Sudan University of Science& Technology

College of Graduate Studies

Formulation of Fermented Peanuts Milk and Millet Milk Blend as a Carrier for *Bifidobacterium longum BB536*

تكوين خلطه مخمره من لبن الفول السوداني ولبن الدخن كحامل للبكتريا الصديقة *Bifidobacterium longum BB536*

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الآيـة

قال تعالي :

(فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَى إِلَيْكَ وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْمًا).

صدق الله العظيم

سورة طه الآية (114)

Dedication

I dedicated this dissertation

To my great parents Rahma and Elzein.

To my sisters, brothers, aunts, uncle and all members of

My big family for their kind helps and support.

It also goes to teachers, scientists, researchers and all

Seekers for knowledge.

Acknowledgement

Alhamdulillah, I finish my dissertation with help and full support of my lord ALLAH, guidance of my supervisor, cooperation of friends and family.

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List of Abreviation

GIT	**Gastrointestinal Tract**
US	**United States**
FAO	**Food Agricultural Organization**
CFU	**Colony forming Unit**
Log	**Logarithm**
(%)	**Percentage**
g	**Gram**
TSS	**Total Soluble Solid**
h	**Hour**
MRS	**de mann- Rogosa –Sharpe**
c^o	**Degree Celsius**
l	**Liter**
et al	**Et Cetera(and company)**
ANOVA	**Analysis of Variance**
ml	**Milliliter**
w/w	**Weight per weight**
N	**Normality**
UNICEF	**United Nations International Children's Emergency Fund**
HDL	**High density lipoprotein**

Abstract

This study was carried out to develop probiotic fermented beverages. The commercial strain *Bifidobacterium longum BB536* was used. The growth of the strain and its related physicochemical changes during fermentation was evaluated. The strain survival during refrigeration storage was also examined. In addition, the physicochemical composition of different fermented beverages at initial and maximum growth of the strain were also determined .Growth medium were formulated from fresh cow milk, pure peanut milk, pure millet milk. As will as, to three different blends based on peanut milk prepared by partial substitution of 15% (A), 30%(B) and 45%(C) with millet milk .Peanut contains high levels of fat and protein. Moreover, levels of carbohydrates, fiber and ash were high in millet. Roasting of peanut increased fat , proteins, fiber, ash and Carbohydrates in ratio of 0.23, 1.43, 0.32, 0.1 and 0.62% respectively, as compared to raw peanut. The results obtained on *B. longum BB536* viable count revealed significant ($P < 0.05$) increases by extended fermentation period in all type of formulated beverages, as compared to strain level at beginning of fermentation. The rate of *B. longum BB536* increases in different fermented beverages were 3.15, 2.9, 2.89, 2.76, 2.43, % and 2.1% in fermented peanut milk, millet milk, cow milk, blend (B), blend (A) and blend (C) ,respectively. In spite of declining in viable count of *B. longum BB536* in all types of fermented beverages at 24h fermentation , the count still above the number required to presence in probiotic food which is at least 6 log CFU/ml fermented products. Except fermented beverage C (5.77 CFU/ml) is not fulfill propiotic requirement in food. During fermentation process with strain *BB536* there were significant ($P<0.05$) decrease in pH levels in all type of beverages by extended fermentation period to 24h .The decreases in pH are due to increased of acids production during fermentation process as a result of fermenting sugar by *Bifidobacterium BB536* . TSS levels decrease in all types of fermented

beverages. The rates of TSS decreases at maximum growth were 0.3, 0.4, 1.3, 0.1and 0.9 in fermented peanut milk, millet milk followed by the blend (a),blend (b) and blend (c) , respectively. Sugars also decrease in all fermented beverages by extended fermentation period to 24 h. The rates of sugar reduction at maximum growth of strain BB536 were 0.15, 0.04, 0.03,0.02 ,and 0.01 in fermented peanut milk, blend (C), blend (B), millet milk and then blend (A) , respectively. During the fermentation of the fermented beverages there were no significant ($p>0.05$) changes in compound of beverages. There were increase in moisture, portion, ash and fiber, tend to decrease in fat, carbohydrate and total soluble solid of fermented of peanut milk .On the other hand, during refrigeration storage of different formulated beverages. There were significant ($p<0.05$) reduction in *B. longum BB536* viable count in all fermented beverages .The rate of reduction in the first week of the refrigeration storage were 2.39, 2.08, 1.84, 1.7, 1.43and 0.77 CFU /ml in fermented millet milk, peanut milk, blend (C), blend (B), blend (A) and cow milk, respectively. Hopefully, the final viable count of *B. longum BB536* in fermented, peanut milk, cow milk and blend (A) was above the minimum number required to presence in probiotic to exert health benefits upon consumption. During two weeks refrigeration there were significant reductions in *B. longum BB536* in all types of fermented beverages, except fermented cow milk. Nevertheless, the number required to presence in probiotic foods, which is 6 log CFU/ml fulfilled in peanut and blend (A) during only one week storage, while achieved in fermented cow milk during two weeks storage. Therefore, they are suitable carrier to deliver *B. longum BB536* to consumer at the same time the fermented beverages provide other essential nutrients such as protein, fat, minerals and fiber.

ملخص البحث

أجريت هذه الدراسة لتطوير مشروبات مخمره باستخدام البكتريا الصديقة (بروبايتك) .البكتريا الصديقة التجارية *Bifidobacterium longum BB536* تم استخدامها. تقيم نمؤها والتغيرات الفيزيوكيميائيه خلال عملية التخمير.وتم حساب عدد بكتريا *BB536* والتغيرات الفيزيوكيميائيه أثناء عملية التخمير والتخزين في الثلاجة وأيضا تم تقدير التغيرات الكيميائية عند بداية وأقصي نمؤ للبكتريا في كل من لبن الفول السوداني ,لبن الدخن, لبن البقر الطازج , بالإضافة إلى ثلاثة خلطات علي أساس لبن الفول السوداني ونسب 15 % (أ),30% (ب) و45% (ج) من لبن الدخن .

يحتوي الفول السوداني علي نسب عالية من البروتين والدهون والدخن احتوي علي نسب أعلي من الكربوهيدريتات ,الألياف والرماد. عملية تحميص الفول السوداني أدت لزيادة معنوية ($P < 0.05$) في نسب الدهون,البروتين,الألياف,الرماد والكربوهيدريتات بنسب 0.23 , 1.43 , 0.32 , 0.1 و 0.62 % علي التوالي .وضحت النتائج التي تم الحصول عليها أن هناك زيادة معنوية ($P < 0.05$) في النمؤ الميكروبي عند أقصي نمو لبكتريا *BB536* في المشروبات المخمرة مقارنة بعددها عند بداية عملية التخمير .وكان معدل زيادة نمؤ بكتريا *BB536* 3.15, 2.9, 2.89, 2.76 , 2.43% و2.1 % في كل من لبن الفول السوداني,لبن الدخن,لبن البقر, الخلطة (ب), الخلطة (أ) و الخلطة (ج) علي التوالي. وعلي الرغم من انخفاض عدد نمؤ بكتريا *BB536* بتمديد التخمير لأربعة وعشرين ساعة في كل أنواع المشروبات المخمرة لا يزال عدد البكتريا الحية أعلي من العدد المطلوب وجوده في أغذية البروبيتك والتي من المفترض أن تحتوي علي (log 6 CFU/ml) ماعدا المشروب المخمر في الخلطة (ج) كان يحتوي علي (5.77 CFU/ml) لا يفي بمتطلبات أغذية البروبيتك. أثناء عملية التخمير بالبكتريا *BB536* كان هناك انخفاض معنوي ($P < 0.05$) في الرقم الهيدروجيني pH في جميع المشروبات المخمرة هذا الانخفاض صوحب بزيادة في الحموضة و انخفاض في مستوي السكريات لتخميرها بواسطة البكتريا *BB536* . والجوامد الكلية الصلبة الذائبة في كل أنواع المشروبات المخمرة مع تقدم عملية التخمير. وكان معدل النقصان في الجوامد الكلية الصلبة الذائبة عند أقصي نمؤ لبكتريا *BB536* 0.4,0.3 ,0.1 ,1.3 ,و 0.9 % في كل من الفول السوداني ,الدخن والخلطة (أ), الخلطة (ب) و الخلطة (ج) علي التوالي. وكان معدل نقصان مستوي السكريات 0.15, 0.04 ,0.03 ,0.02 و 0.01 % في كل من لبن الفول السوداني, الخلطة (ج), الخلطة (ب),لبن الدخن والخلطة (أ) علي التوالي .خلال عملية تخمير المشروبات هنالك زيادة معنوية ($P < 0.05$) في الرطوبة,البروتين,الرماد والألياف و نقصان في الدهون, الكربوهيدريتات والجوامد الكلية الصلبة الذائبة في لبن الفول السوداني المخمر. من ناحية أخرى أثناء عملية التخزين في الثلاجة للمشروبات المخمرة هناك انخفاض

معنوي ($P < 0.05$) في نمؤ بكتريا *BB536* في جميع المشروبات وكان معدل الانخفاض في الأسبوع الأول من التخزين (2.39, 2.08 , 1.84, 1.7, 1.43 و 0.77 CFU /ml) خليه بكتيرية في كل مل من اللبن المخمر للدخن,الفول السوداني, الخلطة (ج), الخلطة (ب), الخلطة (أ) ولبن البقر علي التوالي. وكان عدد خلايا بكتريا *BB536* في كل من لبن الفول السوداني ,الخلطة (أ) ولبن البقر يفي بالعدد المطلوب وجوده في أغذية البروبيتك. وخلال الأسبوع الثاني من التخزين كان هناك انخفاض معنوي ($P < 0.05$) في جميع المشروبات المخمرة ماعدا حليب البقر المخمر وعلي الرغم من ذلك كان العدد المطلوب وجوده في أغذية البروبيتك توفر في لبن الفول السوداني المخمر و الخلطة (أ) المخمرة أثناء التخزين في الأسبوع الأول فقط . بينما تحقق في لبن البقر المخمر خلال أسبوعين التخزين لذلك يعتبر كل منهم ملائم لحمل بكتريا *BB536* للمستهلكين وفي نفس الوقت هذه المشروبات المخمرة تقدم عناصر غذائية أساسيه مثل البروتينات, الدهون, المعادن والألياف.

CHAPTER ONE

INTRODUCTION

Fermentation is one of the oldest known uses of biotechnology. All over the world, fermented foods continue to constitute an important part of our diet and together with beverages are estimated to present some 20-40% of our food supply world-wide (Campbell-Platt, 1994). Particularly in developing countries, where refrigeration is not always an option, the fermentation process is widely used. Fermentation prolongs the shelf-life of foods in addition to improving the nutritional value and reducing the risk for food borne illness (Campbell-Platt, 1994) .Cereal and legumes are mostly used to develop fermented beverages. Fermented foods can even have beneficial health effects, when microorganisms used possess probiotic activity. The word probioticis derived from Greek and means “for life” (Metchnikoff, 1907). One of the more detailed current definitions of probiotics is; “a microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract”. Mainly specific strains of lactobacilli, *Bifidobacterium*, enterococci and yeast are today used commercially as probiotics (Naidu *et al*., 1999; Holzapfel and Schillinger, 1995; Saxelin *et al*., 2005). *Bifidobacterium* are considered as important probiotics and used in the food industry to relieve and treat many intestinal disorders. *Bifidobacterium* exert a range of beneficial health effects, including the regulation of intestinal microbial homeostasis, the inhibition of pathogens and harmful bacteria that colonize and/ or infect the gut mucosa, the modulation of local and systemic immune responses, the repression of procarcinogenic enzymatic activities within the microbiota, the production of vitamins, and the bioconversion of a number of dietary compounds into bioactive molecules (Mayo and van Sinderen, 2010).

Bifidobacteruim longum may be considered the most common species of *Bifidobacterium*, being found both in infant and adult feces (Bivati *et al.*; 1984). Potential benefits from consumption of *B. longum* include: antagonistic action toward intestinal pathogens, improved lactose utilization, anticarcinogenic action and control of serum cholesterol levels. Many scientific studies showed the benefits offered by *Bifidobacterium longum BB536* (Kojima *et al.*, 1996; Namba *et al.*, 2003). Thus there is considerable interest in incorporating these heaths promoting *bifidobacterium* into food. On the other hand, dairy products are the main carriers of probiotic bacteria to human, as these products provide a suitable environment for probiotic bacteria that support their growth and viability. However, with an increase in the consumer vegetarianism throughout the developed countries, there is also a demand for alternative carrier for beverage. The development of new nondairy probiotic food products is very much challenging, as it has to meet the consumer's expectancy for healthy benefits (Stanton *et al.*, 2003). Nevertheless, there were no many studies regarding application of probiotic *bifidobacterium* into fermented Sudanese foods. In previous investigation, (Kabeir *et al.*, 2005) successfully incorporated *B. longum BB536* into Sudanese cereal beverage Medida.

Legumes (*Arachis hypogaea* L.) groundnut has a potential role in combating malnutrition are a major source of edible oil and protein meal and therefore considered to be highly valuable in human and animal nutrition (Nwokalo, 1996). It's rich in protein, energy and other nutrient. Peanut- based formulated food can be developed to for a therapeutic purposely and to aid in famine relief .There for the present low level in peanut consumption, especially in the developing countries, should be increased. It is, therefore, necessary to direct research into the possibility of peanut processing into other useful and edible products. Fermentation of groundnut milk may serve as one such effort that

can increase the protein availability and consumption (Roberts-Sunny *et al.*, 2004).

On the other hand, millet is the sixth most important grain in the world. Millet is equal or superior to grain of wheat, corn sorghum and rice in protein and oil content, it contains similar amount of calcium (Ca) and phosphorus (P), more iron (Fe) than the cereals grains (Marwa, 2005). Millets have an alkaline pH and are the only grains that keep their alkaline properties even after being cooked. As another plus, millet is a gluten free grain and thus, is ideal for people with wheat/gluten allergies or intolerance (Baltensperger and Cai, 2004).

In this respect, the use of peanut milk and millet blend will complement nutrients same time can be a successful non-dairy carriers for *Bifidobacterium* strain .There for the objective of this study are to:

1- Evaluate the growth of *B. longum BB536* and its related physio - chemical changes during fermentation of different formulated beverages.
2- Determine the nutritional value of the different *Bifidobacterium BB536* fermented beverages.
3- Evaluate the survival of *B. longum BB536* during refrigeration storage of different fermented beverages.

CHAPTER TWO

LITERATURE REVIEW

2.1. History of probiotics

The idea that some bacteria contained in our food may have beneficial effects is much older than the term probiotic. At the beginning of the 20th century, the Russian Nobel Prize Laureate Elie Metchnikoff associated the observed longevity of Bulgarian peasants with their high consumption of live microbes in fermented milk products, as he reported in his book.The prolongation of life (Metchnikoff, 1907). In1930, the Japanese scientist Minoru Shirota isolated a lactic acid bacterium from the feces of a healthy infant. Five years later, one of the first fermented milk drinks thought to support intestinal health was produced with the strain he developed and was named "Yakult". (Metchnikoff , 1907).

2.2. Definition of probitics

The word ‘probiotic’, derived from the Greek language, means ‘for life’ (Fuller, 1989) and has had many definitions in the past. Definitions such as ‘substances produced by protozoa that stimulate the growth of another’ or ‘organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance’ were used. These general definitions were unsatisfactory because ‘substances’ include chemicals such as antibiotics. The definition of probiotics has since then been expanded to stress the importance of live cells as an essential component of an effective probiotic. Most recently, Huis- Veld and Havenaar (1991) broadened the definition of probiotics as being ‘a mono- or mixed culture of live microorganisms which, applied to man or animal (e.g. as dried cells or as a fermented product), beneficially effects the host by improving the properties

of the indigenous micro flora. This definition implies that probiotic products, for example fermented milk, contain live microorganisms and improve the health status of the host by exerting beneficial effects in the gastrointestinal tract.

2.3.Probiotic strains

Probiotic cultures naturally occur in certain fermented foods. Below is a list of different strains of probiotic bacteria.

- Bacillus coagulans GBI-30, 6086
- Bifidobacterium animalis subscp. lactis BB-12
- Bifidobacterium longum subsp. BB536
- Lactobacillus acidophilus NCFM
- Lactobacillus paracasei St11
- Lactobacillus johnsonii La1
- Lactobacillus plantarum 299v
- Lactobacillus reuteri ATCC
- Lactobacillus reuteri Protectis

Saccharomyces boulardi (Rosander *et al*., 2008)

2.4. Characteristics of probitics microorganism

Characteristics of successful probiotics determine their ability to survive the upper digestive tract and to colonize in the intestinal lumen and colon for an undefined time period. Probiotics are safe for human consumption and no reports have found on any harmfulness or production of any specific toxins by these strains (Von Wright, 2000., Salminen *et al*., 1998).

In addition, some probiotics could produce antimicrobial substances like bacteriocins. Therefore, the potential health benefit will depend on the characteristic profile of the probiotics. Some probiotic strains can reduce intestinal transit time, improve the quality of migrating motor complexes, (Husebye, 2001) and temporarily increase the rate of mitosis in enterocytes (Banasaz, 2002, Halvorsen , 2000).

The most common probiotics are Lactobacillus and Bifidobacterium. In general most probiotics are gram-positive, usually catalase-negative, rods with rounded ends, and occur in pairs, short, or long chains (Von Wright, 2000). They are non-flagellated, non-motile and non-spore-forming, and are intolerant to salt. Optimum growth temperature for most probiotics is 37°C but some strains such as L. casei prefer 30 °C and the optimum pH for initial growth is 6.5-7.0 (Von Wright, 2000). L. acidophilus is microaerophilic with anaerobic referencing and capability of aerobic growth.

Bifidobacterium are anaerobic but some species are aero-tolerant. Most probiotics bacteria are fastidious in their nutritional requirements (Desmazeaud, 1983., Marshall, 1984) .With regard to fermentation probiotics are either obligate homofermentative (ex. L. acidophilus, L. helvelicas), obligate heterofermentative (ex. L. brevis, L. reuteri), or facultative heterofermentative (ex. L. casei, L. plantarum), (Barrangou, 2011). Additionally, probiotics produce a variety of beneficial compounds such as antimicrobials, lactic acid, hydrogen peroxide, and variety of bacteriocins (Gorbach, 2002).

Probiotics should have the ability to interact with the host micro flora and competitive with microbial pathogens, bacterial, viral, and fungal (Gorbach, 2002).

2.5. Functional Properties of probiotics

In spite of research progress in recent years the understand of gut of ecosystem is still fragmentary and consequently limits our comprehension of a normal or balanced microbial population. Thus, the impact of a functional property of the strain on composition and function of the intestinal population is still difficult to a certain (Holzapfel *et al.*, 1995; Mercenier and Pvan, 2002).Never the less, numerous beneficial functions have been suggested for probiotic bacteria:

- Nutritional benefits of probiotics includes:
  - Vitamin production, availability of minerals and trace elements.
  - Production of important digestive enzymes. Such as- Production of β-glycosidase of alleviation of factors in tolerance of lactose.
- Barrier , restoration , antagonistic effects against:
- Infectious diarrhea.
- Antibiotic –associated diarrhea, irradiation –associated diarrhea.
- Cholesterol - lowering effect.
- Stimulation and improvement of the immune system.
- Enhancement of bowel motility, relief from constipation.
- Anti-carcinogenic effects in the colon.
- Maintenance of mucosal integrity.
- Reduction of inflammatory allergic reactions.
- Adherence and colonization resistance.
- Antioxidative activities (Kullisaar *et al.*, 2002).

2.6. Criteria of Selection of appropriate probiotic

Different aspects have to be considered in probiotic selection Safety criteria for any successful probiotic have been defined in several reviews (Lee and Salminen, 1995; Donohue and Salminen, 1996; Adams, 1999) include the following specifications:

1. Strains uses are preferably of human origin.

2. They are isolated from healthy human GI tract.

3. They have a history of being non-pathogenic.

4. They have no history of association with diseases such as infective endocarditic or GI disorders.

5. They do not deconjugate bile salts (bile salt deconjucation or dehydroxylation would be a negative trait in the small bowel (Marteau *et al.*; 1995).

6. They do not carry transmissible antibiotic resistance genes.

While in selecting a preferable probiotic strain several aspects of functionality have to be considered:

1. Acid tolerance and tolerance to human gastric juice.

2. Bile tolerance (an important property for survival in the small bowel).

3. Adherence to epithelial surfaces and persistence in the human GI-tract.

4. Immunostimulation, but no pro-inflammatory effect.

5. Antagonistic activity against pathogens such as *Helicobacter pylori*, *Salmonella sp., Listeria monocytogenes* and *Clostridium difficile*.

6. Antimutagenic and antigarcinogenic properties.

Feeding trials with different probiotic strains have shown that the probiotic strain usually disappears from the GI-tract within a couple of weeks after the ingestion is discontinued (Fukushima *et al.,* 1998; Johansson *et al.,* 1998; Alander *et al.;* 1999; Donnet-Hughes *et al.,* 1999). The role of the probiotic persistence in the human GI-tract has therefore been questioned. However, even temporary persistence, which has been noted for several ingested probiotic strains, may enhance their chances for beneficial functions in the GI-tract, and is therefore considered a desirable trait. Necessary safety and functional criteria the aspects related to probiotic production and processing are also of utmost importance, such as:

1. Good sensory properties.

2. Phage resistance.

3. Viability during processing.

4. Stability in the product and during storage.

Good viability and activity of probiotics are considered prerequisites for optimal functionality. However, several studies have shown that non-viable probiotics can have beneficial effects such as immune modulation and carcinogen binding in the host (Ouwehand and Salminen, 1998; Salminen *et al.*; 1999).

2.7.Application of probiotic culture into food

Probiotic bacteria are applied in many different products worldwide. In addition to food products, probiotic cultures are also used in pharmaceuticals and animals feed. Most definitions of probiotics are based on live bacteria that confer a health benefit for consumer. The application of probiotics in food products depends on factors like water activity, processing and storage temperature, shelf life, oxygen content, pH and mechanical stress, salt content and content of the other harmful or essential ingredient (Goktepe *et al* ., 2006). Probiotic bacteria have been applied in fermented dairy products for many years, fruits juices have been shown to be suitable carriers for probiotics there is growing interest in applying probiotics to fermented meat products, vegetable based probiotic products, probiotics bacteria are also applied to infant nutrition powder and powdered milk drinks (Goktepe *et al* ., 2006). Probiotics bacteria are also applied to Cereal products have health-benefiting microbes and potentially prebiotic fibers. The development of new functional foods which combine the beneficial effects of cereals and health promoting bacteria is a challenging issue. Nevertheless, cereal-based products offer many possibilities. Indeed, numerous cereal-based products in the world require a lactic fermentation, often in association with yeast or molds. Cereals are good substrates for the growth of probiotic strains and due to the presence

of non-digestible components of the cereal matrix may also serve as prebiotics (Charalampopoulos, 2002).

Due to the complexity of cereals, a systematic approach is required to identify the factors that enhance the growth of probiotic in cereals (Kedia *et al.*, 2007).

2.8. *Bifidobacterium* as probiotics

Bifidobacteria is the predominant species of human colonic and faucal micro biota .It has been extensively introduced in the food industry and pharmaceutical applications (Guarner and Malagelada, 2003). The prevalence of bifidobacteria in the feces of breast fed infant may have been a major reason for selecting strains of this group for use as probiotics (Lilly and Stillwell, 1965).

2.8.1. Species of Bifidobacterium

B. angulatum; B. animalis; B. asteroides; B. bifidum; B. boum; B. breve; B. catenulatum; B. choerinum; B. coryneforme; B. cuniculi; B. dentium; B. gallicum; B. gallinarum; B indicum; B. longum; B. magnum; B. merycicum; B. minimum; B. pseudocatenulatum; B. pseudolongum; B. psychraerophilum; B. pullorum; B. ruminantium; B. saeculare; B. scardovii; B. simiae; B. subtile; B. thermacidophilum; B. thermophilum; B. urinalis; B. sp. (Holzapfel *et al*., 1995)

2.8.2. *Bifidobacterium longum BB536*

Bifidobacterium longum is one of the *bifidobacterium* species found mainly in human faeces and it may be considered as the most common species of *bifidobacterium*, being found both in infant and adult. Potential benefits from consumption of *B. longum* include: antagonistic action toward intestinal pathogens, improved lactose utilization, anticarcinogenic action and control of serum cholesterol levels. Scientific studies showed the benefits offered by *Bifidobacterium longum BB536* (Kojima *et al*., 1996; Namba *et al*., 2003).

Thus there is considerable interest in incorporating these healths's promoting *bifidobacterium* into food. Nevertheless, probiotic strains, particularly *bifidobacterium* are rarely used outside the diary based industry. The scarcity of animal milk in many countries makes it difficult to provide adequate *bifidobacterium* intake

2.9.Peanut

2.9.1.Scientific classification

Peanut was classified by Alper (2003) as follows:

Kingdom:	***Plantae***
Family:	***Fabaceae***
Genus:	***Arachis***
Species:	***A. hypogaea***

2.9.2. History of peanut

The domesticated peanut is an amphidiploid or allotetraploid, meaning that it has two sets of chromosomes from two different species, thought to be A. duranensis and A. ipaensis. These likely combined in the wild to form the tetraploid species A. monticola, which gave rise to the domesticated peanut .This domestication might have taken place in Paraguay or Bolivia, where the wildest strains grow today. Many pre-Columbian cultures, such as the Moche, depicted peanuts in their art (Seijo *et al.*, 2007) .

Archeologists have dated the oldest specimens to about 7,600 years, found in Peru .Cultivation spread as far as Mesoamerica, where the Spanish conquistadors found the tlalcacahuatl (the plant's Nahuatl name, whence Mexican Spanish cacahuate and French cacahuète) being offered for sale in the marketplace of Tenochtitlan (Mexico City). The plant was later spread worldwide by European traders (Seijo *et al.*, 2007) .

In the United States, a US Department of Agriculture program to encouraged agricultural production and human consumption of peanuts which was instituted in the late 19th and early 20th centuries. George Washington Carver is well known for his participation in that program in which he developed hundreds of recipes for peanuts.

2.9.3. Usage of peanut

Peanuts are often a major ingredient in mixed nuts because of their relative cost compared to Brazil nuts, cashews, walnuts, and so on (Bonnie, 1988). Although peanut butter has been a tradition on camping trips because of its high protein content and because it resists spoiling for long periods of time, the primary use of peanut butter is in the home. Large quantities are also used in the commercial manufacture of sandwiches, candy, and bakery products. Boiled peanuts are a preparation of raw, unshelled green peanuts boiled in brine and often eaten as a snack. More recently, fried peanut recipes have emerged – allowing both shell and nut to be eaten. Peanuts are also used in a wide variety of other areas, such as cosmetics , nitroglycerin, plastics, dyes and paints (Bonnie, 1988).

2.9.4. Nutrition value of peanut

Peanuts are rich in nutrients, providing over 30 essential nutrients and phytonutrients. Peanuts are a good source of niacin, folate, fiber, vitamin E, magnesium and phosphorus. They also are naturally free of trans-fats and sodium, and contain about 25% protein (a higher proportion than in any true nut. Peanuts are used to help fight malnutrition, because they are high-protein, high-energy and high nutrient. Lopes *et al.*, (2011) .Peanut-based pastes developed to be used as a therapeutic food to aid in famine relief. The World Health Organization, UNICEF, Project Peanut Butter and Doctors Without

Borders have used these products to help save malnourished children in developing countries.

2.9.5. Protective Nutrients of peanut

People who eat peanuts tend to take in more key nutrients critical to health. In more than 15,000 people who consumed peanuts and peanut products, it was found that levels of vitamin A, vitamin E, folate, magnesium, zinc, iron, calcium, and dietary fiber were higher than those who did not consume peanuts (Griel, 2004). Peanuts also provide unique bioactive components that act as antioxidants and have been shown to be disease preventative. Arginine, an amino acid that is high in peanuts, is a precursor to nitric oxide, which helps expand blood vessels and can decrease blood pressure (Griel, 2004). Reveratrol, also found in grapes and wine, improves longevity and performance, and reduces inflammation.

Peanuts also have significant levels of phytosterols. Phytosterols are well known for their ability to reduce cholesterol and new research showed that they are cancer-preventative. Flavonoids are a class of compounds also found in peanuts that reduce inflammation and inhibit platelets from sticking to arteries (Griel, 2004)

2.9.6. Composition of peanut

Protein, fats, and fiber are the major components that make up peanuts. The good news is that these major components are all the healthy types when it comes to peanuts. The protein is plant-based; the fat is unsaturated, and the fiber is the main type of complex carbohydrate in peanuts. It makes sense that three healthy components come together in peanuts with their help benefits (Johnston *et al*., 2005).

2.9.6.1. Vitamins and Minerals

Peanuts and peanut butter contain numbers of vitamins and minerals that we need daily in our diets (Swainet *et al.*, 2008) integral to growth, development, metabolism, and immunity. All of the nutrients in peanuts through multiple mechanisms are likely to have synergistic effects toward improving health status (Johnston *et al.*, 2005).

2.9.6.2. Bioactives component of peanut

Research has identified numerous compounds in peanuts and in their skins that may have added health benefits beyond basic nutrition. Peanuts have been touted as a functional food with numerous functional components. These bioactive components have been recognized for having disease preventative properties and some are antioxidants while other are thought to promote longevity. They are together with vitamins, minerals, and healthy fats, protein, and fiber promotes health. Therefore peanuts are bioactive food in a shell (Francisco, 2008)

2.9.6.3. Antioxidant Capacity of the bioactives component

The numerous bioactive components in peanuts contribute to good health by their antioxidant capacity. Compared to well-known foods like green tea and red wine, peanuts have higher antioxidant capacity .When peanuts are consumed with their skins, their antioxidant capacity doubles. And roasting can at times actually increase this capacity as well Roasted peanuts with skins, for example, have higher antioxidant capacity than blueberries (Francisco, 2008). When you eat a handful of cocktail peanuts, you can be assured that your body is taking in a myriad of unique compounds to help in disease prevention.

2.9.7. Peanut and Disease Prevention

Peanuts have provided complex nutrition to many diets and improve health. Peanuts, peanut butter, and peanut oil all help to prevent chronic diseases including heart disease, diabetes, and cancer. Peanuts, peanut butter, and peanut oil have potent lipid lowering effects and may act to reduce inflammation, which is one of the underlying mechanisms that trigger chronic disease. The unique nutrient profile and bioactive components of peanut play a beneficial role in many areas of health and disease prevention (Jiang *et al.*, 2006)

2.10. Millets

Millets are a group of highly variable small-seeded grasses, widely grown around the world as cereal crops or grains for both human food and fodder. They do not form ataxonomic group, but rather a functional or agronomic one. Millets are important crops in the semi-arid tropics of Asia and Africa (especially in India, Nigeria, and Niger), with 97% of millet production in developing countries (Donough *et al.*, 2000). The crop is favored due to its productivity and short growing season under dry, tolerance of high temperature conditions.

2.10. 1.Millet varieties

1-Finger millet (*Eleusine coracana*).

2- Proso millet (*Penicum miliaceum*).

3- Pearl millet (*Pennisetum glaucum*).

4- Foxtail millet (*Setaria italic*).

5- Kodo millet (*Paspalum setaceum*).

6- Little millet (*Panicum sumatrense*).

7- Barnyard millet (*Echinochloa utilis*).

2.10. 2. History of Millet in the Sudan

The cultivation of area millet (dukhn) is about 5 million feddans (about 2.1 million hectars) located mostly in the lighter soils of western Sudan. The millet production in season 2000/01 is estimated at 479 thousand tones, averaging 92 kg per feddan. Around 93% of millet crop is produced by the traditional rainfed sector, of which 66% and 24 % come from Darfur and Kordofan respectively. Darfur and Kordofan are classified as marginal lands where rainfall is in the vicinity of 400mm/ anum, creating an invironment unsuitable and un favourable for the cultivation of other crops. (Hazell, 1986).

2.10.3. Nutritional value of millet

Nutritional quality of food is a key element in maintaining human overall physical well being because nutritional well being is a sustainable force for health and development and maximization of human genetic potential. Therefore, for solving the problem of deep-rooted food insecurity and malnutrition, dietary quality should be taken into consideration (Singh and Raghuvanshi, 2012). In addition to their cultivating advantages, millets were found to have high nutritive value and comparable to that of major cereals such as wheat and rice (Parameswaran and Sadasivam, 1994). It has also been reported that millet proteins are good sources of essential amino acids except lysine and threonine but are relatively high in methionine. Millets are also rich sources of phytochemicals and micronutrients (Mal *et al*., 2010and Singh *et al* ., 2012).

For example, pearl millet was found significantly rich in resistant starch, soluble and insoluble dietary fibers, minerals, and antioxidants (Ragaee *et al* ., 2006). It contains about 92.5% dry matter, 2.1% ash, 2.8% crude fiber, 7.8% crude fat, 13.6% crude protein, and 63.2% starch (Ali *et al*., 2003). Also,

foxtail millet protein characterization showed that its protein concentrate is a potential functional food ingredient and the essential amino acid pattern suggests possible use as a supplementary protein source to most cereals because it is rich in lysine (Mohamed *et al*., 2009). The protein content of proso millet (11.6% of dry matter) was found to be comparable with that of wheat and the grain of proso millet was significantly richer in essential amino acids (leucine, isoleucine and methionine) than wheat protein (Kalinova and Moudry, 2006). Thus, the presence of all the required nutrients in millets makes them suitable for large-scale utilization in the manufacture of food products such as baby foods, snack foods, and dietary food. Increasingly, more millet products have entered into the daily lives of people, including millet porridge, millet wine, and millet nutrition powder from both grain and flour form (Subramanian and Viswanathan 2007; Liu *et al* ., 2012). Millets are rich in vitamin B and also in minerals like potassium, phosphorous, Iron, copper, magnesium, manganese and zinc .Millets have a higher oil content of 4.2% of which 50% is polyunsaturated. Millets are also a rich source of non-nutritional components like phenols, tannins, phytates and flavonoids (Pradeep and Guha, 2010). These compounds serve as antioxidants and millets could also be used as a source of extremely beneficial photochemical in the pharmaceutical and food industry (Pradeep and Guha, 2010).

2.10.4.Health benefits of millets

Millet is more than just an interesting alternative to the more common grains. The grain is also rich in phytochemicals, including phytic acid, which is believed to lower cholesterol, and phytate, which is associated with reduced cancer risk (Coulibaly *et al*., 2011). These health benefits have been partly attributed to the wide variety of potential chemo preventive substances, called phytochemicals, including antioxidants present in high amounts in foods such as millets (Izadi *et al*., 2012). Millet is gluten-free, therefore an excellent option for people suffering from celiac diseases often irritated by the gluten

content of wheat and other more common cereal grains. It is also useful for people who are suffering from atherosclerosis and diabetic heart disease (Gélinas *et al*., 2008). Choi *et al*.(2005) and Park *et al*. (2008) reported that protein concentrate of Korean foxtail millet and proso millet significantly elevated plasma adiponectin and HDL cholesterol levels and caused major decreases in insulin levels relative to a casein diet in type 2 diabetic mice. Furthermore, proso millet also improved glycemic responses and plasma levels (Park *et al*., 2008). In addition, proso millet protein concentrate has protective effects against D-galactosamin-induced liver injury in rats (Ito *et al*., 2008). Choi *et al.(* 2005) and Park *et al*.(2008) concluded that proso millet protein could be a potential therapeutic intervention in type 2 diabetes. Devi *et al*. (2011) review the nature of polyphenols and dietary fiber of finger millet and their role with respect to the health benefits associated with millet. Chandrasekara and Shahidi (2010) reported in their studies on free-radical quenching activity of Finger millet (Eleusine coracana), that nonprocessed brown Finger millet had the highest radical quenching activity than the processed one and postulated that tannins and phytic acid were responsible for the activity (Devi *et al*., 2011 and Quesada *et al*., 2011; Kamara *et al*., 2012). Millets extract from the seed coat where reported to have shown high antibacterial and antifungal activity compared to whole flour extract due to high polyphenols content in seed coat (Viswanath *et al*., 2009). Free radical quenching potential of different millets kodo millet, finger millet, little millet, foxtail millet and barnyard millet), great millet and their white varieties were revealed to have significant antioxidant activity by 1, 1, Diphenyl -2 picrylhydrazyl (DPPH) method (Devi *et al*., 2011; Quesada *et al*., 2011 and Kamara *et al*., 2012). Moreover, Kamara *et al*. (2012) reported different radical scavenging activities of fractionated foxtail millet protein hydrolyses.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Raw Materials

The red-skinned peanut seeds (*Arachis hypogaea*) (*V. Natal*) were purchased from a local crops market in Bahri, Sudan. Care was taken to ensure that good quality and mould-free seeds were selected. The yellow millet (*Panicum miliaceum)* (*V. Proso*) was purchased from Alzraiga village in Eldwaim, Sudan. Fresh cow milk control was obtained from Department of Animal Science, Collage of Agriculture Studies, Sudan University of Science and Technology (SUST).

3.2. Methods

3.2.1. Preparation of peanut milk

Peanut milk was prepared by a similar method to the one reported by Salunkhe and Kadam (1989) with slight modifications. Sorted peanut seeds were roasted at 100°C for 20 min in an oven ((Baird & Tatlock (London) LTD. Chadwell – Heat. Essex. England).The roasting process was found to improve nutrient component, facilitate the removal of the crust and decrease the peany flavor of peanut .The roasted peanut were then de-skinned and weighed before being soaked in water for at least 12 h. The de-skinned roasted peanut kernels were then washed with clean distill water. The roasted kernels were then mixed with water in a ratio of 1:5w/w [peanuts (200g): water (1L)] and transferred to a blender (Panasonic – MX – 101 SP2). Where they were blended for 5 min at medium speed .The slurry formed was filtered using a double layered cheese cloth to prepare the peanut milk.

3.2.2. Preparation of millet milk

3.2.2.1. Malting of millet

The yellow millet (*V. Proso*) was malted following the procedure reported by Kabeir *et al* .(2005). Cleaned millet were washed and soaked in twice its volume with distilled water in 2l beakers, and placed in a temperature-controlled water bath (Scott- Science UK. Model LWB – 122D –Serial N O. 06122858) at 30°C for 12 h. Water was renewed every 6 h during the soaking period to avoid fermentation. For germination, the millet were spread on aluminum dishes and incubated for 48 h at 30°C. During the germination period the millet were turned and rinsed every 12 h with distilled water to promote aeration and prevent mould development. Germinated millet were dried in an oven at 50°C for 48 h, after that the roots of the germinated millet were removed and the malted millet were ground into a flour and sieved through a 355-µm screen. The flour was packed in a plastic container and kept at refrigeration temperature until used.

3.2.2.2. Preparation of millet milk

Yellow millet (*V. Proso*) milk was prepared according to procedure by Kabeir *et al(2005)*, with some modifications. 200g cleaned yellow millet was weighted, washed and soaked in twice its volume with water in 2l beaker, and placed at room temperature for about 7h .Water was drained and millet was blended with 800ml clean water at medium speed for 5 minutes. The slurry formed was filtered using a double layered cheese cloth and boiled in hot plate at 70˚C for 3 min magnetic stirrer was used for mixing .Malted millet flour was added in ratio 1:5 w/w after cooling at 37 ˚C and maintain for 14 min to prepared millet milk with low viscosity and flowing characteristics in addition TSS was high recording values around (6).

3.2.3. Preparation of fermentation inoculums

B.longum BB536 was obtained from the stock culture of microbiology laboratory (Department of Food Science Technology, Collage of Agriculture Studies, SUST. The strain was maintained at -20 ˚C in 20% glycerol solution. Stock culture was prepared by activation of the strain in skim milk, incubate an aerobically at 37 ˚C for 24h. The obtained culture was reactivated again under the same conditions to prepare enough stock for the experiment. The working culture was prepared by twice successive transformation in 10% sterilized skim milk (121˚C for 15 min) and incubation at 37 ˚C for 24h.

3.2.4. Growth medium and fermentation conditions

Growth medium were formulated from fresh cow milk, pure peanut milk, pure millet milk in addition to three different blends based on peanut milk prepared by partial substitution of (A), (B), (C) with millet milk. Formulated medium were sterilized (121˚C for 15 min) and inoculated with a 3% active culture of *B. longum BB536* followed by incubation at 37 ◦C for 24h.

3.2.5. Enumeration of viable cell

MRS medium was used to enumerate *B. longum BB536* of different fermented as beverages using the plate count technique. Fermented Samples were drawn at initial and every 6h intervals during fermentation. One ml of fermentation broth was diluted in peptone water, followed by plating on Rogosa agar (MRS) supplement with 0.05% L- cystiene. The plates were incubated an aerobically at 37 ˚C for 48 h. The growth was calculated as Colony Forming Unit per ml (CFU/ml).

3.2.6. Physico-Chemical compostion

3.2.6.1 Determination of reducing sugars

Ten gram of sample was weighted in volumetric flask. The volume of the solution was completed to 100 ml in conical flask. Burrete (50 ml) was filled with the prepared sugar solution. Ten milliliters of sugar solution was transferred into a conical flask containing 10 ml Fehling's solution representing 5 ml of Fehling A (6.928 gm CuSo4.5 H_2O per 100ml distilled water) and 5 ml Fehling B (34.6 sodium potassium titrate and 10 gm NaOH per 100 ml distilled water) mixed well and then heated moderately to boiling on an electrical hot plate heater. The liquid was kept boiling for about 2 minutes then 3 drops of methylene blue indicator (1%) was added. The titration was then completed by the addition of sugar solution drop by drop until the color of the indicator disappeared and red brick color appeared.
The reducing sugar was calculated from the following equation according to, Schneider (1979).

$$\text{Reducing sugar (\%)} = \frac{\text{mg}\frac{\text{of sugar}}{100\text{ml}}\text{solution} \times \text{dilution factor} \times 100}{1000 \times \text{weight of the sample}}$$

3.2.6.2. Determination of titratable acidity

The titratable acidity (TA) of the different fermented beverages was determined according to AOAC method (1990). Ten ml of sample were weighted into a conical flask. Distilled water was added until the volume in the flask was 150 ml. The sample was then vigorously agitated and filtered. Twenty five milliliters of the filtrate were pipette into a porcelain dish, five drops of phenolphthalein added, and the sample was titrated against 0.1N NaOH till a fain pink color that lasted for at least 30 seconds was obtained. Acidity of different beverage samples was calculated from the following equation:

$$\text{Titratable acidity} = \frac{(\text{N NaoH}) \times (\text{mls NaOH}) \times 0.9}{\text{Weight of sample}} \times 100$$

Were:

N = Normality of NaOH.

0.9 = Factor of lactic acid.

3.2.6. 3. Determination of total soluble solids (TSS)

Total soluble solids (TSS) of the fermented beverages were determined at room temperature using digital refractmeter with degree Brixº scale 0-100 according to AOAC (1990).

3.2.6.4. Determination of pH value

The pH value of the different fermented beverages was determined using a pH-meter (model HI 8521 microprocessor bench PH/MV/C˚ meter. Romania). Two standard buffer solution of pH 4.00 and 7.00 were used for calibration of the pH meter at room temperature. The pH meter was allowed to stabilize for one minute and then the pH of the fermented samples was directly measured.

3.2.7. Proximate Analyses

3.2.7.1 Determination of moisture content

Moisture was determined according to the modified method of AOAC (1990). Five grams of the sample was weight in sensitive balance, after weighting the dishes was transferred to an oven (Kat-NR. 2851, Electrohelios, Sweden) at 105 ± 0.1˚C for 6 hours. Afterwards, the dish with sample was transferred to desiccators and allows to cool at room temperature before reweighting. Moisture content was calculated according to the following formula:

Moisture content (%) =

$$\frac{M2 - M3}{M2 - M1} \times 100$$

Where:

M_1 = mass of dish + cover.

M_2 = mass of dish + cover + sample before drying.

M_3 = mass of dish + cover + sample after drying.

3.2.7.2. Determination of fat content

Fat content was determined according to the official method of AOAC (1990). A sample of 5g was weighed into an extraction thimble and covered with cotton, and then extracted with hexane. The thimble containing the sample and a pre-dried weight extraction flask containing about 100 ml hexane was attached to the extraction unit. The extraction process was conducted for 16h. At the end of the extraction period, the flask was disconnected from the unit and the solvent was evaporated. Later, the flask with the remaining crude hexane extracted was put in an oven, cooled to room temperature reweight and the dried extract was registered as fat content.

Crude fat content (%) =

$$\frac{W2 - W1}{\text{Sample of weight}} \times 100$$

Where: w1= The weight of the empty extraction flask.

W2= The weight of the extraction flask after the extraction process.

3.2.7.3 Determination of protein content

Protein content of different fermented beverages was determined by Kjeldhal method according to the AOAC (1990) method as follow:

1. **Digestion**: two gram of the different fermented products were weighed in a crucible and transferred to a digestion flask with two tablets catalyst (mercury). 25 ml of concentrated sulphuric acid were added to the samples, the flask was placed on the digestion apparatus, heated until the mixture was colour less. Than the flasks were allowed to cool.
2. **Distillation**: 25 ml of boric acid and three drop of bromocresol green+ methyl red indicator were added to each receiving flask. The digested samples were transferred from the digestion flask to volumetric flask and the volume was completed to 100 ml by distilled water. The receiving flask was placed on the distillation rack with the tip of the condenser extended below the surface of the acid. Immediately 5 ml of the diluted samples were added from the funnel of the distillation apparatus, then 10 ml NaOH (40%) was gently added. The distillation was continued until the volume in the receiving flasks were 7 ml, then the flask were removed from the distillatory.
3. **Titration**: The samples in the receiving flask were titrated against 0.1 N HCL. The colour was change from green to purple. The nitrogen content was calculated as follows:

$$\mathbf{N\%} = \frac{\boldsymbol{ml\ HCL \times Normality\ of\ HCL}(\mathbf{0.1}) \times \mathbf{0.014} \times \mathbf{100}}{\mathbf{Sample\ weight}}$$

Protein (%) = (N %) × 6.25

Where N = Nitrogen content.

0.014=molecular weight of nitrogen/1000

3.2.7.4. Ash content

The ash content of samples was determined according to the AOAC (1990) method. A 2g of the deferent fermented beverages were weighed into a clean dry porcelain crucible and placed in muffle furnace (model Tipoforon Z A No 18203 Get Ran 1002) at 600˚C for 6 hours. The Crucible was transferred to

desiccators, cooled to room temperature and weighed .The ash content was calculated as follows:

$$\text{Ash content (\%)} = \frac{W1-W2}{\text{Weight of sample}} \times 100$$

Where:

W1 = Weight of crucible with ash.

W2 = Weight of empty crucible.

3.2.7.5. Determination of crude fiber

Fiber was determined according to official method of AOAC(1990) .About 2g of a defatted sample was placed into a conical flask containing 200ml of H_2SO_4(0.26N). The flask was fitted to a condenser and allowed to boil for 30 minutes. At the end of the digestion period, the flask was removed and the digestate was filtered through a proclaim filter crucible (No.3). After that, the precipitate was repeatedly rinsed with distilled boiled water followed by boiling in 200ml NaOH (0.23N) solution for 30 min under reflux condenser and the precipitate was filtered. Rinsed with hot distilled water, 20 ml ethyl alcohol (96%) and 20ml diethyl ether.

Finally, the crucible was dried at 105 C^o until a constant weight was obtained and the difference in weight was considered a crude fiber.

Crude fiber % =

$$\frac{[(\text{Dry residue + crucible(g)- (ignited residue +crucible (g))}]}{\text{Sample weight}} \times 100$$

3.2.7.6. Calculation of carbohydrates

Carbohydrates were calculated by difference according to the following:

Total carbohydrates = 100% - [Moisture (%) + Protein (%) +Fat (%) + fiber (%) and Ash (%)].

3.2.8. Determination of minerals

Potassium (K) and calcium (Ca) were determined by flame photometer (Sherwood Flame Photometer i410, Sherwood Scientific Ltd. Cambridge, UK) according to procedure of AOAC (1990). The knob of flame photometer was adjusted to potassium and calcium respectively and reading was set to zero using deionized water. Blank solution was run and reading was again set to zero. Standard solution of each mineral was run and recorded the reading of flame photometer. The reading of potassium and calcium in beverages sample was taken by running the sample one by one. Standard solution was run after every samples .The standard curves (appendix I, II,) were obtained by plotting absorbance values of standards against appropriate concentrations of these two elements. One gram of dried beverage samples was subjected to wet digestion method as described by Richards (1968). Then analysis was conducted through absorption spectrophotometer (Varian AA 240, Victoria, Australia) for determination of minerals (Mg and Fe) using standard curve. To determine phosphorus content in beverage samples, colorimetric estimation method was used as described by Kitson and Mellon, (1944).

3.2.9. The storage of the fermented products

Fermented products were held at refrigerator for a period of 2 weeks. During the storage period, viable counts of *B.longum BB536*, pH, TSS, acidity, moisture, minerals and sugars of the fermented beverages were determined. Analysis for samples carried out at initial (0 days), after 1 week and after 2 weeks**.**

3.2.10 Statistical analysis

One- way ANOVA and two sample paired test were performed to examine significant differences between normally distributed data of replicated measurement. Probability level of less than 0.05 was considered significant ($p<0.05$). All data were analyzed using vision 16 MINITAB statistical software for windows (2006).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Physico-chemical composition of the raw peanut and Millet

Referring to our results in Table1, the level of raw peanut were 0.13, 2.24, 2.77 and 2.76 respectively, levels of moisture, fat, protein and carbohydrates and while lower levels fiber (6.42%) and ash (1.37%) recorded compared to reference value of raw peanut in food composition table. In general, these variations might be due to the variety of peanut species, produce, storage and harvesting phase.

Results of the proximate composition of peanut roasted were presented in Table (1) too. It shows that moisture content of raw peanut was 6.13 which decreased to 3.42 after roasting processes. It can be noticed that moisture content decreased significantly. These results are in agreement with those found by Damame *et al*. (1990), Abayomi *et al*. (2002) and Adegoke *et al.* (2004). Roasting of peanut also increased fat, proteins, fiber, ash and Carbohydrates in ratio of 0.23, 1.43, 0.32, 0.1 and 0.62% respectively. These results on composition of roasted peanut are in agreement with those reported by Abayomi *et al.* (2002) and Adegoke *et al.* (2004).

Results of the proximate composition of millet are presented in the same (Table 1). Millet contained high levels of portion and carbohydrate were 2.11and 8.25 respectively .While the decrease levels of moisture, fat, fiber and ash were 3.19, 0.25, 6.25 and 0.36, respectively.

Data of chemical composition of raw peanut and millet were given in same table (1).Raw peanut contains high levels of fat and protein as compared to levels of raw millet. While the levels of moisture, fiber, ash and carbohydrate were high in raw millet than raw peanut. These variations in levels of

chemical components between raw peanut and millet is due to sources variation.

4.2. The growth of *Bifidobacterium longum* BB536 during fermentation of different formulated beverages

Comparative growth of *Bifidobacterium longum BB536* cultured in different beverages (cow milk, peanut milk, millet milk and different blends) is shown in table 2.

There were significant ($p<0.05$) increases in *B. longum BB536* viable count by extended fermentation period in all type of formulated beverages, as compared to strain level at beginning of fermentation. The maximum growth of *B. longum BB536* was attained at 18h in all type of fermented beverages, except in fresh cow milk it was attained at 12 h fermentation. After the maximum growth all the strain declining was observed in all types of fermented beverages (Table2)

The rate of *B. longum BB536* increases in different fermented beverages were 3.15, 2.9, 2.89, 2.76, 2.43 % and 2.1%in fermented peanut milk, millet milk, cow milk, blend (B), blend (A), and blend (C), respectively. These variations in growth rate of *B. longum BB536* could be attributed to variances in availability of nutrients required for growth in the different fermented beverages. Peanut contains almost the essential nutrient for strain growth. Combination of peanut with millet could complement the nutrient component of growth medium. However, the growth of strain *B. longum BB536* was affected by supplementation with millet milk (table2).This decline in rate of growth could be due to increase viscosity of beverages by supplementation with millet milk. However at 24hour of fermentation there was reduction in number of *B. longum BB536* in all fermented beverages. That could be due to the accumulation of acids or reduction of availability of nutrient required for the growth as stated by Kabeir *et al.*, (2005). In spite of declining in viable

count of *B. longum BB536* in all types of fermented beverages at 24h fermentation, the count still above the number required to presence in probiotic food which is at least 6 log cfu/ml fermented product, (Viderola and Reinheimer, 2000). Table 2 shows, the viable count of the strain in blend C (5.77 cfu/ml) is not fulfill propiotic requirement in food.

4.3. changes in beverages during fermentation process with *Bifidobacterium longum* BB536

4.3.1 pH changes

During fermentation process with strain BB536 there were significant ($P<0.05$) decrease in pH levels in all types of beverages by extended fermentation period to 24 h (Table 3).The decreases in pH are due to increased acids production during fermentation process as a result of fermented sugar by *Bifidobacterium BB536,* which to produce acetic to lactic acid in ratio of 1.5:1as reported by De Vries *et al.* (1967). Moreover, the accumulated acids produced by *bifidobacterium* strain, reported to have antibacterial activity such as prevention of the proliferation of pathogens (Bullen *et al*., 1976)**.** The rate of pH decreases at maximum growth of strain BB536 were .0.67, 0.64, 0.60,0.60 , 0.57 and 0.37in fermented blend (B), peanut, blend (C), blend (A) ,millet and then the cow milk respectively. These variances in pH reduction are not expected based on variations of strain *BB536* in growth level in different beverages (Table2). Therefore, millet milk may offer some buffer properties to fermentation medium, explained by initial pH of different formulated beverages (Table2). On other hand levels of pH attained at maximum growth (18h) and end of fermentation at (24h) was above 5, and this is more suitable for consumption, further expected to have potential as carrier for the strain survival during the storage period.

Table 1: Proximate composition (%) of raw peanut and millet*

Components (%)	Raw Peanut	Roasted peanut	Peanut reference values**	Raw Millet	Millet reference values **
Moisture	6.13 ± 0.21	3.42 ± 0.18	6.00 ± 1.70	8.41 ± 0.023	11.6 ± 0.7
Fat	48.14 ± 0.25	48.37 ± 0.31	45.90 ± 3.00	3.85 ± 0.32	4.1 ± 0.7
Proteins	25.17 ± 0.31	26.60 ± 0.44	22.40 ± 1.60	13.01 ± 0.08	10.9 ± 1.00
Fiber	2.08 ± 0.15	2.40 ± 0.20	8.50 ± 7.70	2.25 ± 0.09	8.8 ± 1.00
Ash	1.13 ± 0.13	1.23 ± 0.15	2.30 ± 0.10	1.64 ± 0.06	2.00 ± 1.9
Carbohydrates	17.36± 1.047	17.98 ± 0.58	14.60 ± 0.10	70.85 ± .46	62.6 ± 0.00

*Values are mean ± SD for replicate independent runs.

**Values from food composition table (Barbara *et al.*, 2012)

Table 2: The viable count of *Bifidobacterium longum* BB536 log (CFU/ml) during fermentation period of different beverages*

Time(h)	*Bifidobacterium longum BB536* growth in beverages					
	Cow milk	**Peanut milk**	**Millet milk**	**A**	**B**	**C**
0	4.8 ± 0.12^{a}	5.68 ± 0.10^{d}	$4.89 \pm .06^{d}$	$5.51 \pm .05^{e}$	4.84 ± 0.08^{d}	4.53 ± 0.07^{d}
6	5.84 ± 0.15^{b}	6.96 ± 0.04^{c}	5.61 ± 0.18^{c}	5.89 ± 0.02^{d}	5.78 ± 0.07^{c}	4.92 ± 0.04^{c}
12	7.69 ± 0.14^{c}	7.85 ± 0.056^{a}	6.89 ± 0.06^{a}	$7.66 \pm .11^{b}$	6.95 ± 0.04^{b}	5.77 ± 0.09^{b}
18	6.86 ± 0.11^{d}	8.83 ± 0.07^{b}	7.79 ± 0.06^{b}	$7.94 \pm .05^{a}$	7.60 ± 0.08^{a}	$6.63 \pm .04^{a}$
24	6.03 ± 0.01^{d}	7.60 ± 0.08^{b}	6.68 ± 0.11^{b}	$6.85 \pm .02^{c}$	6.77 ± 0.09^{b}	5.77 ± 0.01^{b}

* Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letter in the same Colum are significantly different at $p<0.05$.

A=Blend1 was prepared using 85% peanut milk and 15% millet milk.

B= Blend 2 was prepared using 70% peanut milk and 30 % millet milk.

C= Blend 3 was prepared using 55% peanut milk and 45% millet milk.

Table3: pH changes during the growth of *Bifidobacterium longum* BB536 in beverages*

Beverages / Time (h)	PH					
	Peanut	**Millet**	**Cow**	**A**	**B**	**C**
0	6.88 ± 0.02^{a}	6.72 ± 0.09^{a}	6.24 ± 0.49^{a}	6.80 ± 0.04^{a}	6.84 ± 0.04^{a}	6.71 ± 0.07^{a}
6	6.35 ± 0.05^{b}	6.46 ± 0.11^{ab}	6.42 ± 0.54^{a}	6.37 ± 0.02^{bc}	6.49 ± 0.01^{b}	6.58 ± 0.01^{b}
12	6.34 ± 0.04^{b}	6.28 ± 0.15^{b}	6.05 ± 0.64^{a}	6.52 ± 0.05^{b}	6.37 ± 0.05^{b}	6.11 ± 0.01^{c}
18	6.24 ± 0.02^{b}	6.15 ± 0.04^{b}	6.00 ± 0.22^{a}	6.20 ± 0.03^{c}	6.17 ± 0.07^{c}	6.11 ± 0.01^{c}
24	$5.15 \pm .04^{c}$	5.06 ± 0.06^{c}	5.83 ± 0.11^{a}	5.90 ± 0.12^{d}	5.69 ± 0.04^{d}	5.58 ± 0.01^{d}

*Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letter in the same Colum are significantly different at $p<0.05$.

A= Blend 1 was prepared using 85% peanut milk and 15% millet milk.

B= Blend 2 was prepared using 70% peanut milk and 30 % millet milk

.C= Blend 3 was prepared using 55% peanut milk and 45% millet milk.

4.3.2 Total Soluble Solids

Table (4) shows the changes in TSS during fermentation of different formulated beverages with *B. longum* BB536.

There were significant ($P<0.05$) decrease in TSS levels in all types of fermented beverages by extended fermentation period to 24 h. The rates of TSS decreases at maximum growth were 0.3, 0.4, 1.3, 0.1and 0.3in fermented peanut milk, millet milk followed by the three different blends, respectively. At 24 h of fermentation the amount of TSS reductions were 1.75, 0.75, 0.7, 0.45 and 0.35 in the fermented millet milk, peanut milk, blend (C), blend (A) and blend (B), respectively. Enzymatic activity of the strain plays a vital role in rate of TSS fermentations.

4.3.3 Reduction of sugars

There were significant ($P<0.05$) decrease in sugars levels of all fermented products by extended fermentation period to 24 h (Table 5). *Bifidobacterium* strain ferment sugars and produce organic acids mainly acetic, lactic, probunic, butyric and other organic acids.

The rates of sugar reduction at maximum growth of strain *BB536* were 0.15, 0.04, 0.03,0.02 , and 0.01 in fermented peanut milk, blend(C), blend (B), millet milk and then blend (A) respectively. Moreover, after 24h of fermentation, the maximum reductions in sugar were 0.19, 0.11, 0.1, 0.08 and 0.03 in fermented, blend (C), blend (A), millet milk, blend (B), and then fermented peanut milk, respectively. These variances in invert sugar reduction refer to the strain activity in different fermented beverages, and correlated well with rate of maximum growth of the strain. There was linear relationship between growths of sugars reduction.

Table 4: TSS changes during the growth of the strain *Bifidobacterium longum* BB536 in different beverages *

Beverages / Time(h)	TSS (%)				
	Peanut	**Millet**	**A**	**B**	**C**
0	1.55 ± 0.07^{a}	6.15 ± 0.07^{a}	3.35 ± 0.07^{a}	3.10 ± 0.00^{ab}	4.10 ± 0.14^{a}
6	1.25 ± 0.07^{a}	5.50 ± 0.14^{b}	3.20 ± 0.14^{a}	3.40 ± 0.14^{a}	3.55 ± 0.07^{bc}
12	0.80 ± 0.14^{b}	4.95 ± 0.21^{c}	3.05 ± 0.07^{a}	3.20 ± 0.14^{a}	3.20 ± 0.14^{c}
18	1.25 ± 0.07^{a}	5.75 ± 0.07^{ab}	2.05 ± 0.07^{b}	3.00 ± 0.00^{ab}	3.80 ± 0.14^{ab}
24	0.50 ± 0.14^{b}	4.50 ± 0.00^{c}	1.60 ± 0.14^{c}	2.65 ± 0.21^{b}	3.10 ± 0.00^{c}

*Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letter in the same Colum are significantly different at $p<0.05$.

A= Blend 1 was prepared using 85% peanut milk and 15% millet milk.

B= Blend 2 was prepared using 70% peanut milk and 30 % millet milk.

C= Blend 3 was prepared using 55% peanut milk and 45% millet milk

Table 5: Reducing of sugars (mg /100ml) % during the growth of *Bifidobacterium longum BB536* in different beverages*

Beverages / Time(h)	Reducing of sugars (%)				
	Peanut	**Millet**	**A**	**B**	**C**
0	0.16 ± 0.01^{a}	0.23 ± 0.02^{a}	0.15 ± 0.03^{a}	0.16 ± 0.01^{a}	0.24 ± 0.01^{a}
6	0.03 ± 0.01^{c}	0.18 ± 0.02^{ab}	0.10 ± 0.00^{ab}	0.15 ± 0.03^{a}	0.18 ± 0.01^{c}
12	0.09 ± 0.00^{b}	0.16 ± 0.01^{bc}	0.09 ± 0.01^{b}	0.11 ± 0.01^{ab}	0.14 ± 0.01^{d}
18	0.10 ± 0.00^{ab}	0.21 ± 0.01^{ab}	0.14 ± 0.01^{ab}	0.13 ± 0.01^{a}	0.20 ± 0.00^{b}
24	0.07 ± 0.03^{bc}	0.11 ± 0.01^{c}	0.03 ± 0.01^{c}	0.05 ± 0.00^{b}	0.01 ± 0.00^{e}

*Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letter in the same Colum are significantly different at $p<0.05$.

A=Blend 1 was prepared using 85% peanut milk and 15% millet milk.

B=Blend 2 was prepared using 70% peanut milk and 30 % millet milk.

C=Blend 3 was prepared using 55% peanut milk and 45% millet milk.

4.3.4 Titratable acidity changes

Table 6 shows the titratable acidity of different fermented beverages. There, were significant ($p<0.05$) increase in titratable acidity by extended fermented period to 24h. At maximum growth of strain *BB536* (18h), the rates of increase were 0.2, 0.09, 0.08,0.08 and 0.07 in fermented peanut milk, blend (A) , blend (B) , millet milk and blend (C) ,respectively. The increased in acidity is explained by accumulation of lactic acid and other organic acids produced during fermentation of the formulated beverages (Sefa –Dedeh *et al*., and Afoakwa,2003).

4.4. Chemical composition of different beverages fermented with growth of *Bifidobacterium longum BB536* time

Table 7 shows the chemical composition of peanut milk and millet milk beverages fermented with *B. longum BB536* at initial (0h) and maximum growth time (18h). The result, revealed that there were no significant ($p>0.05$) changes in compound of beverages .In fermented peanut milk the moisture, portion , ash and fiber increased while the fat, carbohydrate and total soluble solids were decreased in peanut milk .

The result presented in table 7 shown the chemical compound of the blends increase in protein, ash, fiber carbohydrate and total soluble solids, while decreases in moisture and fat in millet milk. Moreover, the result presented in table 8, revealed there were significant ($p<0.05$) decrease in fats and TSS (blend A), as well as decreased in protein content (blend C). Significantly ($p<0.05$) there were increase in moisture, fiber and protein of blends A, B and C, respectively. There are no significant difference in increase of blend of (A), also, moisture of blend (B) and (c). In addition, there are no significant ($p\leq0.05$) differences in decrease of carbohydrate of blend (A), fat. Ash, fiber, carbohydrate and TSS of blend (C), fat, protein, ash , carbohydrate and total soluble solids of blend (B).

Table 6: Titratable acidity (%) during the growth of the strain *Bifidobacterium longum BB536* in different beverages*

Beverages / Time(h)	Titratable acidity (%)				
	Peanut	**Millet**	**A**	**B**	**C**
0	0.02 ± 0.00^{d}	0.18 ± 0.01^{a}	0.13 ± 0.00^{b}	0.15 ± 0.05^{a}	0.17 ± 0.01^{b}
6	0.11 ± 0.00^{c}	0.20 ± 0.00^{a}	0.16 ± 0.06^{ab}	0.15 ± 0.00^{a}	0.18 ± 0.00^{b}
12	0.18 ± 0.01^{b}	0.21 ± 0.01^{a}	0.17 ± 0.02^{ab}	0.18 ± 0.01^{a}	0.20 ± 0.00^{ab}
18	0.22 ± 0.00^{ab}	0.26 ± 0.04^{a}	0.22 ± 0.00^{ab}	0.23 ± 0.03^{a}	0.24 ± 0.04^{ab}
24	0.25 ± 0.02^{a}	0.27 ± 0.04^{a}	0.26 ± 0.02^{a}	0.24 ± 0.03^{a}	0.27 ± 0.02^{a}

*Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letter in the same Colum are significantly different at $p<0.05$.

A=Blend 1 was prepared using 85% peanut milk and 15% millet milk.

B=Blend 2 was prepared using 70% peanut milk and 30 % millet milk.

C=Blend 3 was prepared using 55% peanut milk and 45% millet milk.

Table 7: Chemical composition of peanut and millet beverages fermented with *Bifidobacterium longum BB536* growth time*

Component	peanut milk		millet milk	
	Initial growth time	Maximum growth time	Initial growth time	Maximum growth time
Moisture (%)	87.37 ± 0.37[a]	88.00 ± 0.01[a]	92.41 ± 0.27[a]	91.87 ± 2.50[a]
Fat content (%)	2.90 ± 0.04[a]	2.83 ± 0.08[a]	1.54 ± 0.18[a]	1.18 ± 0.02[a]
Protein content (%)	3.11 ± 0.13[a]	3.450 ± 0.14[a]	1.90 ± 0.02[a]	1.95 ± 0.05[a]
Ash content (%)	0.19 ± 0.01[a]	0.21 ± 0.01[a]	0.09 ± 0.00[a]	0.18 ± 0.02[a]
Total soluble solid (%)	12.63 ± 0.20[a]	12.00 ± 0.07[b]	7.59 ± 0.27[a]	8.13 ± 2.50[a]
Carbohydrates (%)	6.53 ± 0.18[a]	5.48 ± 0.104[a]	3.91 ± 0.45[a]	4.66 ± 2.49[a]
Fiber (%)	0.017 ± 0.00[a]	0.032 ± 0.00[a]	0.15 ± 0.03[a]	0.16 ± 0.01[a]

* Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letters in the same raw of each specific beverage are significantly different at $p<0.05$

4.5. Minerals of different fermented beverages

Table 9 and 10 shows the minerals content (Ca, K, P, Mg and Fe) of different fermented beverages with *B. longum BB536.* At initial (0h) and mat maximum growth time (18h). there were no significant ($p>0.05$) increase in Ca, p and Fe and significant ($p<0.05$) decrease in K and Mg in peanut milk, also an increase in Ca and Mg, as well as decrease in P, K and Fe in millet milk .These results in table10 as increase (Ca, K, P, Mg and Fe) of all beverage but only one decrease (Fe) in blend (A). To some extent are similar to those reported by Isanga and Zhang (200).

4.6. survival of *Bifidobacterium longum* BB536 log (CFU/ ml) during the storage of different fermented beverages

Table 11 shows the viable counts of *B. longum* BB536 during refrigeration storage of different formulated beverages. There were significant ($p<0.05$) reduction in *B. longum* BB536 viable count in all fermented beverages. The rate of reduction in the first week of the refrigeration storage were 2.39, 2.08, 1.84, 1.7, 1.43 and 0.77 CFU /ml in fermented millet milk, peanut milk, blend (C), blend (B), blend (A) and cow milk, respectively . Hopefully, the final viable count of *B. longum* BB536 in fermented peanut milk, cow milk and blend (A) was above than the minimum number required to presence in probiotic to exert health benefits upon consumption, which was 6 log CFU/ml. While Alkaline *et al*. (2004) noted a significant reduction on *B. longum BB536* in yogurt after one week at refrigeration.

Table 8: Chemical composition of different blends beverages fermented with *Bifidobacterium longum* BB536 growth time *

Component	A		B		C	
	Initial growth time	Maximum growth time	Initial growth time	Maximum growth time	Initial growth time	Maximum growth time
Moisture (%)	88.83±0.14[a]	89.96±0.20[b]	89.90±0.05[a]	91.39±0.58[a]	90.15±0.12[a]	93.28±0.88[a]
Fat content (%)	2.78±0.05[a]	2.20±0.02[b]	2.60±0.09[a]	2.32±0.00[a]	2.17± 0.01[a]	2.14±0.03[a]
Protein content (%)	2.70±0.16[a]	2.83±0.01[a]	2.72± 0.0[a]	2.67±0.07[a]	2.34 ±0.05[a]	2.27±0.07[b]
Ash content (%)	0.17± 0.00[a]	0.18±0.00[a]	0.18±0.02[a]	0.17±0.02[a]	0.18± 0.01[a]	0.17±0.00[a]
Fiber (%)	0.08±0.00[a]	0.13±0.05[a]	0.09±0.00[a]	0.17±0.00[b]	0.19±0.00[a]	0.18±0.00[a]
Carbohydrates (%)	5.43±0.07[a]	4.70±0.24[a]	4.52±0.066[a]	3.30±0.48[a]	4.93± 0.08[a]	1.955±0.90[a]
Total soluble solid (%)	11.17±0.14[a]	10.04± 0.2[b]	10.10±0.049[a]	8.61±0.58[a]	9.85 ±0.12[a]	6.718±0.88[a]

* Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letter in the same raw of each specific beverage are significantly different at $p<0.05$.

A=Blend 1 was prepared using 85% peanut milk and 15% millet milk.

B= Blend 2 was prepared using 70% peanut milk and 30 % millet milk.

C= Blend 3 was prepared using 55% peanut milk and 45% millet milk.

Table 9: Minerals contents (mg/100 ml) of fermented peanut and millet beverages fermented with *Bifidobacterium longum* BB536 growth time*

Mineral	Peanut milk		Millet milk	
	Initial growth time	Maximum growth time	Initial growth time	Maximum growth time
Ca	82.24±0.60[a]	85.04± 2.43[a]	56.91±7.79[a]	60.04 ±1.81[a]
K	170.02±2.40[a]	116.35±2.87[b]	136.22±7.07[a]	134.83±3.52[a]
P	94.04±6.05[a]	116.97±0.70[a]	103.32±9.90[a]	99.22±0.77[a]
Mg	116.50±8.24[a]	93.37± 8.55[b]	72.90±9.22[a]	77.40±0.12[a]
Fe	1.049±0.372[a]	8.848±0.18[b]	7.858±1.22[a]	7.400±1.02[a]

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw of each specific beverage are significantly different at $p<0.05$.

Table 10: Minerals content (mg/100ml) of different blended beverages fermented with *Bifidobacterium longum* BB53 growth time*

Mineral	A		B		C	
	Initial	**Maximum**	**Initial**	**Maximum**	**Initial**	**Maximum**
Ca	75.08± 3.39[a]	77.40±1.43[a]	68.40± 0.82[a]	75.82± 4.95[a]	92.32±7.07[a]	114.05±4.83[b]
K	174.82±9.19[a]	181.84±2.73[a]	176.57±6.71[a]	182.40±2.85[a]	184.32±2.83[a]	185.87±0.64[a]
P	82.03±0.91[a]	115.98±3.77[b]	111.52±0.43[a]	113.04±0.39[b]	127.29±6.41[a]	118.87±2.19[a]
Mg	118.05±0.61[a]	125.04±3.42[a]	119.23±5.04[a]	127.12±0.43[a]	169.86±2.19[a]	173.25±4.47[a]
Fe	1.87± 0.08[a]	1.43± 0.27[a]	2.165± 0.36[a]	2.541±0.31[a]	3.35±0.19[a]	3.607±0.30[a]

*Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw of each specific beverage are significantly different at $p<0.05$.

A= blend 1 was prepared using 85% peanut milk and 15% millet milk.

B=blend 2 was prepared using 70% peanut milk and 30 % millet milk.

C= blend 3 was prepared using 55% peanut milk and 45% millet milk.

Furthermore, the reductions in the second week at the refrigeration storage were 1.21, 0.99, 0.98, 0.89, 0.86 and0.16 in the fermented blend (B), peanut milk , blend (A), blend (C), millet milk and cow milk , respectively (Table11). However, Kabeir *et al* .,(2005) reported that survivability of *B. longum BB536* under refrigeration storage of fermented Sudanese Media beverages was not affected for a period of 2 week. While, Nakalin *et al*. (2004) noted a significant reduction of *B. longum BB46* in yogurt after only one week refrigeration. This indicates that the viability of *Bifidobacterium* in fermented products was dependent on the carrier type and pH of the fermented products during the storage .Overall most strains of *bifidobacteria* are sensitive to pH values below 4.6. Therefore, for practical application, a pH value of the final product must be maintained above 4.6 to prevent the decline of bifidobacteria populations (Tamime a Robinson, 1985 and Modler *et al*., 1990and Laroia a Martin, 1991a). The survival of probiotic bacteria in fermented dairy bio-products depends on such varied factors as the strains used, interaction between species present, culture conditions, chemical composition of the fermentation medium (e.g. carbohydrate source), final acidity, milk solids content, availability of nutrients, growth promoters and inhibitors, concentration of sugars (osmotic pressure). As well as, dissolved oxygen (especially for *Bifidobacterium sp*.), level of inoculation, incubation temperature, fermentation time and storage temperature. The variances in survival were interpreted by the metabolic activity of *Bifidobacterium* in different fermented products, which might be affected by the composition and availability of nitrogen and carbon sources in growth media as stated by Chou and Hou (2000).

4.7 Changes in fermented beverages during the refregeation storage

4.7.1 Reducing of sugars

Table 12 shows the sugars content of the different fermented beverages during refrigeration storage.

There was significant ($p<0.05$) reduction in reducing sugars of different beverages. The rate of decreasing in all fermented beverages except in blend (A) and blend (B).The amount of sugars decrease in the first week were 0.06, 0.04, 0.03, 0.03 and 0.02 % in fermented peanut milk, fermented blend (A), fermented blend (B) , the fermented millet milk and blend (C), respectively (Table12). The amount of sugars reductions in the second weak were 0.08, 002, and 0.02 in the fermented blend (C), millet milk and peanut milk, respectively. While there an increase in the amount of reducing sugars recorded in fermented blend (A) of 0.03% and the fermented blend (B) of 0.05%.

4.7.2 Changes of pH

Table 13 shows the pH measurement of the different fermented beverages during the refrigeration storage.

There was significant ($p<0.05$) reduction in pH of all types of fermented products during the two weeks at refrigeration temperature ($4c^{o}$). The rate of pH reductions in the first week were 0.53, 0.41, 0.28, 0.24, 0.31and 0.19 in fermented cow milk, peanut milk, millet milk, blend (C), blend (A) and the blend (B), respectively. While the reductions recorded in the second week at refrigeration storage were 0.64, 0.57, 0.22, 0.2, 0.11and 0.03 in fermented blend (A), cow milk, blend (B) , millet milk , blend (C) and peanut milk , respectively . The reduction of pH is mainly due to the fermentation of sugars and accumulation of acid. That is why *Bifidobacterium* maintain a relatively acid pH in large intestine, thus preventing the proliferation of pathogens. It produces lactic acid, acetic acid, hydrogen peroxide, and bactericides. They are known to inhibit the development of pathogenic bacteria it was also reported that lactic acid and acetic acid in fermented dairy product have antibacterial effect (Bullen *et al.,* 1976).Sakai and coworkers reported that low PH and storage temperature were the most important determinations in *Bifidobacterium* mortality (Sakai *et al*., 1987)

Table 11: The survival of *Bifidobacterium longum* BB536 log (CFU/ ml) during the storage of different fermented beverages *

Type of fermented beverages **	At initial storage	After 1 week	After 2 week
peanut milk	8.83 ± 0.07^{a}	6.75 ± 0.09^{b}	5.76 ± 0.03^{c}
Millet milk	7.79 ± 0.06^{a}	5.58 ± 0.14^{b}	4.72 ± 0.09^{c}
Cow milk	7.69 ± 0.72^{a}	6.92 ± 0.46^{a}	6.76 ± 0.58^{a}
A	7.94 ± 0.05^{a}	6.51 ± 0.05^{b}	5.53 ± 0.04^{c}
B	7.60 ± 0.08^{a}	5.90 ± 0.05^{b}	4.69 ± 0.12^{c}
C	6.63 ± 0.04^{a}	4.79 ± 0.07^{b}	3.9 ± 0.03^{c}

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw are significantly different at $p<0.05$.

A=Blend 1 was prepared using 85% peanut milk and 15% millet milk.

B=Blend 2 was prepared using 70% peanut milk and 30 % millet milk.

C=Blend 3 was prepared using 55% peanut milk and 45% millet milk.

Table12: Reducing of sugar(mg /100ml) % of the different fermented beverages during refrigeration storage*

Type of fermented beverages **	At initial storage	After 1 week	After 2 week
peanut milk	0.099 ± 0.00^{a}	0.044 ± 0.01^{b}	0.018 ± 0.00^{c}
Millet milk	0.206 ± 0.01^{a}	0.175 ± 0.00^{b}	0.158 ± 0.01^{b}
A	0.139 ± 0.01^{a}	0.101 ± 0.00^{a}	0.130 ± 0.05^{a}
B	0.127 ± 0.01^{ab}	0.110 ± 0.01^{b}	0.154 ± 0.01^{a}
C	0.201 ± 0.00^{a}	0.167 ± 0.01^{a}	0.094 ± 0.10^{a}

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw are significantly different at $p<0.05$.

A=Blend 1 was prepared using 85% peanut milk and 15% millet milk.

B= Blend 2 was prepared using 70% peanut milk and 30 % millet milk.

C=Blend 3 was prepared using 55% peanut milk and 45% millet milk.

Table 13: pH of the different fermented beverages during refrigeration storage*

Type of fermented beverages **	At initial storage	After 1 week	After 2 week
peanut milk	6.24 ± 0.02^{a}	5.83 ± 0.07^{b}	5.8 ± 0.02^{b}
Millet milk	6.15 ± 0.04^{a}	5.87 ± 0.01^{b}	5.67 ± 0.00^{c}
Cow milk	6.05 ± 0.65^{a}	5.52 ± 0.79^{a}	4.95 ± 0.57^{a}
A	6.20 ± 0.03^{a}	5.89 ± 0.01^{b}	5.25 ± 0.11^{c}
B	6.17 ± 0.07^{a}	5.98 ± 0.00^{b}	5.67 ± 0.00^{c}
C	6.11 ± 0.01^{a}	5.87 ± 0.01^{b}	5.76 ± 0.00^{c}

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw are significantly different at $p<0.05$.

A=Blend 1 was prepared using 85% peanut milk and 15% millet milk.

B=Blend 2 was prepared using 70% peanut milk and 30 % millet milk.

C= Blend 3 was prepared using 55% peanut milk and 45 % millet milk.

4.7.3 Changes in TSS during the storage of different fermented beverages

Table 14 shows TSS of different fermented beverages**.** There were significant ($p<0.05$) decreases in TSS of all types of fermented beverages under refrigerated storage for two weeks. The amounts of reductions in the first week of refrigerated storage of different formulated beverages (blend (A), blend (C), millet milk, blend (B), and peanut milk) were 1.0, 0.4, 0.15, 0.15and 0.00%, respectively. The amounts of reduction in the second week of refrigerated storage of millet milk, peanut milk, blend (C) and blend (B) were 2.7, 0.55, 0.4, and 0.35, respectively. While there was increase in blend (A).

4.7.4 Changes in moisture during the storage of different fermented beverages

Table 15 shows moisture of different fermented beverages. There were significant ($p<0.05$) increases in moisture of different fermented beverages by extended storages period for two weeks. The amount of moisture increases in fermented blend (B), peanut milk, blend (C), blend (A) and millet milk were 1.01, 0.75, 0.69, 0.41 and 0.21% respectively. Over all levels, the moisture content of fermented beverages stored under refrigeration temperature ($4c^{o}$) was increased as compared to their initial value. This increase in moisture might indicate to enzymatic activity that break down the macro component into simple and release more water.

4.7.5 Changes in titratable acidity

Table 16 shows the titratable acidity of different fermented beverages throughout storage period. Titratable acidity of the different fermented products increased by extended storage period for the two weeks .The rates of titratable acidity were 0.08, 0.04, 0.04, 0.03 and 0.02% in fermented blend (C), the peanut milk , blend(B), millet milk , and the blend (A) , respectively . While the rate recorded at second week were 0.12, 0.1, 0.08, 0.03 and 0.01% in fermented blend (C), the peanut milk, millet milk, blend (A) and the blend (B), respectively . The amount of the titratable acidity was significant ($p<0.05$) increased gradually till the end of storage except in blend (A) and blend (C).

Table 14: TSS of the different fermented beverages during refrigeration storage*

Type of fermented beverages **	At initial storage	After 1 week	After 2 week
peanut milk	1.25 ± 0.07^{a}	1.25 ± 0.07^{a}	0.70 ± 0.14^{b}
Millet milk	5.75 ± 0.07^{a}	5.60 ± 0.14^{a}	2.90 ± 0.00^{b}
A	2.05 ± 0.07^{b}	1.05 ± 0.21^{c}	2.95 ± 0.07^{a}
B	3.00 ± 0.00^{a}	2.85 ± 0.07^{ab}	2.50 ± 0.14^{b}
C	3.80 ± 0.14^{a}	3.40 ± 0.14^{ab}	3.00 ± 0.00^{b}

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw are significantly different at $p<0.05$.

A=Blend 1 was prepared using 85% peanut milk and 15% millet milk.

B=Blend 2 was prepared using 70% peanut milk and 30 % millet milk

C=Blend 3 was prepared using 55% peanut milk and 45 % millet milk.

Table 15: Moisture %of the different fermented beverages during refrigeration storage*

Type of fermented beverages **	At initial storage	After 1 week	After 2 week
peanut milk	89.27 ± 0.49[a]	89.96 ± 0.05[a]	90.02 ± 0.00[a]
Millet milk	93.73 ± 0.13[a]	93.94 ± 0.08[a]	94.00 ± 0.01[a]
A	89.959 ± 0.20[a]	90.365 ± 0.53[a]	90.465 ± 0.53[a]
B	91.39 ± 0.58[a]	92.40 ± 0.57[a]	92.48 ± 0.53[a]
C	93.28 ± 0.88[a]	94.03 ± 0.36[a]	94.03 ± 0.36[a]

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw are significantly different at $p<0.05$.

A=Blend 1 was prepared using 85% peanut milk and 15% millet milk.

B=Blend 2 was prepared using 70% peanut milk and 30% millet milk.

C=Blend 3 was prepared using 55% peanut milk and 45 % millet milk.

Table 16: Titratable acidity of the different fermented beverages during refrigeration storage*

Type of fermented beverages **	At initial storage	After 1 week	After 2 week
peanut milk	0.25 ± 0.02^{ab}	0.29 ± 0.02^{a}	0.21 ± 0.00^{b}
Millet milk	0.26 ± 0.04^{b}	0.29 ± 0.01^{b}	0.39 ± 0.01^{a}
A	0.26 ± 0.02^{a}	0.28 ± 0.06^{a}	0.25 ± 0.04^{a}
B	0.24 ± 0.03^{a}	0.28 ± 0.02^{a}	0.29 ± 0.01^{a}
C	0.24 ± 0.04^{b}	0.36 ± 0.03^{a}	0.24 ± 0.01^{b}

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw are significantly different at $p<0.05$.

A=Blend 1 was prepared using 85% peanut milk and 15% millet milk.

B=Blend 2 was prepared using 70% peanut milk and 30% millet milk.

C=Blend 3 was prepared using 55% peanut milk and 45 % millet milk.

4.8. Changes in minerals content during storage of different fermented beverages

Table 17 shows the minerals content of different fermented beverages**.** There were no significant ($p>0.05$) changes in minerals content except Fe and K in (peanut milk) and P in (millet milk) of fermented beverages by extended storage period for the two weeks. In the first week the rates of decreases in Ca, Mg and Fe were 2.9, 17.18 and 7.96 %, respectively. There were increases in K (11. 8%) and P (4.84%) in formula peanut milk. Moreover, in formula millet milk the increase in K, P, Mg and Fe were 0.04, 17.6, 0.32 and1.55%, respectively. While, there was decrease in Ca (3.15%). In the second weeks the rates of increase of Ca, Mg, Fe, K and P in formula millet milk were 5.47, 6.5, 2.28, 4.37 and17.74%, respectively. While, in formula peanut milk there was increase in Ca (2.83 %) and K (7.49%). However, in the second week Mg (0.5%), P (27.57%) and Fe (8.24) decreased in formula peanut milk.

Table 18 shows the minerals content of the different blend fermented beverages during first refrigeration storage, the increase of Ca, Fe and k (blend A) were 0.26 ,5.67 and 0.25% , respectively. while there are decreases in P(5.44%) and Mg(2.15%).The rates decrease of(Ca, K, Mg and Fe) in blend(B) were 6.94 ,5.18,6.21and 0.71% respectively. while increase of P(7.93%).In blend (C),the rates increase of (Ca, K and P) were10.24,4.38 and5.43% respectively. While decrease the rates of Mg (2.84% and Fe (0.32%).In the second week, the rate increase of Ca and P, while decrease of K, Mg and Fe in blend (A). In blend (B) the rates increase of (Ca, K, Mg and Fe), while decrease of P. In blend (C) the rates decrease of (Ca, K and Mg), while increase in P and Fe. Antony and Chandra,(1998) , reported that mineral contents of finger millet were (Ca) 313.1, (P) 467.2, (Fe) 6.53, (Cu) 1.01, (Zn) 2.02 and (Mg) 9.86 mg/100g on dry weight. Arora et al., (2003) reported that content of pearl millet seeds were 123.67, 15.90, 2.13, 0.84, 0.50 and 0.35 mg/100g, on dry matter basis of phosphorous, calcium, iron, zinc, copper and manganese, respectively.

Table 17: Minerals content (mg/100ml) of the fermented peanut milk and millet milk during refrigeration storage *

Mineral	Peanut milk			Millet milk		
	Initial storage	After 1 week	After 2 week	Initial storage	After 1 week	After 2 week
Ca	85.04± 2.43[a]	82.14± 0.25[a]	87.87± 2.05[a]	60.04±1.81a	56.89± 4.42[a]	65.78 ±1.46[a]
Mg	93.37± 8.55[a]	75.55± 8.15[a]	92.87± 6.29[a]	77.40± 0.12[a]	77.72± 0.85[a]	83.90± 3.65[a]
Fe	8.85 ±0.18[a]	0.98 ± 0.01[b]	0.612± 0.03[b]	7.40±1.02[a]	8.95 ± 0.25[a]	10.28±0.65a
K	161.35 ±2.87[a]	173.33± 4.38[b]	180.82± 0.70[b]	134.83± 3.52[a]	134.87± 3.47[a]	139.20± 0.31[a]
P	116.97 ±0.70[a]	121.81±13.42[a]	89.40 ±0.01[a]	99.22±0.77[b]	116.82± 0.84[a]	118.96 ±0.48[a]

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw of each specific beverage are significantly different at $p<0.05$.

Table 18: Minerals content (mg/100ml) of the different fermented formulate blend beverages during refrigeration storage*

Mineral **	A			B			C		
	Initial storage	After 1 week	After 2 week	Initial storage	After 1 week	After 2 week	Initial storage	After 1 week	After 2 week
Ca	77.40± 1.43[b]	77.66±1.72[b]	98.02± 0.85[a]	75.82± 4.95[b]	68.88± 1.22[b]	97.57± 0.35[a]	114.05± 4.83[a]	124.87±8.85[a]	121.49±0.46[a]
K	181.84± 2.73[a]	187.51±6.80[a]	180.25± 1.51[a]	182.40±2.85[a]	177.22±13.33[a]	186.76±0.78[a]	185.87± 0.64[a]	190.25±1.32[a]	188.72±1.27[a]
P	115.98± 3.77[a]	110.54±0.50[a]	119.82 ±1.98[a]	113.04±0.39[b]	120.43±0.94[a]	120.25± 2.11[a]	118.87± 2.19[a]	124.30±7.09[a]	126.98±2.04[a]
Mg	125.04± 3.42[a]	122.89±2.22[a]	97.97± 0.51[b]	127.12±0.43[a]	120.91± 3.16[a]	124.72± 4.11[a]	173.25± 4.47[a]	170.41±1.40[a]	170.27±1.95[a]
Fe	1.43± 0.[27a]	1.68±0.09[a]	1.43±0.29[a]	2.54±0.31[a]	1.83± 0.09[a]	2.39± 0.10[a]	3.61± 0.[30a]	3.29±0.15[a]	3.65±0.27[a]

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw of each specific beverage are significantly different at $p<0.05$.

A =Blend 1 was prepared using 85% peanut milk and 15% millet milk.

B=Blend 2 was prepared using 70% peanut milk and 30 % millet milk.

C= Blend 3 was prepared using 55% peanut milk and 45 % millet milk.

CHAPTER FIVE

CONCLOUSION AND RECOMMENDATION

5.1 Conclusion:

Our investigation on physic-chemical properties of select peanut and millet , indicated that they are rich in many nutrients and they could contribute to improve the nutritional content of the rural and cities in Sudan. Furthermore, peanut and millet can be considered as source of protein and carbohydrates supplements. The millet has high minerals value in one formula produce complementary product provide both nutritional and health benefits. Fermentation of the peanut, millet and blend beverages with *Bifidobacterium longum BB536*, peanut and blend (A). They were the best medium for strain growth. Sufficient numbers of *Bifedobacterium longum BB536* were obtained in different types of fermented peanut, millet milk and formulated blends. The viable number of the strain during fermentation was above 6 Log CFU/ml in all fermented beverages. Therefore, these products can be called probiotics. In addition the levels of pH are decreased while there was increase in acidity. This study can facilitate the development of new, fermented, non-dairy, nutritionally well-balanced food products with unique physical properties and cheap compared to fresh dairy milk which is high prices in today's food markets of Sudan.

5.2 Recommendations

1- Encourage the incorporation of *Bifidobacterium* into non-dairy based cereal and nuts.
2- More researches to be conducted on sensory characteristics nutritional values and functional properties of the fermented beverages to explore their health benefits and consumer preferences.

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