بسم الله الرحمن الرحيم

# Sudan University of Science& Technology

## College of Graduate Studies

## Microbiological and physiochemical Study on *Bifidobacterium infantis* 20088 Fermented Peanut Milk and Rice Milk and their Blends

دراسة مايكروبيلوجية وفيزيوكيميائية لألبان الفول السوداني والأرز ومزيجهما المخمره ببكتريا

## *Bifidobacterium infantis* 20088

A thesis Submitted to Sudan University of Science and Technology in Partial Fulfillment of the Requirements of the Degree of Master of Science in Food Science and Technology

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(اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (١)    خَلَقَ الإِنْسَانَ مِنْ عَلَقٍ (٢) اقْرَأْ وَربُّكَ الأَكْرَمُ (٣) الَّذِي عَلَّمَ بِالْقَلَمِ (٤) عَلَّمَ الإِنْسَانَ مَا لَمْ يَعْلَمْ (٥)

صدق الله العظيم

سورة العلق الآيات (١-٥)

# Dedication

*I dedicated this dissertation*

*To my great  parents mother Elatia Osman and father*
*Hashim Mohammed El Tahir.*

*To my husband Yasser Mustafa Saran and my little sons*
*Ammar , Osman and Alshazali.*

*To my sisters, brothers, aunts, uncles 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 .*

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# LIST OF ABREVIATION

| | |
|---|---|
| WHO | World Health Organization |
| FAO | Food Agricultural Organization |
| USDA | United States Department of Agriculture |
| CFU | Colony forming Unit |
| Log | Logarithm |
| (%) | Percentage |
| G | Gram |
| TSS | Total Soluble Solid |
| h | Hour |
| MRS | Sharpe-Rogosa-Demann |
| ℃ | Degree Celsius |
| *et al* | Cetera ct / company |
| ANOVA | Variance Analysis of |
| Ml | Milliliter |
| w/w | Weight per weight |

# Abstract

This study was carried out to develop probiotic fermented beverages. The commercial strain *Bifidobacterium infantis* 20088 was used. The growth of the strain and its related physiochemical changes during fermentation was evaluated. The strain survival during refrigeration storage was also examined. In addition the chemical composition of different fermented beverages at initial and maximum growth of the strain were also determined. Growth media were formulated from reconstituted skim milk, pure peanut milk, rice milk in addition to three different blends based on peanut milk prepared by partial substitution of 15% (A), 30% (B), 45% (C) with rice milk .peanut contains high levels of fat, fiber and protein. Moreover levels of carbohydrates were high in rice. Roasting of peanut also increased fat, proteins, fiber and ash in ratio of 0.71, 1.41, 0.32, 0.2 and 0.62% respectively. While decrease carbohydrate (1.73%) as compared to raw peanut (4.65%). The results obtained on *B. infantis* 20088 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. infantis 20088* increases in different fermented beverages were 3.71, 3.01,2.95, 2.16,2.00, and 1.93 in fermented peanut milk, blend ( B),blend( A), skim milk ,blend(C) and rice milk respectively.  In spite of declining in viable count of *B. infantis* 20088 in all types of fermented beverages at 48h 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 blend C (5.89) and rice milk (5.78 CFU/ml) strain viable count dose  not fulfill propiotic requirement in food. During fermentation process with strain  *B. infantis* 20088   there were significant (P<0.05) decrease in pH levels in all type of beverages by extended fermentation period to 36h .The decreases in pH were due to increased acids production during fermentation process as a result of fermenting sugar by *B. infantis* 20088

.TSS levels decrease in all types of fermented beverages. The rates of TSS decreases at maximum growth were 0.8, 1.1, 0.5, 1.05 and 1.25 in fermented peanut milk, rice milk, followed by the blend (A),blend (B) and blend (C) respectively . Sugar also decrease in all fermented beverages with extended fermentation period to 48 h. The rates of sugar reduction at maximum growth of strain *B. infantis* 20088 were 0.4, 0.31, 0.28, 0.29 ,and 0.31 in fermented peanut milk, rice milk, blend (A),blend(B) and blend (C) respectively. During the fermentation of the fermented beverages there were no significant ($p > 0.05$) changes in compound of beverages. Fermentation of peanut milk increase in moisture, portion, ash and fiber while decrease in fat, carbohydrate and total solid in peanut milk .On the other hand during refrigeration storage of different formulated beverages. There were significant ($p < 0.05$) reduction in *B. infantis* 20088 viable count in all fermented beverages .The rate of reduction in the first week of the refrigeration storage were 1.05, 1.1, 1.28 , 1.17, 0.97 and 0.27 CFU /ml in the fermented peanut milk , rice milk , blend(A) ,blend(B), blend(C) and skim milk respectively. Hopefully, the final viable count of *B. infantis* 20088 in fermented, peanut milk, skim milk , blend (A) and blend(B) 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. infantis* 20088 in all types of fermented beverages , except fermented peanut milk, skim milk and blend(A) . Therefore they are suitable carrier to deliver *B. infantis* 20088 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 infantis 20088 تم استخدامها. ثم تقيم نموؤها والتغيرات الفيزيوكيميائيه خلال عملية التخمير. وتم حساب عدد بكتريا B.infantis 20088 والتغيرات الفيزيوكيميائيه أثناء عملية التخمير والتخزين في الثلاجة وأيضا تم تقدير التغيرات الكيميائية عند بداية وأقصي نموؤ للبكتريا في كل من لبن الفول السوداني ،لبن الأرز،اللبن منزوع الدسم ، بالإضافة إلى ثلاثة خلطات علي أساس لبن الفول السوداني ونسب١٥ % (أ) ،٣٠،% (ب) و٤٥% (ج) من لبن الأرز.

يحتوي الفول السوداني علي نسب عالية من البروتين والدهون والارز احتوي علي نسب أعلي من الكربوهيدريتات ،الألياف والرماد. عملية تحميص الفول السوداني أدت لزيادة معنوية (P < 0.05) في نسب الدهون،البروتين،الألياف،الرماد بنسب 0.71،1.41 ، 0.32 ، 0.2 و 0.62 % علي التوالي .ونقصان في الكربوهيدريتات (1.73%).وضحت النتائج التي تم الحصول عليها أن هناك زيادة معنوية (P < 0.05) في النمؤ الميكروبي عند أقصي نمو لبكتريا B.infantis 20088 في العصائر المخمرة مقارنة بعددها عند بداية عملية التخمير. وكان معدل زيادة نموؤ بكتريا B.infantis 20088 3.71،3.01، 2.95 ، 2.16 ،2.00% و 1.93% في كل من لبن الفول السوداني، الخلطة (ب)، الخلطة (أ) ،اللبن منزوع الدسم، الخلطة (ج) ولبن الأرز علي التوالي. وعلي الرغم من انخفاض عدد نمؤ بكتريا B. infantis 20088 بتمديد التخمير ثمانيه واربعين ساعة في كل أنواع العصائر 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 العصير المخمر في الخلطة (ج) كان يحتوي علي ( CFU/ml (5.89)) ولبن الارز (5.78 CFU/ml) لا يفي بمتطلبات أغذية البروبيتك. أثناء عملية التخمير بالبكتريا B.infantis 20088 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 B.infantis 20088. والجوامد الكلية الصلبة الذائبة في كل أنواع العصائر المخمرة مع تقدم عملية التخمير. وكان معدل النقصان في الجوامد الكلية الصلبة الذائبة عند أقصي نمؤ لبكتريا B.infantis 20088 ،0.8 ،1.1 ،0.5 ،1.05 ،و1.25% في كل من لبن الفول السوداني،الأرز والخلطة (أ) ، الخلطة (ب) و الخلطة (ج) علي التوالي. وكان معدل نقصان مستوي السكريات 0.4، 0.31،0.28 ، 0.29 و 0.31 % في كل من لبن الفول السوداني ،لبن الأرز، الخلطة (أ)، الخلطة (ب) والخلطة( ج) علي التوالي. خلال عملية تخمير العصائر هنالك زيادة معنوية (P < 0.05) في الرطوبة،البروتين،الرماد والألياف ومالت للنقصان 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 *B. infantis 20088* في جميع العصائر وكان معدل الانخفاض في الأسبوع الأول من التخزين (1.05،1.1، 1.28،1.17 ، 0.97 و $0.27$ CFU /ml) خليه بكتيرية في كل مل من اللبن المخمر للفول السوداني، لبن الأرز،الخلطة (أ)، الخلطة (ب)، الخلطة (ج) واللبن منزوع الدسم علي التوالي. وكان عدد خلايا بكتريا *B. infantis 20088* في كل من لبن الفول السوداني ،الخلطة (أ) ،الخلطة (ب) واللبن منزوع الدسم يفي بالعدد المطلوب وجوده في أغذية البروبيتك. وخلال الأسبوع الثاني من التخزين كان هناك انخفاض معنوي ($P < 0.05$) في جميع المشروبات المخمرة ماعدا لبن الفول السوداني، و الخلطة (أ) واللبن منزوع الدسم المخمر. لذلك يعتبر كل منهم ملائم لحمل بكتريا *B. infantis 20088* للمستهلكين وفي نفس الوقت هذه المشروبات المخمرة تقدم عناصر غذائية أساسيه مثل البروتينات، الدهون، المعادن والألياف.

# CHAPTER ONE

# INTRODUCTION

The growing interest in health and diet has recently produced the concept of functional foods . By definition , functional foods are normal foods and parts of the daily diet , but they contain a component that benefits some particular physiological function and reduce the risk of diseases (Salovaaro, 1999). Nowadays , the wide applications of functional food are in form containing probiotics and non-digestible carbohydrate known as prebiotics (Fuller and Gibson, 1997) .

Reorganization of probiotic effect dates back to the 19$^{th}$ century when the French scientist Louis Pasteur (1822 – 1895) postulated the importance of microorganisms in human life , this was further reinforced by work done by 1908 Nobel Prize-winner Elie Metchnikoff, which led to the concept of probiotics.

Strain of *Bifidobacterium* , *lactobacillus* and nonpathogenic yeast such as *Saccharomyces boulardii* are principally used individually or in combination as probiotics  (Tomasik and Tomasik, 2003).

Bifidobacteria is the predominant species of human colonic and fecal microbiota .It has been extensively introduced in the food industry and pharmaceutical applications (Guarner and Malagelada, 2003).

*Bifidobacterium infantis* is a probiotic bacterium that inhabits the intestines of both infants and adults. This type of bacteria is considered beneficial because of the acids it produces. The acids produced by *Bifidobacterium infantis* may help impede the growth or colonization of harmful bacteria within the colon (Sanders, 2007).  According to medical news today, *Bifidobacterium infantis* is "normal, friendly bacteria that play an important role in basic digestion, proper metabolism and overall well-being. It works within the digestive

system to restore intestinal balance and maintain normal digestive health. "according to a study by ( Sanders, 2007). The strain may be beneficial to those that suffer from symptoms of irritable bowel syndrome (IBS) including bloating, gas, diarrhea, constipation, urgency and abdominal discomfort (Gibson and Shepherd, 2010).

However , most human origin probiotics are fastidious when used alone , they are characterized by low growth capability in food mediums including the dairy , the main recommended carrier of probiotics to human (FAO/WHO, 2001) .

For ages it has been well known that peanut and peanut milk products have nutritional benefits for young and old people because of their extreme richness in protein, minerals and essential fatty acids such as linoleic and oleic acids, which are considered to be highly valuable in human nutrition. It is extensively used in India and other developing countries by vegetarians and more recently by children allergic to cow milk proteins (Kouane *et al*., 2005).

Cereal grains are utilized as food worldwide. Among cereal grains, rice has the highest calorie content (Watt and Merrill, 1963). In addition, rice contains a better balance of essential amino acids. Thus its lysine content can be supplemented to an optimum level at a lower cost than for wheat, corn, millet, or sorghum.

In Sudan, the majority of foods are consumed in the form of naturally fermented products ( Dirar, 1993) . In addition , the use of rice and peanut as carrier for probiotic is approval aproved  and the process claimed to improve overall protein quality of fermented beverages  (Kabeir *et al*., 2005, and Kabeir *et al.,* 2005 ).

However, the use of peanut milk and rice milk blend  as a complementally non-dairy  based carrier for *Bifidbacterium*  is not explored. There for, the objective of this study are to :

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1- To evaluate the growth of *B.infantis20088* in peanut milk and rice blends and its related physio-chemical changes during fermentation.

2-  To determine the nutritional value of the different fermented blends.

3- To evaluate the survival of *B.infantis 20088* during refrigeration storage of different fermented the blends .

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1. Definition of probiotics

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 in't 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 microflora. 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.

## 2.2. 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,, . The concept of probiotics was already successful in Asia for many years when the first probiotics fermented milk products were eventually introduced in Europe (Metchnikoff, 1907).

## 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. infantis 35624
- Lactobacillus acidophilus NCFM
- Lactobacillus paracasei St11
- Lactobacillus johnsonii La1
- Lactobacillus plantarum 299v
- Lactobacillus reuteri ATCC
- Lactobacillus reuteri Protectis
- Saccharomyces boulardii

## 2.4. Characteristics of probiotics microorganism

Probiotics microorganism  have to be alive when administered. (Kligler and Cohres, 2008) One of the concerns throughout the scientific literature resides in the viability and reproducibility on a large scale of the observed results, as well as the viability and stability during use and storage and finally the ability to survive in the intestinal ecosystem.(Neish, 2009)

Probiotics must have undergone controlled evaluation to document health benefits in the target host. Only products containing live organisms in reproducible human studies confer of a health benefit claimed as a probiotic. (Petrof, 2009). However, represents a major challenge because several difficulties arose , such as variability of probiotics specially used  in the site (oral, vaginal, intestinal) and mode of its application. (Sethi, 2009)

The probiotic candidate must be a taxonomically defined microbe or combination of microbes (genus, species and strain level). It is commonly admitted that most effects of probiotic are strain-specific and cannot be extended to other probiotics of the same genus or species.( Kligler and Cohres, 2008) .This calls for a precise identification of the strain, i.e. genotypic and phenotypic characterization of the tested microorganism. (Hempel , *et al* 2011).

## 2.5. Functional Properties of probiotics

 In spite of research progress in recent years our 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 strain on composition and function of the intestinal population is still difficult to ascertain (Holzapfel *et al.*, 1998; Mercenier and Pavan, 2002). 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.
  - 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 use is 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 endocarditis 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. Immuno stimulation, but no pro-inflammatory effect.

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

6. Antimutagenic and anticarcinogenic 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). Thus, for certain probiotic strains it might be sufficient that they grow well during initial production steps (to obtain high enough numbers in the product) but they do not necessarily need to retain good viability during storage.

## 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. Thus, it is considered as important that

probiotic products contain an effective dose of living cell during their whole shelf life . 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 ( 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 (Seludar 2013) , there is growing interest in applying probiotics to fermented meat products , probiotics bacteria are also applied to infant nutrition powder and powdered milk drinks (Goktepe *et al*, 2006).

## 2.8. *Bifidobacterium* as probiotics

Bifidobacteria is the predominant species of human colonic and faecal microbiota. 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).

Bifidobacteria are considered as important probiotics and used in the food industry to relieve and treat many intestinal disorders . Bifidobacteria 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 a number of dietary compounds into bioactive molecules (Mayo and van Sinderen, 2010).

Strains of this genus *Bifidobacterium infantis* are normal in habitants of the human and animal gastrointestinal ecosystems , where they are found in relatively in high number (Biavati *et al.*, 2000) . They are thought to be important in maintaining the microbial balance necessary for health through their metabolic , trophic and protective activities (Guarner and Malagelada,

2003) . In addition , consumption of *Bifidobacterium ifantis* without any toxicity or adverse health effects is well proved,(Frece *et al*,2005) .

## 2.9. 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.*

## 2.10. *Bifidobacterium Infantis*

*Bifidobacterium infantis* is a probiotic bacteria that inhabits the intestines of both infants and adults. This type of bacteria is considered beneficial because of the acids it produces. The acids produced by *Bifidobacterium infantis* may help impede the growth or colonization of harmful bacteria within the colon. According to Medical News Today, *Bifidobacterium infantis* is "normal, friendly bacteria that play an important role in basic digestion, proper metabolism and overall well-being. *Bifidobacterium infantis* works within the digestive system to restore intestinal balance and maintain normal digestive health."( Medical News Today, July 2006) According to  a study by (Sanders, 2007), *Bifidobacterium infantis* may be beneficial to those suffer from symptoms of irritable bowel syndrome (IBS) including bloating, gas, diarrhea, constipation, urgency and abdominal discomfort.

The digestive process begins when an individual chews food, thereby breaking it down into smaller food particles that are more susceptible to digestive enzymes. This breakdown not only makes the food more susceptible to digestive enzymes but it also allows the food particles to travel more easily through the digestive tract.(Sanders, 2007) The large intestine has a high number of microbes present that help complete the process of food digestion.

Microbes are tiny, living organisms usually too small to be seen with the naked eye and are also commonly referred to as microorganisms. These microbes or microorganisms include bacteria, viruses, fungi, algae, and protozoa. *Bifidobacterium infantis,* which is a bacteria, falls into the microbe or microorganism category.

*Bifidobacterium infantis* is considered a "good" or beneficial bacterial. According to some research "there are advantages in skewing the balance of bacteria toward beneficial ones because the metabolic end products of their growth are organic acids (lactic and acetic acids) that tend to lower the pH of the intestinal contents, creating conditions less desirable for harmful bacteria."( Sanders, 2007) For these reasons, *Bifidobacterium infantis* may help provide relief to individuals suffering from IBS symptoms.

## 2.11.Peanut

**Scientific classification (**Alper and Mattes, 2003)
Kingdom:           ***Plantae***
(unranked):      ***Angiosperms***
(unranked):      ***Eudicots***
(unranked):      ***Rosids***
Order:            ***Fabales***
Family:           ***Fabaceae***
Subfamily:      ***Faboideae***
Tribe:            ***Dalbergieae***
Genus:           ***Arachis***
Species:        ***A. hypogaea***
**Binomial name:**
      ***Arachis hypogaea L.***

## 2.12. 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.(Lavia and Aveliano, 2007) 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. (Berrin and Larco, 1997)

Archeologists have dated the oldest specimens to about 7,600 years, found in Peru. (Dillehay, 2007) 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.

Although the peanut was mainly a garden crop for much of the colonial period of North America, it was mostly used as animal feed stock until the 1930s.( Putnam, 1991 ) 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.

## 2.13. Usage of peanut

Peanuts have much usage. They can be eaten raw, used in recipes, made into solvents and oils, medicines, textile materials, and peanut butter, as well as many other uses. Popular confections made from peanuts include salted peanuts, peanut butter (sandwiches, peanut candy bars, peanut butter cookies, and cups), peanut brittle, and shelled nuts (plain/roasted). Salted peanuts are usually roasted in oil and packed in retail-size plastic bags or hermetically sealed cans. Dry roasted salted peanuts are also marketed in significant quantities. 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 spoilage 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, plastics, dyes and paints nitroglycerin, (Bonnie, 1988).

## 2.14. Protective Nutrients of peanut

The fat, protein, and fiber in peanuts are all healthy and are plant-based. Research on peanuts shows that all of these components promote health and reduce the risk of chronic disease (Alper and Mattes, 2003).

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 *et al.,* 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 *et al.,* 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 *et al.,* 2004).

### 2.14.1. 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 and Buller, 2005).

### 2.14.2. Vitamins and Minerals

Peanuts and peanut butter contain   numbers of vitamins and minerals that we need daily in our diets (Swain, Carron, Hamilton, Sacks, Appel, 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 and Buller, 2005).

### 2.14.3. 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 and Resurreccion, 2008).

### 2.14.4. 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 .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 (Francisco and Resurreccion, 2008).

## 2.15. 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 triggers 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).

## 2.17. Rice

Rice, *Oryza sativa* is the world's second most important cereal crop. Rice is the major caloric source. Nearly 2.5 billion people depend on rice as their main food (FAO, 2004). Rice is cultivated mostly in developing countries and it's primary source of income for employment for more 100 million households in Asia and Africa (FAO, 2004). Globally, no food grain is more important than rice from nutritional perspective, a food security perspective, or an economic perspective.

Rice is whole some nutritious cereal grain and it has qualities, which make it ideally suited for specific need. It is used almost exclusively as direct human food. Rice grain's containing 100% amylo pectin is called glutinous or waxy rice. A complex carbohydrate with no cholesterol or sodium, rice is nearly fat-free, rich in vitamins and minerals, and very easy to digest. Rice contains a

very high percentage of carbohydrates, which reach 76% in decorticated rice. The protein in rice is well balanced because all essential amino acids are present and in proper proportion (10%). Rice contains only traces of fat and ash (1%).

## 2.18. History of rice in the Sudan

In the Sudan, there is a plenty of land suitable for rice production, which was estimated by 300000 hectares in White Nile, Bahr Elgazal, South Darfur, Gadarif and Blue Nile State. Rice in Sudan has been grown since 1905, but on a very limited acreage and information about methods of production are lacking (Farah, 1981). Swamp and Upland varieties were first tried at the Gezira Research Farm in 1951. Later, extensive rice trials were carried out at Malakal and several varieties were selected at the Gezira Research Station. Although rice cultivation in the Sudan was known for some times, especially in Southern Sudan and White Nile areas, large –scale production started only in the 1950 in the upper Nile Province (Malakal), and in 1960 in Aweel, but for security reasons production was abandoned. Rice production was started once again along the White Nile at Abu Gassaba (Awok *et al.,* 1996).

## 2.19. Nutritional value of rice

A detailed analysis of nutrient content of rice suggests that the nutrition value of rice varies based on a number of factors. It depends on the strain of rice, that is between white, brown, black, red and purple varieties of rice – each prevalent in different parts of the world. It also depends on nutrient quality of the soil rice is grown in, whether and how the rice is polished or processed, the manner it is enriched, and how it is prepared before consumption (Juliano, 1993).

Rice is consumed as milled rice after dehulling process and whitening (removal of pericarp, bran layer and embryo from brown rice). After

whitening parts of rice were removed which has very low starch content but has high percentages of oil, protein, vitamins and minerals, ( El-Hissewy *et al.,* 2002 ). Rice is a great source of complex carbohydrate which gives us the energy we need. According to FAO, rice provides 20% of the world's dietary energy supply

# CHAPTER THREE

# MATEIALS AND METHODS

## 3.1. Raw Materials

The red-skinned peanut seeds (*Arachis hypogaea*) (*v. Ashford*) were purchased from a local crops market in Bahri (Khartoum state, Sudan). Care was taken to ensure that good quality and mould-free seeds were selected. Paddy rice (*Oriza sativa* OM 8923) grown in Eldwaim was obtained from Department of Crops production, Collage of Agricultural Studies, Bakht Elred University, Eldwaim, Sudan.

## 3.2. Methods

### 3.2.1. Preparation of peanut milk

Peanut milk was prepared by following the method of 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). Roasting found to improve nutrient composition (protein, fiber and fat) and decrease the peany flavor of peanut .The seeds were then de-skinned and weighed before being soaked in water for at least 12 h. The de-skinned peanut kernels were then washed with clean water. The kernels were then mixed with water in a ratio of 1:5 [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. Malting of baddy rice

Malting of baddy rice was carried to the procedure reported by Kabeir *et al.,* (2005). Cleaned baddy rice 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 baddy rice were spread on aluminums dishes and incubated for 48 h at 30°C. During the germination period the baddy were turned and rinsed every 12 h with distilled water to promote aeration and prevent mould development. Germinated baddy were dried in an oven at 50°C for 48 h, after that the roots of the germinated baddy were removed and the remaining portion of the baddy rice were manually dehusked and ground into a flour, sieved through a 355-µm screen. The flour was packed in a plastic container and kept at refrigerated temperature until used.

### 3.2.3. Preparation of rice milk

Rice milk was prepared according to procedure by Kabeir *et al., (2005)* .Cleaned brown rice 200g, washed and soaked in twice its volume with water in 2L beakers, and placed at room temperature for about 4h. The water drained and brown rice was blended (Panasonic- MX-101SP2) with 800ml clean water of medium speed for 5 minutes. The slurry formed was filtered using a double layered cheese and boiled at 70˚C for 3 min; magnetic stirrer was used for mixing. Rice malt flour was added in rate of 1:5 w/w after cooling at 37 ˚C and maintain for 14 min to prepared rice milk with low viscosity and flowing characteristics in addition TSS was high recording values around (6).

### 3.2.٤. Preparation of fermentation inoculums

*B.infantis* 20088 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, incubated an aerobically condition 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.

### 3.2.5. Growth media and fermentation conditions

Growth media were formulated reconstituted skim milk, pure peanut milk, pure rice milk in addition to three different blends  based on peanut milk prepared by partial substitution of (A), (B), (C) with rice milk. Formulated medium were sterilized (121˚C for 15 min) and inoculated with a 3% active culture of *B.infantis* 20088   followed by incubation at 37 ˚C for 48 hours.

### 3.2.6. Enumeration of viable cell

MRS medium was used to enumerate *B.infantis* 20088 of different fermented as beverages using the plate count technique. Fermented Samples were drawn at 0time and every 12h intervals during fermentation.1ml 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 unaerobically at 37 ˚C for 48 h. The growth was calculated as Colony Forming Unit per ml (CFU/ml).

### 3.2.7. Determination of  reducing sugars

The reducing sugar of the fermented beverages was determined according to the method described by Lane and Eynon's (1923) .Ten grams of sample were 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 of ml Fehling's solution (5 ml of Fehling A) (6.928 gm Cu So4.5H2O per 100ml distilled water) and 5 ml Fehling B (34.6 sodium

potassium tartrate and 10 gm NaoH per 100 ml distilled water), mixed well and then heated moderately to boiling point 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 *et al.,* (1979).

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

## 3.2.8. Determination of titrable acidity

The titrable acidity (TA) of the different fermented beverages was determined according to AOAC method (2006). Ten ml of sample were weighted into a conical flask. Adistilled 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 pipetted into porcelain dish , five drops of phenolphthalein added, and the sample was titrated against 0.1N NaOH till a faint pink colour that lasted for at least 30 seconds was obtained . Acidity of different beverages was calculated from the following equation:

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

Were:

N = Normality of NaoH.

0.9 = Factor of lactic acid.

### 3.2.9. Determination of total soluble solids (TSS)

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

### 3.2.10.  Determination of pH value

The pH value of the different fermented products 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 beverage samples was directly measured.

### 3.2.11. Chemical composition

### 3.2.11.1 Determination of moisture content

Moisture was determined according to the modified method of AOAC (1990). Five grams of the sample was weight using in sensitive balance, after weighting the empty dishes and then transferred to an oven (Kat-NR.2851, Electrohelios, Sweden) at $105 \pm 0.1$°C for 6 hours .Afterwards, the dish with sample was transferred to dessicator and allow to cool to room temperature before reweighting. Moisture calculated according to the following formula:

Moisture content (%)   =

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

  Were:

$M_1$ = mass of dish + cover .

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

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

### 3.2.11.2. Determination of fat content

Fat content was determined according to the official method of AOAC (1990). A sample of 5g was weighed in extraction thimble and covered with cotton, and then extracted with hexane. The thimble containing the sample and a pre-dried weight sample and 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 (%)  =

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

Where: W1= the weight of the empty extraction flask.

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

### 3.2.11.3 Determination of protein content

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

1. **Digestion**: two grams 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:

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

Protein (%) = (N %) × 6.25

Where N = Nitrogen content.

0.014=molecular weight of nitrogen/1000

### 3.2.11.4. Ash content

The ash content of the sample was determined according to the AOAC (1990) method. Two grams of the deferent fermented products were weighed into a clean dry porcelain crucible and placed in muffle furnace (model Tipoforon Z A No 18203 Get Ran 1002. England) at 600˚C for 6hours. The Crucible was transferred to a desiccator, cooled to room temperature and weighed .The ash content was calculated as follows:

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

Where:

W1 = Weight of crucible with ash.

W2 = Weight of empty crucible.

### 3.2.11.5. Determination of crude fiber

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 (0.23) solution for 30 min under reflux condenser and the precipitate was filtered. Rinsed with hot distilled water, 20ml ethyl alcohol (96%) and 20ml diethyl ether.

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

Crude fiber % =

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

### 3.2.11.6. Calculation of carbohydrates

Carbohydrates were calculated by difference according to the following:

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

### 3.2.11.7. 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).

### 3.2.12. 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.infantis* 20088, 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**.**

### 3.2.13 .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. Chemical composition of the raw peanut and raw rice

Referring to our results in Table1, in raw peanut contains lower levels of carbohydrates, fiber, Ash, moisture and higher levels of fat and protein as 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 are presented in same table (Table 1). It shows that moisture content of raw peanut was 5.75 which decreased to 3.04 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.* (2002and Adegoke *et al.* (2004). Roasting of peanut also increased fat, proteins, fiber and ash in ratio of 0.71, 1.4, 0.32, 0.2 and 0.62% respectively. While decrease carbohydrate (1.73%).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 rice are presented in same (Table 1) it revealed that rice contained high levels of moisture and carbohydrate were 0.19 and 4.06 respectively .While the decrease levels of fiber, fat, ash and portion were 1.75, 1.1, 0.97 and 0.45 respectively

Data on chemical composition of raw peanut and rice were given in same table (1).peanut contains high levels of fat, protein and fiber as compared to their levels in raw rice. While the levels of moisture, ash and carbohydrate were high in raw rice than in raw peanut. These variations in levels of chemical components between raw peanut and rice is due to sources variation.

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

| Components (%) | Raw Peanut | Roasted peanut | Peanut reference values** | Raw Rice | Rice reference values ** |
|---|---|---|---|---|---|
| Moisture | 5.75 ± 0.21 | 3.04 ± 0.19 | 6.00±1.7 | 12.09 ± 0.38 | 11.9±2.00 |
| Fat | 49.14 ± 0.25 | 49.85 ±  0.29 | 45.90±3.0 | 1.10 ± 0.14 | 2.2±0.60 |
| Proteins | 35.07 ± 0.31 | 36.48 ± 0.39 | 22.40±1.6 | 7.350 ± 0.212 | 7.8±2.00 |
| Fiber | 5.22 ± 0.15 | 5.54 ± 0.20 | 8.50±7.7 | 1.25 ± 0.07 | 3.00±1.00 |
| Ash | 0.17 ± 0.13 | 2.17 ± 0.05 | 2.30±0.1 | 0.33 ± 0.02 | 1.30±1.00 |
| Carbohydrates | 4.65± 1.047 | 2.92 ± 0.04 | 14.60±0.1 | 77.89 ± 0.78 | 73.8±0.00 |

*Values are mean ± SD for replicate independent runs.

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

**4.2. The growth of *Bifidobacterium.infantis*20088 during fermentation of different formulated beverages**

Comparative growth of *B.infantis* 20088 cultured in different beverages (skim milk, peanut, rice and different blend is shown in table 2.

There were significant (p<0.05) increases in *B.infantis*20088 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.infantis*20088 in all type of fermented beverages was attained at 36 h fermentation. After the maximum growth all the strain growth declined with time 48h was observed in all type of fermented beverages (Table2)

The rate of *B.infantis*20088 increases in different fermented beverages were 3.71, 3.01,2.95, 2.16,2.00, and 1.93 CFU/ml in fermented peanut milk, blend B, blend A, skim milk ,blend C and rice milk respectively as compared to strain level at 0time at the beginning of formulated . This variation in growth rate of *B.infantis*20088 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 Rice could complement the nutrient component of growth medium. However the growth of strain *B.infantis*20088 was affected by supplementation with rice milk. (Table2).This decline in rate of growth could be due to increase viscosity of beverages by supplementation with rice milk. However at 48hour of fermentation there was reduction in number of *B.infantis*20088 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 decreased in viable count of *B.infantis*20088 in all types of fermented beverages at 36h fermentation, the count still above the number required to presence in probiotic food which is at least 6 log cfu/ml fermented products, ( Viderola and Reinheimer, 2000).

**Table 2: The viable count of *Bifidobacterium.infantis20088* log (CFU/ml) during fermentation    of different beverages***

| Fermented time(h) | Beverages | | | | | |
|---|---|---|---|---|---|---|
| | Counts CFU/ml | | | | | |
| | Skim Milk | Peanut | Rice | A | B | C |
| 0 | $6.11\pm0.03^a$ | 5.84±0.05e | 4.74 ±0.06d | 5.84 ±0.09d | 4.83 ±0.07d | 4.57 ±0.13c |
| 12 | $8.11\pm0.05^b$ | 6.74±0.06d | 5.86 ±0.06 bc | 6.57 ±0.13c | 5.97 ±0.03c | 5.75 ±0.09b |
| 24 | $8.14\pm0.01^b$ | 7.96±0.04c | 5.97 ±0.02b | 6.96 ±0.04c | 6.59 ±0.16b | 5.95 ±0.04b |
| 36 | $8.27\pm0.02^c$ | 9.55±0.37a | 6.67 ±0.02a | 8.79 ±0.13a | 7.84 ±0.14a | 6.57 ±0.13a |
| 48 | $8.07\pm0.07^b$ | 8.72±0.09b | 5.78 ±0.06c | 7.65 ±0.07b | 6.87 ±0.03b | 5.89 ±0.07b |

* 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% rice milk.

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

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

## 4.3. pH changes during fermentation of different beverages with *Bifidobacterium.infantis20088*

During fermentation process with strain *B.infantis20088* there were significant ($P<0.05$) decrease in pH levels in all types of beverages by extended fermentation period to 36 h (Table 3).The decrease**s** in pH are due to increased acids production during fermentation process as a result of fermented sugar by *Bifidobacterium.infantis*20088, 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 at (36) of strain *B.infantis*20088 were 1.84, 1.83, 1.72 , 1.65, 0.9 and 0.3in fermented blend A, blend C, blend B, rice milk, peanut milk and skim milk respectively. Level of acidity increased by extended fermentation period and thus caused reduction in pH. The acid produced are beneficial reported to have antibacterial such as prevent the proliferation of pathogens (Bullen *et al.* 1976).

**Table3: pH change during the growth of *Bifidobacterium.infantis*20088 in beverages***

| Fermented time (h) | Beverages | | | | | |
|---|---|---|---|---|---|---|
| | pH | | | | | |
| | Peanut | Rice | Skim milk | A | B | C |
| 0 | $6.80 \pm 0.01^a$ | $6.82 \pm 0.00^a$ | $6.30 \pm 0.28^b$ | $6.99 \pm 0.035^a$ | $6.86 \pm 0.057^a$ | $6.85 \pm 0.10^a$ |
| 12 | $6.57 \pm 0.02^b$ | $6.06 \pm 0.06^b$ | $6.00 \pm 0.00^a$ | $6.40 \pm 0.11^b$ | $6.30 \pm 0.021^b$ | $6.11 \pm 0.01^b$ |
| 24 | $6.39 \pm 0.02^c$ | $5.55 \pm 0.09^{bc}$ | $6.00 \pm 0.00^a$ | $5.91 \pm 0.11^c$ | $5.95 \pm 0.092^c$ | $5.78 \pm 0.04^c$ |
| 36 | $5.90 \pm 0.01^d$ | $5.17 \pm 0.30^c$ | $6.00 \pm 0.00^a$ | $5.06 \pm 0.07^d$ | $5.14 \pm 0.035^d$ | $5.02 \pm 0.01^d$ |
| 48 | $5.22 \pm 0.01^e$ | $4.07 \pm 0.014^d$ | $5.9 \pm 0.00^c$ | $4.76 \pm 0.16^d$ | $4.31 \pm 0.021^e$ | $4.20 \pm 0.03^e$ |

* 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=Blend1 was prepared using 85% peanut milk and 15% rice milk.

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

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

## 4.4. Total Soluble Solid changes during fermentation of different beverages with *Bifidobacterium.infantis20088*

Table (4) shows changes in total soluble solid  (TSS) during fermentation of different formulated beverages with *Bifidobacterium.infantis*20088.

There were significant (P<0.05) decreases in TSS levels in all types of fermented beverages by extended fermentation period to 48 h.  The rates of TSS decreases at maximum growth were 0.8, 1.1, 0.5, 1.05 and 1.25 in fermented peanut milk, rice milk, and then their three different blend (A,B,C) respectively. Enzymatic activity of the strain play a vital role in rate of TSS reduction . Also found similar decreases in TSS in traditional processing microbial and physicochemical changes during fermentation of malwa by (Muyanj *et al.,* 2010).

## 4.5. Reduction sugars during fermentation of different beverages with *Bifidobacterium.infantis*20088

There were significant (P<0.05) decrease in sugar levels of all fermented products by extended fermentation period to 36 h (Table 5). *Bifidobacterium* strain ferment sugar and produce organic acids mainly acetic, lactic, probunic ,  butyric and other organic acids .

The rates of sugar reduction at maximum growth of strain *Bifidobacterium infantis* 20088 were 0.4, 0.31, 0.28, 0.29, and 0.31 in fermented peanut milk, rice milk, blend A , blend B and blend C respectively. Moreover, after 36h of fermentation  , the maximum reductions in sugar were 0.11, 0.6, 0.03, 0.02 and 0.01 recorded in fermented peanut milk, blend( A), blend(B), blend(C) and then fermented rice 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. The  higher the growth the higher rate of sugar reduction.

**Table 4: Total Soluble Solid during the growth of the strain *Bifidobacterium infantis 20088* in different beverages \***

| Fermented time(h) | Beverages | | | | |
|---|---|---|---|---|---|
| | TSS (%) | | | | |
| | Peanut | Rice | A | B | C |
| 0 | $2.45 \pm 0.07^a$ | $6.15 \pm 0.07^a$ | $2.50 \pm 0.00^a$ | $4.00 \pm 0.14^a$ | $4.15 \pm 0.07^a$ |
| 12 | $2.20 \pm 0.14^{ab}$ | $5.95 \pm 0.07^{ab}$ | $2.35 \pm 0.07^{ab}$ | $3.50 \pm 0.14a^b$ | $3.35 \pm 0.07^b$ |
| 24 | $1.95 \pm 0.07^{bc}$ | $5.85 \pm 0.07^b$ | $2.20 \pm 0.14a^{bc}$ | $3.15 \pm 0.07^b$ | $3.25 \pm 0.07^c$ |
| 36 | $1.65 \pm 0.07^{cd}$ | $5.05 \pm 0.07^c$ | $2.00 \pm 0.14^{bc}$ | $2.95 \pm 0.21^b$ | $2.90 \pm 0.00^c$ |
| 48 | $1.60 \pm 0.00^d$ | $3.70 \pm 0.00^d$ | $1.80 \pm 0.14^c$ | $3.05 \pm 0.21^b$ | $3.70 \pm 0.14^d$ |

\*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% rice milk.

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

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

**Table 5: Reducing sugars (mg/100ml) during the growth of *Bifidobacterium infantis* 20088 in different beverages***

| Fermented time(h) | Beverages | | | | |
|---|---|---|---|---|---|
| | Reducing sugars (mg/100ml) | | | | |
| | Peanut | Rice | A | B | C |
| 0 | 0.17± 0.01b | 0.43 ± 0.05a | 0.40 ± 0.03a | 0.46 ± 0.025a | 0.43 ±0.004a |
| 12 | 0.32 ± 0.01a | 0.31± 0.10ab | 0.30 ± 0.002b | 0.30 ± 0.002b | 0.31 ±0.001b |
| 24 | 0.19 ± 0.02b | 0.11 ± 0.01b | 0.26 ± 0.034bc | 0.22 ± 0.014c | 0.18 ±0.025c |
| 36 | 0.13 ± 0.01c | 0.11 ± 0.02b | 0.12 ± 0.021d | 0.17 ± 0.007cd | 0.12 ±0.000c |
| 48 | 0.02± 0.00d | 0.12 ± 0.01b | 0.18 ± 0.013cd | 0.14 ± 0.012d | 0.14 ±0.032c |

*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% rice milk.

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

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

## 4.6. Titratable acidity during fermentation of different beverages with *Bifidobacterium infantis 20088*

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 48h. At maximum growth of strain *B .infantis 20088*  (36h), the rates of  increase were 0.14, 0.16, 0.19,0.20 ,and 0.21 in fermented peanut milk, rice milk, A, C and B respectively. The increased acidity is explained by accumulation of lactic acid and other organic acids produced during fermentation of the formulated beverages (Sefa *et al.,*2003).

## 4.7. Chemical composition of different beverages fermented with growth of *Bifidobacterium infantis* 20088

Table7 shows the chemical composition of peanut milk and rice milk beverages fermented with *B. infantis* 20088 at initial (0h) and maximum growth time (36h). The result presented in table 7, revealed that there were no significant (p>0.05) changes in chemical content of beverages except in fiber. In fermented peanut milk there was increase in moisture, portion, ash and fiber while decrease in fat, carbohydrate and total solid in peanut milk .

The result presented in table 7 shown the chemical compound of the blends increase in moisture, portion, and ash. While decrease in fiber, carbohydrate and total solid in rice milk. Moreover, the result presented in table 8, revealed there were significant (p<0.05) in total solid , moisture  and fat  in  blend(A ), fiber in blend( B) and protein in blend( C ). Increase  in protein ,moisture, ash ,and fiber content  ,while decrease in fat , carbohydrate  and  total solid in blend(A), also increase in moisture and fiber in blend( B ),while decrease in fat ,protein, ash, carbohydrate and total solid   .Blend(C )decrease in all compound while increase in moisture.

**Table 6: Titratable acidity (%) during the growth of the strain *Bifidobacterium infantis* 20088 in different products***

| Fermented time(h) | Beverages | | | | |
|---|---|---|---|---|---|
| | Titratable acidity (%) | | | | |
| | Peanut | Rice | A | B | C |
| 0 | $0.16 \pm 0.01^b$ | $0.13 \pm 0.05^b$ | $0.09 \pm 0.01^c$ | $0.08 \pm 0.00^c$ | $0.08\pm 0.00^d$ |
| 12 | $0.28 \pm 0.04^a$ | $0.13 \pm 0.01^b$ | $0.19 \pm 0.01^b$ | $0.19\pm 0.01^b$ | $0.15 \pm 0.02^c$ |
| 24 | $0.28\pm 0.02^a$ | $0.26 \pm 0.01^a$ | $0.20 \pm 0.00^b$ | $0.22\pm 0.00^b$ | $0.23 \pm 0.00^b$ |
| 36 | $0.30 \pm 0.00^a$ | $0.29 \pm 0.01^a$ | $0.28 \pm 0.02^{ab}$ | $0.29 \pm 0.01^a$ | $0.28 \pm 0.^{01ab}$ |
| 48 | $0.35 \pm 0.04^a$ | $0.34 \pm 0.03^a$ | $0.25\pm 0.03^{ a}$ | $0.34 \pm 0.02^a$ | $0.31 \pm 0.01^a$ |

*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% rice milk.

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

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

**Table 7: Chemical composition of peanut and rice beverages fermented with *Bifidobacterium infantis 20088*growth time***

| Component | peanut milk | | Rice milk | |
|---|---|---|---|---|
| | **Initial growth time** | **Maximum growth time** | **Initial growth time** | **Maximum growth time** |
| Moisture (%) | $87.37 \pm 0.37^a$ | $88.00 \pm 0.01^a$ | $92.11 \pm 0.04^a$ | $92.99 \pm 0.03^b$ |
| Fat content (%) | $2.90 \pm 0.04^a$ | $2.83 \pm 0.08^a$ | $0.18 \pm 0.01^a$ | $1.74 \pm 0.01^b$ |
| Protein content (%) | $3.11 \pm 0.13^b$ | $3.450 \pm 0.14^b$ | $1.87 \pm 0.014^a$ | $3.450 \pm 0.14^b$ |
| Ash content (%) | $0.19 \pm 0.01^b$ | $0.21 \pm 0.01^b$ | $0.42 \pm 0.13^a$ | $0.19 \pm 0.04^b$ |
| Total solid (%) | $12.63 \pm 0.20^c$ | $12.00 \pm 0.07^c$ | $7.89 \pm 0.13^a$ | $7.01 \pm 0.58^b$ |
| Carbohydrates (%) | $6.53 \pm 0.18^c$ | $5.48 \pm 0.104^c$ | $5.40 \pm 0.43^a$ | $1.60 \pm 0.36^b$ |
| Fiber (%) | $0.017 \pm 0.00^a$ | $0.032 \pm 0.00^b$ | $0.02 \pm 0.00^b$ | $0.03 \pm 0.01^b$ |

* 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 $p<0.05$

**Table 8: Chemical composition of different blends beverages fermented with *Bifidobacterium infantis 20088* 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 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% rice milk.

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

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

## 4.8. 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.infantis 20088*. at initial (0h) and at maximum growth time (36h). there were no significant (p>0.05) increase in Ca and P and significant (p<0.05) decrease in K, Fe and Mg in peanut milk, also increase Ca, Fe and P and decrease in Mg and K. These results in table10 as increase (Ca , K, P ,Mg and Fe) of all beverage but decrease of (Mg, k and P) in blend A ,(Mg) in blend (b) and (P) in blend c. To some extent are similar to those reported by Isanga and Zhang (2009).

## 4.9. The counts of *Bifidobacterium infantis 20088* log (CFU/ ml) during the storage of different fermented beverages

Table 11 show the viable counts of *B. infantis 20088* during refrigeration storage of different formulated beverages. There were significant (p<0.05) reduction in *B. infantis 20088* viable count in all fermented beverages. The rate of reduction in the first week of the refrigeration storage were 1.05, 1.1, 1.28 , 1.17, 0.97 and 0.27 CFU /ml in the fermented peanut milk , rice milk , blend(A) ,blend(B), blend(C) and skim milk respectively. It's clear that the rate of reduction did not differ significantly among different fermented beverages. Moreover *B. infantis 20088* reductions were recorded in the second week of the refrigeration storage which were 1.79, 0.65, 0.71, 0.79, 0.93 and 0.77 in the fermented skim milk, peanut milk, the rice milk and blend (B) and blend (c) respectively. Hopefuly, the final viable count of *B. infantis 20088* in fermented, peanut milk, skim milk and blend (A) after two weeks refrigeration storage was above the minimum number required to presence in probiotic to exert health benefits upon consumption, which was 6 log CFU/ml.

Kabeir *et.al* (2005) reported that survivability of *B. longum BB536* under refrigeration storage of fermented Sudanese Madeeda beverages was not

affected for a period of 2 week. While Akalin *et al.* (2004) noted a significant reduction on *B.longum BB46* in yogurt after 1 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 *bifidobacterium*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 bifidobacterium populations (Tamime and Robinson, 1985; Modler *et al.*, 1990; Laroia and Martin, 1991). 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), 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).

**Table 9: Mineral content(mg/100ml) of fermented peanut and rice beverages fermented with *Bifidobacterium .infantis 20088* growth time***

| Mineral | Peanut milk | | Rice milk | |
|---|---|---|---|---|
| | **Initial growth time** | **Maximum growth time** | **Initial growth time** | **Maximum growth time** |
| Ca | 86.2 ±4.2$^a$ | 159.8 ±30.6$^b$ | 171.8± 14.2$^a$ | 198.1±1.1$^b$ |
| K | 159.4± 24.0$^a$ | 157.0± 28.0$^b$ | 155.1±32.7$^a$ | 131.4±0.0$^b$ |
| P | 108.4 ±26.7$^a$ | 148.4 ±48.4$^b$ | 136.5±34.9$^a$ | 168.7± 10.6$^b$ |
| Mg | 107.4±8.6$^a$ | 100.8±15.6$^b$ | 171.6 ±14.5$^a$ | 112.2± 0.8$^b$ |
| Fe | 0.649± 0.35$^a$ | 0.723 ±0.06$^b$ | 0.159±0.02$^a$ | 0.509 ±0.46$^b$ |

* 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: Mineral content(mg/100ml) of different blended beverages fermented with *Bifidobacterium infantis 20088* growth time***

| Mineral | A | | B | | C | |
|---|---|---|---|---|---|---|
| | **Initial** | **Maximum** | **Initial** | **Maximum** | **Initial** | **Maximum** |
| Ca | $86.6\pm 3.2^{a}$ | $151.3\pm 17.7^{b}$ | $121.5\pm 14.5^{a}$ | $165.5\pm 22.4^{b}$ | $142.1\pm 42.1^{a}$ | $186.6\pm 6.7^{b}$ |
| K | $150.12\pm1.84^{a}$ | $162.33\pm7.09^{b}$ | $134.1\pm 25.2^{a}$ | $152.1\pm 15.2^{b}$ | $122.10\pm6.67^{a}$ | $126.54\pm 6.8^{b}$ |
| P | $116.90\pm6.47^{a}$ | $97.87 \pm0.78^{b}$ | $115.2 \pm22.5^{a}$ | $116.6 \pm 7.4^{b}$ | $146.5\pm 35.6^{a}$ | $136.6\pm 49.1^{b}$ |
| Mg | $96.82\pm 2.75^{a}$ | $93.07\pm6.01^{b}$ | $107.6\pm 13.8^{a}$ | $134.9\pm 51.7^{b}$ | $117.61\pm1.42^{a}$ | $121.62 \pm0.3^{b}$ |
| Fe | $0.78\pm 0.13^{a}$ | $0.79\pm0.04^{b}$ | $0.91\pm0.13^{a}$ | $0.93\pm0.05^{b}$ | $0.80\pm0.03^{a}$ | $0.92\pm0.08^{b}$ |

*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% rice milk.

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

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

**Table 11: The counts of *Bifidobacterium infantis 20088* 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 | $9.55 \pm 0.37^a$ | $8.50 \pm 0.04^b$ | $7.85 \pm 0.06^b$ |
| Rice milk | $6.67 \pm 0.02^a$ | $5.57 \pm 0.13^b$ | $4.86 \pm 0.03^c$ |
| Skim milk | $8.85 \pm 0.01^a$ | $8.58 \pm 0.00^a$ | $6.79 \pm 0.05^b$ |
| A | $8.79 \pm 0.13^a$ | $7.51 \pm 0.05^b$ | $6.72 \pm 0.17^c$ |
| B | $7.84 \pm 0.14^a$ | $6.67 \pm 0.09^b$ | $5.74 \pm 0.06^c$ |
| C | $6.57 \pm 0.13^a$ | $5.61 \pm 0.07^b$ | $4.83 \pm 0.02^c$ |

* Values are mean ± SD for replicate independent runs.

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

***Initial storage = 36h.

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

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

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

## 4.10. Reducing sugars during refrigeration storage of different fermented beverages

Table 12 shows reducing 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 (Table12) . The amount of sugar decrease in the first week of were 0.19,0.18,0.14,0.13 and 0.03 in fermented blend(A) , blend( B) , peanut milk , blend(C)and rice milk respectively .The amount of sugar reductions in the second weak were 0.17, 0.04,0.04,0.03 and 0.01 in the fermented blend( C), blend(B) ,rice milk, blend (A) and peanut milk respectively.

## 4.11. Reduction of pH during refrigeration storage of different fermented beverages

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 of refrigeration (table13). The rate of pH reductions in the first week were 1.58, 0.93,0.8, 0.71, 0.8,0.7and0.0 in the fermented peanut milk, blend( B) , the rice milk ,the blend( C ), the blend(A ) and skim milk respectively. While the reductions recorded in the second week of refrigeration were 1.03, 0.55, 0.46, 0.32, 0.2and 0.06 in fermented rice milk, blend (C), the peanut milk, the blend (B), skim milk and blend (A) 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 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).

Shah *et al*. (1995, 2000) also found similar decreases in pH values during storage of commercial yoghurts containing *L. acidophilus* and *B. bifidum*.

**Table12: Reducing sugar of the different fermented beverages during refrigeration storage***

| Type of fermented beverages ** | At initial storage*** | After 1 week | After 2 week |
|---|---|---|---|
| peanut milk | $0.30 \pm 0.01^{a}$ | $0.16 \pm 0.00^{c}$ | $0.13 \pm 0.01^{b}$ |
| Rice milk | $0.52 \pm 0.00^{a}$ | $0.19 \pm 0.00^{b}$ | $0.15 \pm 0.02^{b}$ |
| A | $0.31 \pm 0.00^{a}$ | $0.12 \pm 0.02^{b}$ | $0.11 \pm 0.01^{b}$ |
| B | $0.39 \pm 0.016^{a}$ | $0.21 \pm 0.01^{b}$ | $0.17 \pm 0.01^{b}$ |
| C | $0.42 \pm 0.01^{a}$ | $0.29 \pm 0.00^{c}$ | $0.12 \pm 0.00^{b}$ |

* 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.

***Initial storage = 36h.

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

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

C=Blend 3 was prepared using 55% peanut milk and 45% rice 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 | $6.32 \pm 0.02^a$ | $4.74 \pm 0.02^a$ | $4.28 \pm 0.00^c$ |
| Rice milk | $6.37 \pm 0.01^a$ | $5.57 \pm 0.01^a$ | $4.54 \pm 0.01^c$ |
| Skim milk | $6.20 \pm 0.00^a$ | $6.20 \pm 0.14^a$ | $6.00 \pm 0.14^a$ |
| A | $5.06 \pm 0.07^b$ | $4.36 \pm 0.01a$ | $4.30 \pm 0.01c$ |
| B | $5.14 \pm 0.04^b$ | $4.21 \pm 0.02^a$ | $3.89 \pm 0.01^c$ |
| C | $5.02 \pm 0.01^b$ | $4.31 \pm 0.01^a$ | $3.76 \pm 0.01^c$ |

* Values are mean ± SD for replicate independent runs.

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

***Initial storage = 36h.

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

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

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

## 4.12. Changes in TSS during the storage of different fermented beverages

Table 14 shows TSS of different fermented beverages. There were significant ($p<0.05$) increase in TSS of all types of fermented beverages under refrigerated storage for one week. The amount of increase in the first week of refrigerated storage of different formulated beverages were 0.95, 0.65, 0.6, 0.35and 0.15 in fermented blend (C), rice milk, the peanut milk, the blend (B) and then the blend (A), and peanut milk respectively. The rate of reduction in the second week of refrigerated storage of different beverages were 0.8, 0.2,0.5, and 0.1 in fermented blend (C), rice milk , blend( B ) and blend( A) , respectively. While there is significant different ($p<0.05$) change observed on TSS level of fermented peanut milk at the second week, there was increases and the rate is 0.8. (Table14)

## 4.13. 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 ( A ), blend (B) ,blend(C), rice milk and the peanut milk were 1.81, 0.94 ,0.76, 0.52 and 0.43% respectively. Over all levels of moisture after two weeks refrigeration storage of fermented beverages increased as compared to their initial levels at the beginning of the storage (Table 15) .These increase in moisture might indicate enzymatic activity that break down the macro component into simple and release more water.

**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 | $1.65 \pm 0.07^c$ | $2.25 \pm 0.07^b$ | $3.05 \pm 0.07^a$ |
| Rice milk | $5.05 \pm 0.07^b$ | $5.70 \pm 0.00^a$ | $5.50 \pm 0.14^a$ |
| A | $2.00 \pm 0.14^a$ | $2.15 \pm 0.07^a$ | $1.05 \pm 0.07^b$ |
| B | $2.95 \pm 0.21^a$ | $3.30 \pm 0.14^a$ | $1.80 \pm 0.14^b$ |
| C | $2.90 \pm 0.00^b$ | $3.85 \pm 0.07^a$ | $3.05 \pm 0.07^b$ |

* Values are mean ± SD for replicate independent runs.

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

***Initial storage = 36h.

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

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

C=Blend 3 was prepared using 55% peanut milk and 45 % rice 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 | 88.00 ± 0.01$^a$ | 88.43 ± 0.33$^a$ | 89.64 ± 0.73$^a$ |
| Rice milk | 92.99 ± 0.03$^b$ | 93.51 ± 0.08$^{ab}$ | 93.80 ± 0.28$^a$ |
| A | 87.94 ± 0.06$^b$ | 89.12 ± 0.51$^{ab}$ | 87.94 ± 0.06$^a$ |
| B | 89.55 ± 0.15$^b$ | 90.49 ± 0.21$^a$ | 91.12 ± 0.17$^a$ |
| C | 90.82 ± 0.07$^c$ | 91.58 ± 0.06$^b$ | 92.35 ± 0.19$^a$ |

* Values are mean ± SD for replicate independent runs.

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

***Initial storage = 36h.

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

B=Blend 2 was prepared using 70% peanut milk and 30% rice milk.
 C=Blend 3 was prepared using 55% peanut milk and 45 % rice milk.

## 4.14. Changes in titratable acidity during the storage of different fermented beverages

Table 16 shows the titratable acidity of different fermented beverages. Titratable acidity of the different fermented products increased by extended storage period for the two weeks .The rate of titratable acidity were 0.12, 0.5, 0.02, 0.02 and 0.01 in fermented blend(A), peanut milk , rice milk , blend( C) and blend( B ) respectively .While the rate at recorded at second week were  0.68, 0.66,0.66, 0.63 and 0.51 in fermented rice milk , blend(B), blend( C), peanut milk and blend( A ), respectively (Table16) respectively . The amount of the titratable acidity was significant ($p<0.05$) increased gradually till the end of storage in all fermented beverages.

## 4.15. Changes in minerals content during the storage of different fermented  beverages

Table 17 shows the mineral content of different fermented beverages. Mineral content of the different fermented products increased by extended storage period for the two weeks. The rates of Ca content decreased in fermented peanut milk and rice milk and were 64.14and 15.51 respectively. And the rates of K content in the first week increased in fermented peanut milk (18.83) and decreased in rice milk 5.05 %. And the rates of P content in the first week also decreased in fermented peanut milk and rice milk were 44.42, 44.11 respectively. And the rates of Mg content in the first week increased in fermented blend1, peanut milk (41.25) and rice milk (38.86).  And there is decreased in Fe levels in fermented rice milk, but it's increased in peanut milk. Over all levels of minerals after two weeks refrigeration storage of fermented significantly ($p<0.05$) increase as compared to their initial levels at the beginning of the storage in all minerals except (ca) in peanut milk and (ca, p) in rice 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 | $0.30 \pm 0.01^{b}$ | $0.25 \pm 0.00^{c}$ | $0.88 \pm 0.00^{a}$ |
| Rice milk | $0.29 \pm 0.01^{b}$ | $0.31 \pm 0.00^{b}$ | $0.99 \pm 0.01^{a}$ |
| A | $0.28 \pm 0.02^{b}$ | $0.40 \pm 0.21^{ab}$ | $0.90 \pm 0.01^{a}$ |
| B | $0.28 \pm 0.02^{b}$ | $0.29 \pm 0.01^{b}$ | $0.95 \pm 0.00^{a}$ |
| C | $0.28 \pm 0.01^{b}$ | $0.26 \pm 0.00^{b}$ | $0.92 \pm 0.00^{a}$ |

* Values are mean ± SD for replicate independent runs.

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

***Initial storage = 36h.

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

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

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

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

| Mineral | Peanut milk | | | Rice milk | | |
|---|---|---|---|---|---|---|
| | Initial storage*** | After 1 week | After 2 week | Initial storage*** | After 1 week | After 2 week |
| **Ca** | 159.81 ±30.65[a] | 95.67 ± 8.05 [a] | 83.65 ± 5.90[a] | 198.06 ± 1.06[a] | 182.55 ± 1.51[a] | 192.35 ± 7.11 [a] |
| **Mg** | 100.77 ± 5.63[a] | 142.02 ± 27.44[a] | 111.84 ± 1.37[a] | 112.19 ± 0.81[b] | 151.05 ± 3.01[b] | 126.65 ± 6.75[a] |
| **Fe** | 0.72 ± 0.06[b] | 0.77 ± 0.01 [ab] | 0.95 ± 0.05[a] | 0.51 ± 0.45[a] | 0.1805± 0.00[a] | 0.1880 ± 0.01[a] |
| **K** | 156.99 ± 7.97[a] | 175.82 ±0.00[a] | 184.86 ±9.12[a] | 131.42 ± 0.00 [a] | 126.37 ± 7.43 [a] | 136.79 ± 7.03 [a] |
| **P** | 148.37 ±48.44[a] | 103.95 ± 34.60[a] | 184.86 ± .12[a] | 168.68 ± 10.55[a] | 124.57 ± 9.55[b] | 117.34 ± 7.82 [b] |

\* 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 $p<0.05$.

\*\*\*Initial storage = 36h.

Table 18 shows the mineral content of the different blend fermented beverages during first refrigeration storage, the rates of Ca content decreased in fermented blend (A) , blend B(15.51) and C(13.78) % respectively . And the rates of K content in the first week increased in fermented blend1, blend B blend(C) were 43.18, 22.71, 18.15 respectively. And the rates of P content in the first week also decreased in fermented blend C were 22.92, but increased in blend A( 30.35 ) and blend B (15.72). . And the rates of Mg content in the first week increased in fermented blend A (56.76), blend B( 0.67)  and decreased in blend C(33.07) %. And there is decreased in Fe levels in fermented blend (C) and blend (B), but it's increased in blend A .Over all levels of minerals after two weeks refrigeration storage of fermented significantly ($p<0.05$) decreased as compared to their initial levels at the beginning of the storage .

reported that mineral contents of fermented plain rice beveragewere (Ca) 204.00, (P) 200.00, (Fe) 15.4, (Cu) 1.01, (Zn) 2.7 and (k) 117.5 mg/l.( Amal *et al*, 2012).

**Table 18: Mineral 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 | 151.27±17.67$^a$ | 92.87± 6.29$^b$ | 131.38±14.20$^{ab}$ | 165.50±22.36$^a$ | 151.72±28.42$^a$ | 155.65±61.04$^a$ | 186.56±6.71$^a$ | 147.02±8.06$^a$ | 146.79±35.32$^a$ |
| K | 126.33± 7.09$^b$ | 169.50±2.38$^a$ | 129.77±11.39$^b$ | 154.57±18.74$^a$ | 177.28± 5.88$^a$ | 166.47±21.57$^a$ | 126.54± 6.76$^a$ | 144.69±46.9$^a$ | 158.77±28.35$^a$ |
| P | 97.87± 0.78$^b$ | 128.22±0.64$^a$ | 129.77±11.39$^a$ | 116.57± 7.43$^a$ | 132.29±0.75$^a$ | 166.77±21.15$^a$ | 136.60±49.11$^a$ | 113.64±0.95$^a$ | 158.79±28.39$^a$ |
| Mg | 93.07±6.01$^b$ | 149.83±12.04$^a$ | 118.87± 0.00$^{ab}$ | 134.87±51.69$^a$ | 135.54± 50.6$^a$ | 121.77±0.01$^a$ | 121.62±0.28$^a$ | 88.55±0.80$^b$ | 121.831±0.02$^a$ |
| Fe | 0.78±0.13$^a$ | 0.899±0.11$^a$ | 0.858± 0.09$^a$ | 0.907±0.13$^a$ | 0.804± 0.02$^a$ | 0.778±0.01$^a$ | 0.798 ±0.03$^a$ | 0.750±0.04$^a$ | 0.799± 0.26$^a$ |

* 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 $p<0.05$.

***Initial storage = 36h.

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

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

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

# CHAPTER FIVE

# CONCLOUSION AND RECOMMENDATION

## 5.1 Conclusion

The chemical composition of revealed peanut and rice milk that it's at high nutritional value. It contains high levels of protein, fat and fiber, there for, could contribute to improve the nutritional value of food. Together with rice of high calorie value in one formula produce complementary product provide both nutritional and health benefits to consumers.

During fermentation of different peanut milk rice blend, sufficient numbers of *Bifedobacterium infantis* 20088 were obtained in different types of fermented peanut and rice milk. The viable number of the strain during fermentation and storage were above 6 Log CFU/ml in all fermented products. Therefore, this product fulfills the requirement of probiotics foods to claim health benefit. In addition the levels of pH are decreased while there was increase in the acidity.

It can provide children with enough energy for different activities.  Children 1 - 5 years old would need to ingest at least 1.54 - 2.52L of

This blend (A) to meet the daily energy requirements of  1541.16 – 2521.89 kcal as recommended by FAO/WHO/UNU (2000). On the other hand, fermentation of the peanut and rice milk with *B. infantis* 20088 improves the nutritional value of the products by raising the nutritional value of protein, ash and fiber. In addition, it can provide children with enough energy for different activities. Children 1-5 years old would need to ingest at least 1.54 -2.52L of this blend (A) to meet the daily energy requirements of 1541.16- 2521.89 kcal as recommended by FAO/WHO/UNU (2000). It is possible to utilize the peanut milk and rice milk in manufacture of non-dairy beverages .The

microbial feature of fermented peanut milk and rice milk are influenced by storage period.

## 5.2 Recommendations

1- Encourage the incorporation of *Bifidobacterium* into non-dairy based cereal and grains.

2- More researches to be conducted on nutritional values and functional properties of the developed probiotic fermented product to explore their health benefits.

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