**93**(2): 169-174.

Rao, T. M., Rao, B G, Rao, Y. V. 2012. Antioxidant activity of *Spilanthes acmella* extracts. *International Journal of Phytopharmacology*. **3**(2): 216-220.

Rasic, J. L. 2003. Microflora of the intestine probiotics. In: Caballero, B., Trugo, L., Finglas, P. editors. Encyclopedia of Food Science and Nutrition. Oxford Academic Press.

Rattanachaikunsopon, P, and Phumkhachorn, P. 2010. Lactic acid bacteria: their antimicrobial compounds and their uses in food production. *Annals of Biological Research*.**1**(4): 218-228.

Reber, E.F., Eboh L., Aladeselu A., Brown W.A. and Marshall D.D. 1983. Development of high-protein low-cost Nigerian Foods. *Journal of Food Science*.**48**: 217-219.

Reitman, S. and Frankel, S. 1957. Colorimetric method for determination of serum transaminases. *American Journal of Clinical Pathology*. **28**: 56-61.

Renuka, S.S., Shevali, K., Anjana, K.N. and Navneet, A. 2015. Aggravation of oxidative stress by ethanolic extract of Ocimum gratissimum in DMH induced

colon injury. *International Journal of Toxicological and Pharmacological Research*.**7**(6): 280-285.

Rosenberg, M., Gutnick, D. and Rosenberg, E. 1980. Adherence of bacteria to hydrocarbons: A simple method for measuring cell-surface hydrophobicity. *FEMS Microbiology Letters*.**9**: 29-33.

Saarela, M. G., Mogensen, R., Fonden, J., Mättö, N. and Mattila-Sandholm, T. 2000. Probiotic bacteria: safety, functional and technological properties. *Journal of Biotechnology*.**84**: 197-215.

Sanders, K.M. 1984. Evidence that prostaglandins are local regulatory agents in Canine ilea circular Muscles. *American Journal of Physiology*. **246**(4 Partt 1):G361-371.

Sanni, A.I. and Adesulu, A.T. 2013. Microbiological and physico-chemical changes during fermentation of maize for masa production: *African Journal for Microbiology Resources*. **7**(34): 4355-4362.

Sanoussi, A.F., Dansi, A., Bokossa-yaou, I., Dansi, M., Egounlety, M., Sanni, L.O. and Sanni, A. 2013. Formulation and biochemical characterization of sweet potato (Ipomoeabatatas) based infant flours fortified with soybean and sorghum flours.*International Journal of Current Microbiology and Applied Science.***2**(7): 22-34.

Sarwar, M.H, Sarwar, M.F, Sarwar, M, Qadri, N.A and Moghal, S. 2013. The importance of cereals (Poaceae: Gramineae) nutrition in human health: A review. *Journal of Cereals and Oilseeds*. **4**(3):32-35.

Schleifer, K. and Ludwig, W. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiology Reviews*.**59**: 143-169.

Sengev, A.I., Ariahu, C.C. and Gernah, D.I. 2016. Effects of natural fermentation on the vitamins, amino acids and protein quality indices of sorghum-based complementary foods. *American Journal of Food and Nutrition*. **6**(3): 91-100.

Settanni, L. and Moschetti, G. 2010. Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. *Food Microbiology*. **27**(6): 691-697.

Sharaf, E.F., El-sayed, W.S. and Abosaif, R.M. 2014. Lecithinase-producing bacteria in commercial and home-made foods: Evaluation of toxic properties and identification of potent producers. *Journal of Taibah University for Science*. 03.006.

Sharma, N., Handa, S. and Gupta, A. 2013. A comprehensive study of different traditional fermented food/beverages of himachal Pradesh to evaluate their nutrition impact on health and rich biodiversity of fermenting microorganism. *International Journal of Research in Applied, Natural and Social Sciences*. **3**: 19-28.

Sharma, K. D., Vinod, K. G., Surendra, K., Vivek, J., Ravi, S. K., Mandal, A. G., Bhanu, P., Mamta, S. 2015. Evaluation of Antidiarrheal Activity of Ethanolic Extract of Holarrhena antidysenterica Seeds in Rats. *Veterinary World*. **812:** 1392–1395.

Shiriki, D., Igyor, M.A. and Gernah, D.I. 2015. Nutritional evaluation of complementary food formulations from maize, soybean and peanut fortified with *Moringa oleifera* leaf powder. *Food and Nutrition Sciences*. **6**: 494-500.

Singh, A., Kumar, M., Ghosh, M. and Gangul, A. 2014. Traditional foods and beverages as delivery vehicles for probiotics in: Biotechnology. Volume 8: Novel Drug Delivery B. Singh and Katare O.P. eds. Studium Press LLC ISBN: 9781626990234.

Smid, E.J. and Hegenholtz, J. 2010. Functional genomics of food fermentation processes. *Annual Review in Science and Technology*. **1**: 497-519.

Sobrun, Y., Bhaw-Luximon, A.,Jhurry, D. and Puchooa, D. 2012. Isolation of lactic acid bacteria from sugar cane and production of lactic acid from selected improved strains. *Advances inBioscience and Biotechnology*.**3**: 398-407.

Soro-Yao, A.A., KouakouBrou K., Amani G., Thonart P. and Dje K.M. 2014. The use of Lactic Acid Bacteria Starter Cultures during the processing of fermented cereal-based foods in West Africa: A review. *Tropical Life Sciences Research*. **25**(2): 81-100.

Stanton, C., Desmond, C., Coakley, M., Collins, J.K., Fitzgerald, G. and Ross, R.P. 2003. Challenges facing development of probiotic-containing functional foods. In Handbook of fermented functional foods.Ed. Farnworth, E.R. Florida, CRC press, pp: 27-58.

Syal, P. and Vohra, A. 2014. Probiotic attributes of a yeast-like fungus, *Geotrichum klebahni*. *African Journal of Microbiology Research*. **8**(20): 2017-2043.

Tambekar, D. H. and Bhutada, S. A. 2010. Studies on antimicrobial activity and characteristics of bacteriocins produced by Lactobacillus strains isolated from milk of domestic animals. *The Internet Journal of Microbiology.***8**:1-6.

Tamura, K., Nei, M. and Kumar, S.2004. Prospects for inferring very large phylogenies by using the neighbor-joining method.*Proceedings of the National Academy of Sciences (USA)*.**101**:11030-11035.

Thakkar, P., Modi, H.A. and Prajapati, J.B. 2015. Isolation, characterization and safety assessment of lactic acid bacterial isolates from fermented food products. *International Journal of Current Microbiology and Applied Science*. **4**(4): 713-725.

Thathola, A. and Srivastava, S. 2002. Physichochemical properties and nutritional traits of millet-based weaning food suitable for infants of the Kumoan hills, Northern India. Asia *Pakistan Journal of Clinical Nutrition*.**11**(1): 28-32.

Thorat, P.P., Sadawarte, S.K., Sawate, A.R. and Machewad, G.M. 2017. Studies on effect of fermentation on physico-chemical properties of vegetables and preparation of sauce. *International Journal of Current Microbiology and Applied Science*. **6**(8): 3537-3545.

Ugwuona, F.U., Awogbenja M.D and Ogara J.I., 2012. Quality evaluation of soy-acha mix for infant feeding. *Indian Journal of Science Research*. **3**(1):43-50.

UNICEF 2009. Trading progress on child and maternal nutrition: a survival and development priority. United Nations Children's Fund, World Health Organization, The World Bank, Joint child malnutrition estimates. New York: UNICEF; Geneva: WHO; Washington, DC.

Usman, P. and Hosono, A. 1999. Bile tolerance, taurocholate deconjugation and binding of cholesterol by *Lactobacillus gasseri strains*. *Journal of Dairy Science*.**82**: 956-961.

Valle, N.J., Santos, I, Gigante, D.P, Goncalves, H, Martines, J. and Pelto, G.H. 2003 Household trials with very small samples predict responses to nutrition counseling intervention. *Food Nutrition Bulletin*.**24**(4): 343-349.

Ventura, M., Casas, I.A., Morelli, L. and Callegari, M.L. 2000. Rapid amplified ribosomal DNA restriction analysis (ARDRA) identification of *Lactobacillus* spp. isolated from faecal and vaginal samples. *Systematic and Applied Microbiology*.**23**: 504–509.

Vos, P., Garrity, G., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., Schleifer, K. and Whiteman, W. B. 2009. Bergey's manual of systematic bacteriology Second edition. Vol 3. *The firmicutes*.

Vieira-Dalode, G., Madode Y.E., Hounhouigan J., Jespersen L. and Jakobsen M. 2008. Use of Starter Cultures of Lactic Acid Bacteria and Yeasts as Inoculum Enrichment for the Producition of Gowe, a Sour Beverage from Benin. African.*Journal of Microbiology Research*. **2**: 179-186.

Wakil, S.Mand Onilude, A.A. 2010. Monitoring the effect of fortification on bacterial population dynamics in malted and fermented maize-based weaning foods using PCR-DGGE. *Journal of Applied Biosciences* **26**: 1997-5902.

Wakil, S.M. and Kazeem, M.O. 2012.Quality assessment of weaning food produced from fermented cereal-legume blends using starters. *International Journal of Food Research*. **19**(4): 1679-1685.

Wakil, S.M. and Oriola, O.B. 2012. Qualitative assessment of starter-produced weaning food subejected to different temperature and pH. *AfricanJournal of Food Science*. 6(5): 147-154.

Wakil, S.M. and Alao, O.K. 2013. Microbiological and nutritional qualities of *Moringa oleifera* supplemented and fermented weaning blends.*Journal of Science Research*. **12**: 73-85.

Wakil, S.M.and Alagbe, C.E. 2017. Effect of processing methods on microbiologicaland nutritional qualities of tigernut-fortified weaning food. *Advances in Food Science*. **39**, 48-56.

Wakil, S.M. and Ola, J.O. 2018. Development of maize-tigernut fortified weaning food using starter cultures. *Food and Nutrition Science*. **9**: 1444-1457.

Wakil, S.M. and Olorode, E.M. 2018. Potential probiotic properties of lactic acid bacteria isolated from malted and spontaneously fermented acha (*Digitaria exilis*) flour. *Advances in Research*. **17**(6): 1-12.

Wang, Y., Wu, Y., Wang, Y., Xu, H., Mei, X., Yu, D., Wang, Y. and Li, W. 2017. Antioxidant properties of probiotic bacteria. *Nutrients*. **9**: 521.

William, C.F and Dennis, C.W. 2011. Food Microbiology. Fourth edition. McGraw Hill India. p.330.

World Health Organization. 2009. Diarrhoeal disease. Available at: http://www.who.int/mediacentre/factsheets/fs330/en/index.html. [Accessed 30 April 2011].

World Health Organization, 2012. Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life. Geneva.

World Health Organization (WHO), 2013. Diarrhoea: why children are still dying and what can be done. Archived (PDF) from original on 19 October 2013. Retrieved 12 October 2013.

World Health Organization (WHO), 2013. Diarrhoeal disease Fact sheet No330. Archived from the original on 17 July 2014. Retrieved 9 July 2014.

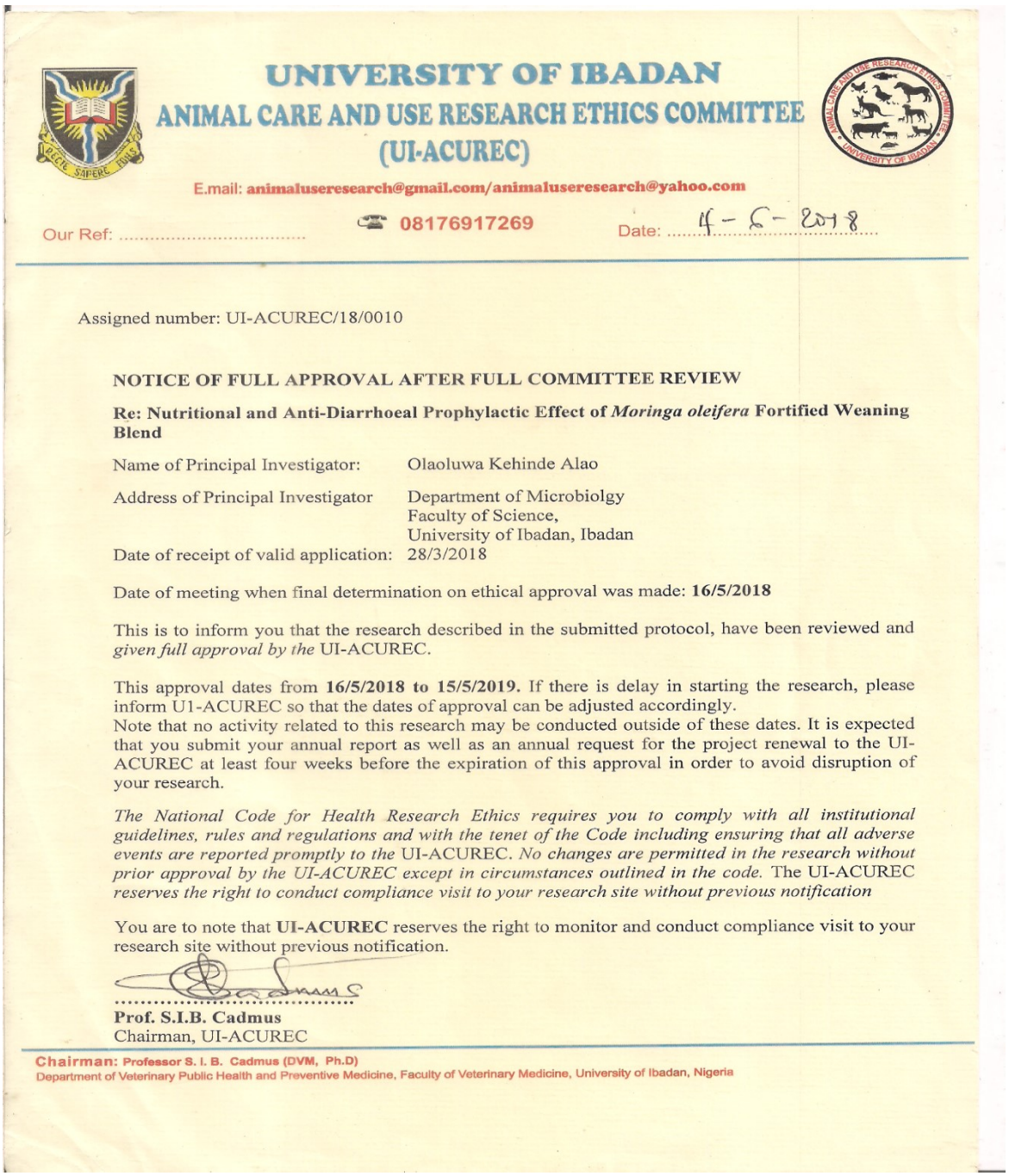
World Health Organization (WHO). 2014. Micronutrient Deficiencies, http://www.who.int/nutrition/topics/ida/ en/index. html.

Yelnetty A., Purnomo, H., Purwadi, A. and Mirah, A., 2014. Biochemical characteristics of lactic Acid Bacteria with proteolytic Activity and Capability as Starter Culture Isolated from Spontaneous Fermented Local Goat Milk. *Journal of Natural Science Research***4**(10):137-146.

Zadak, Z., Hyšpler, A., Tichá, A., Hronek, M., Fikrová, P., Rathouská, J.,Hrnčiariková, D. and Štětina, R. 2009.Antioxidant and vitamins in clinical conditions. *PhysiologicalResearch*. **58**(1): S13-S17.

APPENDICES

Appendix 1: Ethical approval



UNIVERSITY OF IBADAN
ANIMAL CARE AND USE RESEARCH ETHICS COMMITTEE
(UI-ACUREC)

E.mail: animaluseresearch@gmail.com/animaluseresearch@yahoo.com

Our Ref: ..... 08176917269 Date: 4-6-2018

Assigned number: UI-ACUREC/18/0010

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Nutritional and Anti-Diarrhoeal Prophylactic Effect of *Moringa oleifera* Fortified Weaning Blend

Name of Principal Investigator: Olaoluwa Kehinde Alao

Address of Principal Investigator: Department of Microbiolgy, Faculty of Science, University of Ibadan, Ibadan

Date of receipt of valid application: 28/3/2018

Date of meeting when final determination on ethical approval was made: **16/5/2018**

This is to inform you that the research described in the submitted protocol, have been reviewed and *given full approval by the* UI-ACUREC.

This approval dates from **16/5/2018 to 15/5/2019.** If there is delay in starting the research, please inform U1-ACUREC so that the dates of approval can be adjusted accordingly.
Note that no activity related to this research may be conducted outside of these dates. It is expected that you submit your annual report as well as an annual request for the project renewal to the UI-ACUREC at least four weeks before the expiration of this approval in order to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenet of the Code including ensuring that all adverse events are reported promptly to the UI-ACUREC. *No changes are permitted in the research without prior approval by the UI-ACUREC except in circumstances outlined in the code.* The UI-ACUREC *reserves the right to conduct compliance visit to your research site without previous notification*

You are to note that **UI-ACUREC** reserves the right to monitor and conduct compliance visit to your research site without previous notification.

.....
Prof. S.I.B. Cadmus
Chairman, UI-ACUREC

Chairman: Professor S. I. B. Cadmus (DVM, Ph.D)
Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Appendix 2: Sensory Assessment Questionnaire

Department of Microbiology,

University of Ibadan.

Sensory Assessment Questionnaire.

Product: Formulated weaning blends supplemented with *Moringaoleifera*

Instructions: - You are provided with four different samples of formulated weaning blends in plastic transparent cups. Please kindly evaluate them in terms of colour, texture, taste, flavour and overall acceptability using a 7-point hedonic scale as shown below:

Like extremely - 7

Like moderately - 6

Like slightly - 5

Indifferent - 4

Dislike slightly - 3

Dislike moderately - 2

Dislike extremely - 1

-You are also expected to rinse your mouth with the provided portable water between each sample.

Product	Colour	Texture	Taste	Flavour	Overall acceptability
F4 (Control)					
FB1					

Comments:

……..

Thank you for your cooperation.

Olaoluwa Kehinde, ALAO.

Appendix 3: Nucleotide Sequences of isolated Bacteria

Lactobacillus plantarum strain 133 1 16S ribosomal RNA gene sequence

CCTCCCGAAGGGGGATACCCGGAAAGATTTCAATCCGCAACACTGGCCATGG
CCGATTTAAAGATGCTCGGTTCTTTTGGAGGTCCCCGCGGGTTTACGTGATGG
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GAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCC
CGGGCCTAGTACACACCGCCCGTCACACCATAAGAGTTTGTAACACCCAAAGT
CGGTGGGGTAACCTCTTAGGAACCAGCCGCCTAAGGGGGACAGATGATTATG
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Lactobacillus plantarum subsp. *plantarum* strain IMAU11567 (BM52-5) 16S ribosomal RNA gene sequence

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TGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGTTTGAAAGATGGCT
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CGGCTCACCATGGCAATGATACGTAGCCGACCTAAGAGGGTAATCGGCCACA
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TAACCCGCTACT

Lactobacillus pentosus strain: ZU 22 16S ribosomal RNA gene sequence

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ATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGA
GTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGAT
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Lactobacillus plantarum strain BCH-3 16S ribosomal RNA gene sequence

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Lactobacillus pentosus strainLAP1 16S ribosomal RNA gene sequence

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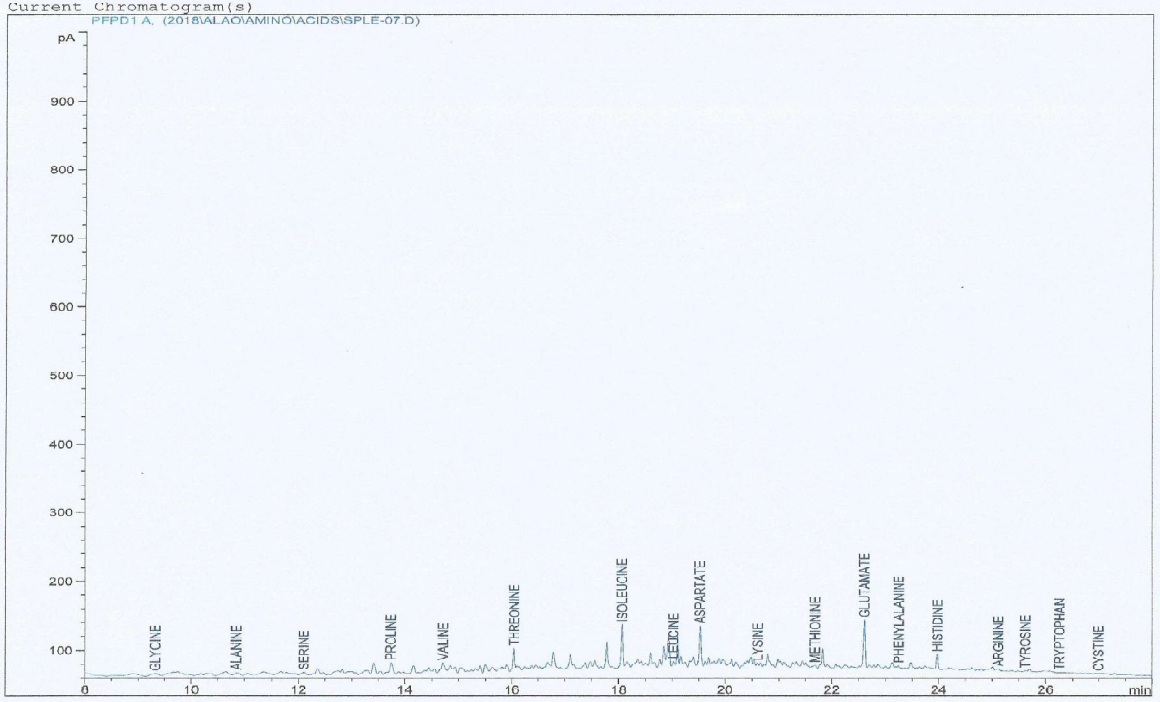
GTAGGGGAAC

Appendix 4: Gas chromatogram for Amino acid analysis

Chromatogram of unfermented 3.0 g *M. oleifera* supplemented cereal legume showing its amino acid profile

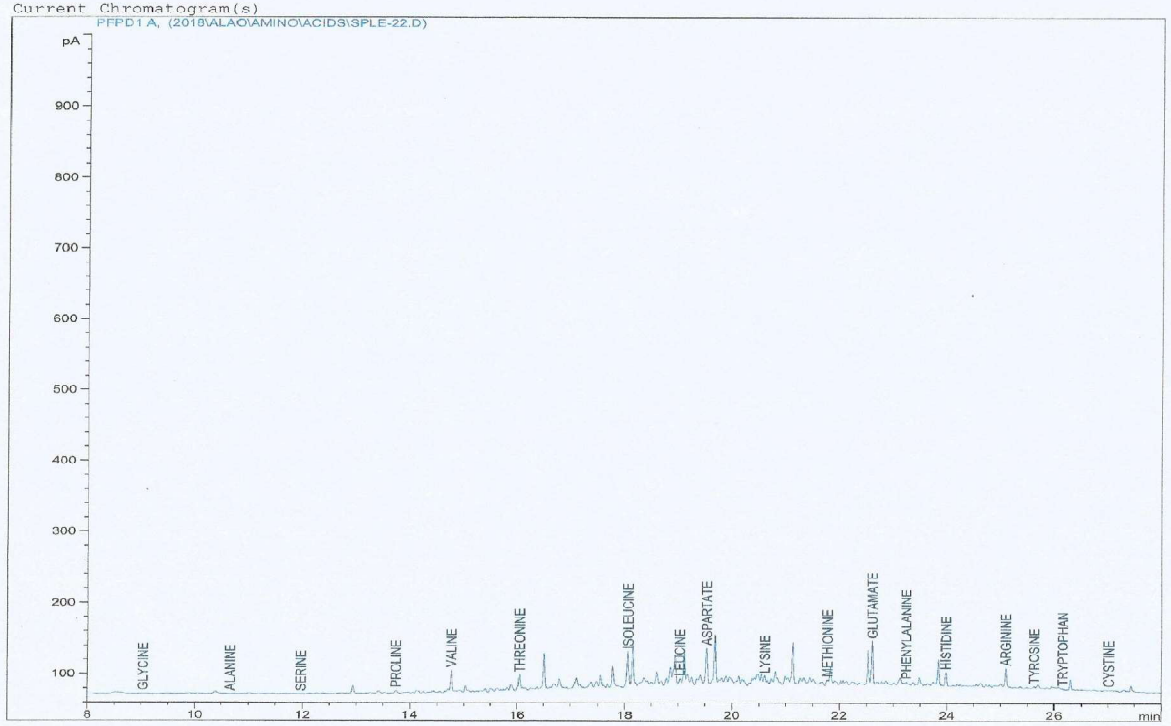
of window 38: Current Chromatogram(s)

Current Chromatogram(s)



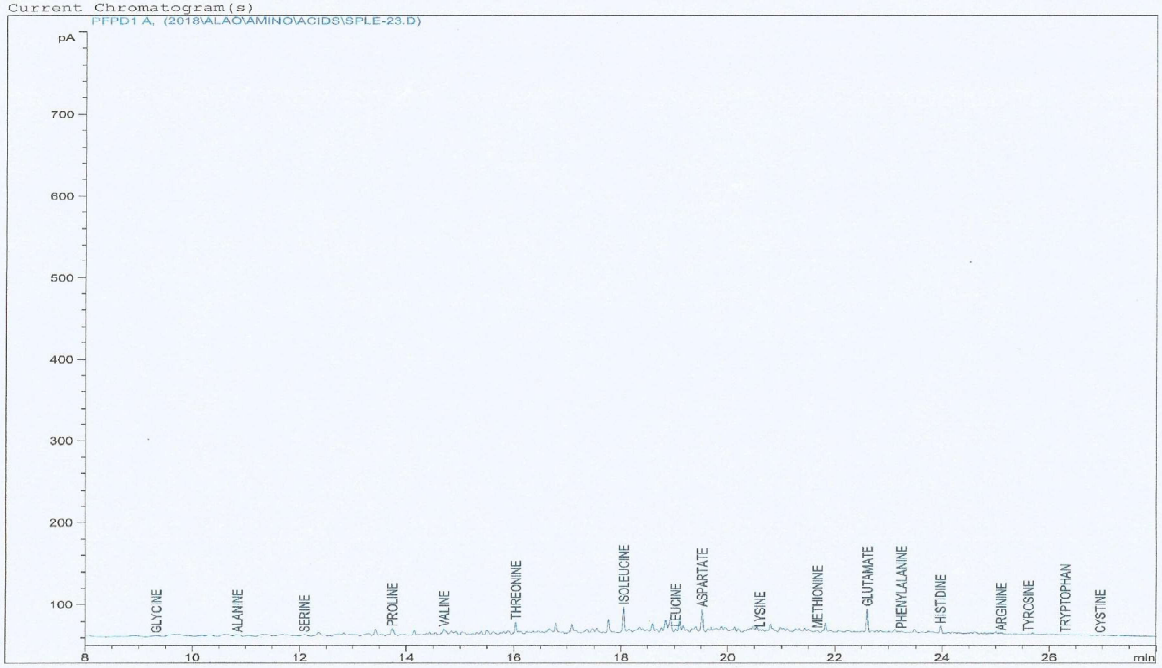
Chromatogram of 1.0 mL *Lactobacillusplantarum* fermented 3 g *M. oleifera* supplemented cereal legume showing its amino acid profile

Current Chromatogram(s)



Chromatogram of 2.0 mL *Lactobacilluspentosus* fermented 3 g *M. oleifera* supplemented cereal legume showing its amino acid profile.

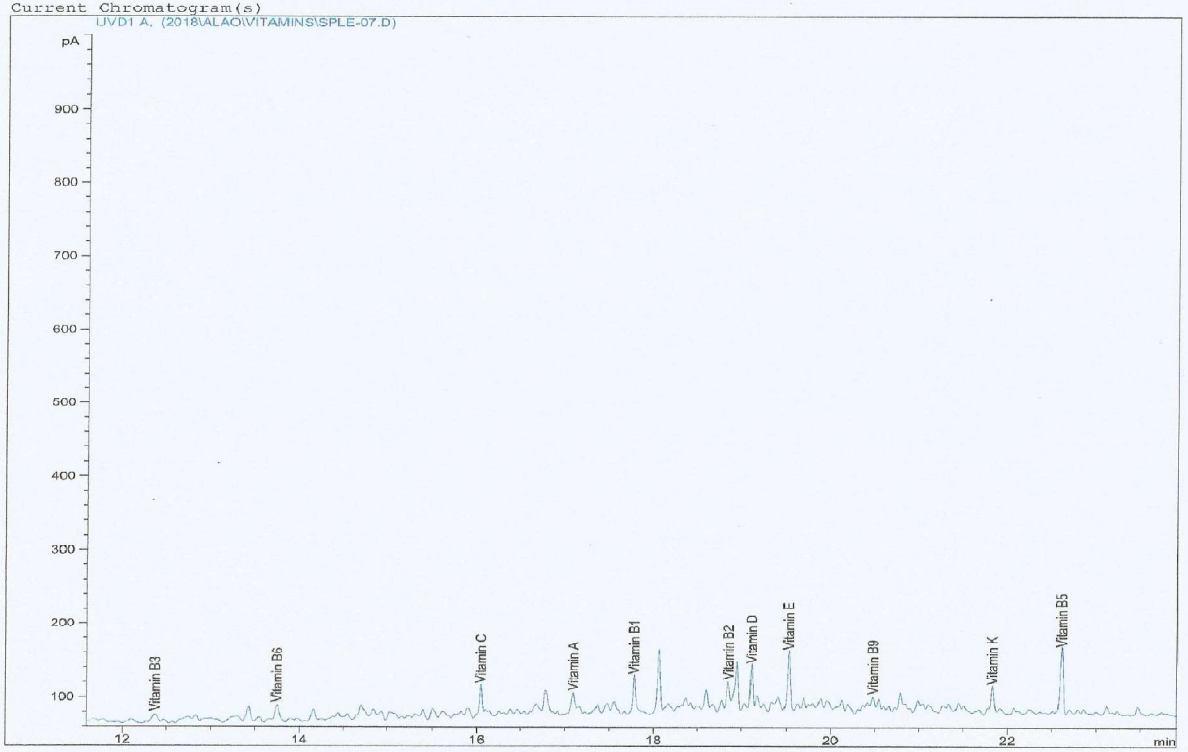
Current Chromatogram(s)



Appendix 5: Gas chromatogram for Vitamin analysis

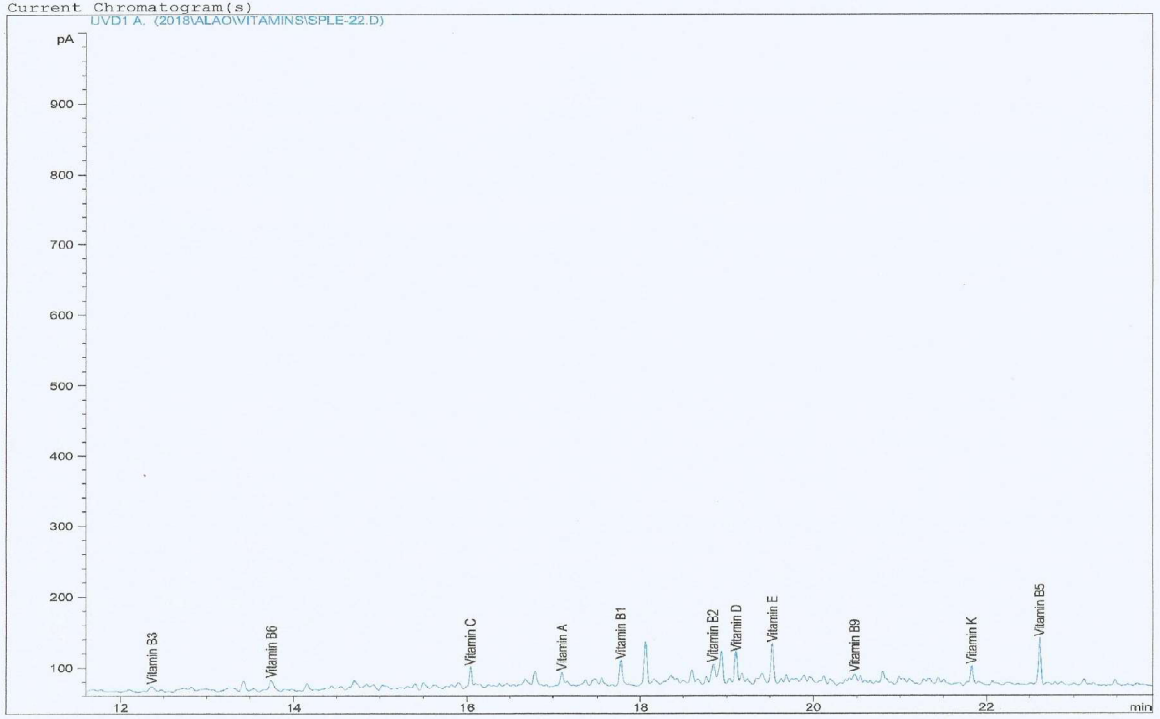
Chromatogram of unfermented 3.0 g *M. oleifera* supplemented cereal legume showing its vitamin profile

Current Chromatogram(s)



Chromatogram of 1.0 mL *Lactobacillusplantarum* fermented 3 g *M. oleifera* supplemented cereal legume showing its vitamin profile

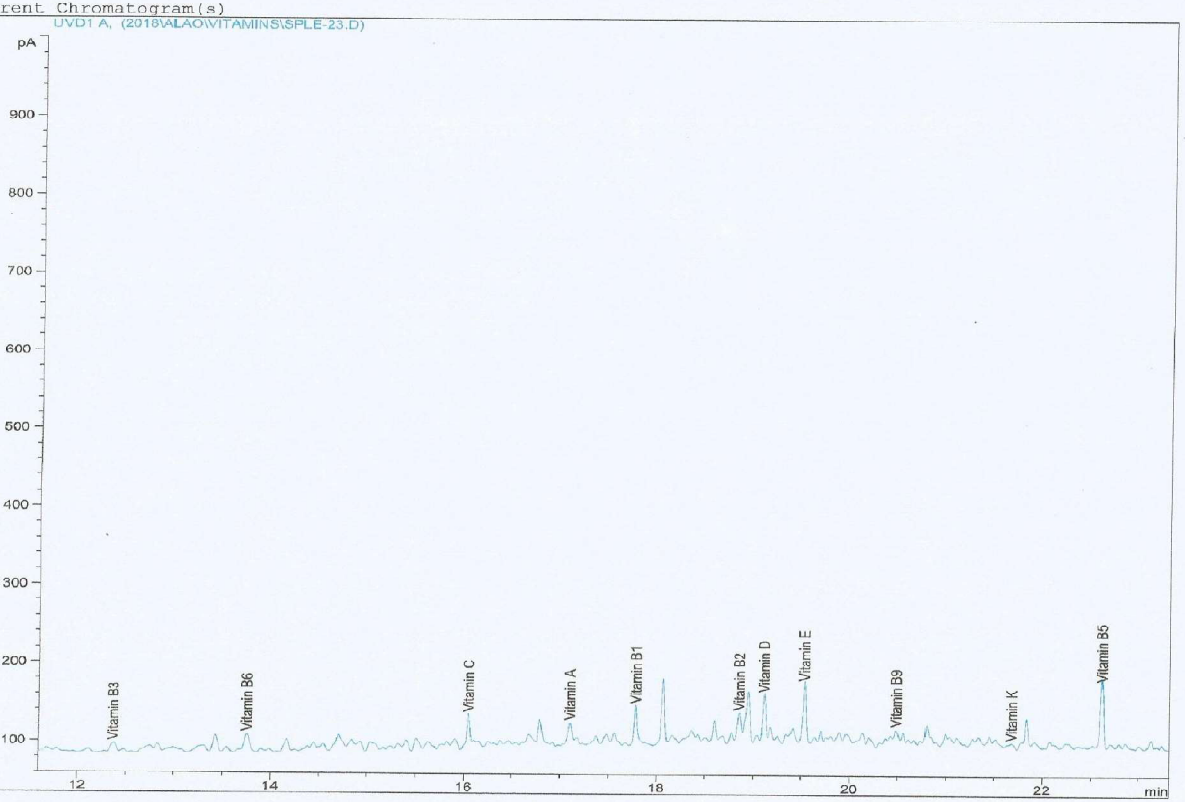
Current Chromatogram(s)



Chromatogram of 2.0 mL *Lactobacillusplantarum* fermented 3 g *M. oleifera* supplemented cereal legume showing its vitamin profile

dow 38: Current Chromatogram(s)

rent Chromatogram(s)



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