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Sudan University of Sciences and Technology

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Food Sciences and Technology

The Growth of *Bifidibacterium infantis 20088* in Different Non-Dairy Based Milk

A Dissertation Submitted to Sudan University of Science And Technology in partial fulfillment for the degree of B.Sc. (Honours) in food Science and Technology.

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الآية

قال تعالى:

[redacted]

(أَمَّنْ هُوَ قَانِتٌ آنَاءَ اللَّيْلِ سَاجِدًا وَقَائِمًا يَحْذَرُ الْآخِرَةَ وَيَرْجُوا رَحْمَةَ رَبِّهِ قُلْ

هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ وَالَّذِينَ لَا يَعْلَمُونَ إِنَّمَا يَتَذَكَّرُ أُولُوا الْأَلْبَابِ)

صدق الله العظيم

سوره الزمر الآية (9)

Dedication

To spirit of my Father.

To my Mother, to my brother, Sister.

To all my teacher in Food Sciences and Technology, Sudan University of Sciences and Technology, College of Agricultural Studies,

To my class mate.

Acknowledgements

- Firstly Thanks to Allah for giving me health and keep me well to finish this work.

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Abstract

This study has been conducted to estimate the growth of *Bifidobacterium infantis 20088* in different Beverage (Brown rice milk, Peanut milk, skim milk, mixture). Peanut was roasted (100C^{o} for 20min). Soaked in water (8h), blended (5min), and filtered to prepare the roasted peanut milk. Brown rice were Soaked in water (12h), blended (5min), and filtered to obtain the brown rice milk. The brown rice and peanut milk were sterilized, after cooling 2g of malted brown rice flour, was added. The different sterilized milk were cooled to 37^{o} and inoculated with 2% *Bifidobacterium infantis 20088* culture and incubated for 48h at 37^{o}. The results revealed that that there were a significant ($p< o.05$) increase in the growth of the strain 20088 in each fermented milk (Brown rice milk, peanut milk, skim milk, the mixture) at maximum growth as compared with strain level at the beginning of the fermentation process. The growth of strain 20088 was high in different types of fermented milk, which was more than 7 log CFU/ml. These total numbers of strain 20088 was found to be quite enough for using these different fermented milk as probiotic, which at least should contain 6- 7 log CFU/ml. These significant increases in strain 20088 during fermentation is associated with decrease in the (pH) and (TSS) with the progress of fermentation time.

ملخص الدراسة

أجري هذا البحث لتقدير نمو باكتيريا إنفانتس في مختلف المشروبات (الفول السوداني , الأرز البني , اللبن المنزوع الدهن , خليط بينهم)

تم تحميص الفول السوداني عند درجه حراره 100م لمده 20 دقيقه ,غمر في الماء لمدة 8 ساعات , خلطه بواسطه خلاط كهربائي لمدة 5 دقائق , الأرز البني غمر في الماء لمدة 12 ساعه خلطه بواسطه خلاط كهربائي لمدة 5 دقايق , تم ترشيحم بإستخدام مصفاة .المصفاة تجحز الفول السوداني المحمص والأرز البني , مشروب كل من الفول السوداني والأرز البني يغلي في درجه حراره 70 درجه مئوية لمده 3 دقايق, ويبرد في درجه حراره الغرفة.

النتائج أظهرت وجود زيادة معنوية في نمو باكتيريا إنفانتس في المشروبات المخمره عند مقارنتها بعددها عند بداية عملية التخمير, نمو باكتيريا إنفانتس عالية في مختلف أنواع المشروبات (لبن الأرز البني , لبن الفول السوداني , اللبن المنزوع الدهن , الخليط) ولاحظ أن العينة الخليط كانت تحتوي علي أعلي نسبه من باكتيريا إنفانتس, هذه الأعداد الكلية من باكتيريا إنفانتس كافية تماماً لإستخدام هذه المشروبات مضادة .

هذه الزياده المعنوية كانت مصحوبة بإنخفاض في الرقم الهيدروجيني والجوامد الصلبة الذائبة مع تقدم عملية التخمير.

CHAPTER ONE

INTRODUCTION

The word probiotic derived from Greek and mean "for life" **(Metchnikoff, 1770)**. one of the more detailed current definition of probiotic is "microbial dietary intestinal tract" mainly specific strain of lactobacilli , Bifidobacteium , enterococci and yeasts today used commercially as probiotic, According to medical news friendly bacteria that play an important role in basic digestion, proper metabolism and overall well – being. However, most human origin probiotic are they are characterized by low growth capability in food medium including the dairy. The main recommended carrier of probiotic to human **(Naidu *et al*., 1999; Holzapfel *et al*., 1995; Saxe in *et al*., 2005).**

Bifidobacterium are considered as important probiotic and used in the food industry to relieve and treat many intestinal disorders. Bifidobacterium exacts a range of beneficial health effects, including the regulation of intestinal of pathogens and or in fact the gut mucosa, the modulation of local and systemic immune responses , the repression of vitamins and the bioconversion of a Number of dietary compounds into bioactive molecules **(Mayo and Van Sincere .2010)**.

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 Beverage are estimated to present Some20-40% of our food Supply

World widow **(Compbell-Patt, 1994)**

in Sudan, the majority of food, are consumed in the form of naturally fermented products .In addition, the use of rice and peanut as carrier for probiotics is approved and the process claimed to improve overall protein quality of fermented beverage **(Kabeir *et al.*, 2005)** however the use of peanut milk and brown rice milk blend as a complementally non-dairy based carrier for *bifidobacteriaum infantis* is not explored. Therefore, the objectives of this study are

1. To examine the growth of *Bifidobacterium infantis* 20088 in different sources of milk (brown rice milk, peanut milk, skim milk, mixture of milk).
2. To determine physicochemical prosperities (TSS, pH and moisture) of the different milk during the growth of the strain 20088.

CHAPTER TWO
LITERATURE REVIEW

2.1 Brown Rice:

2.1.1 History of Brown rice:

Human have long history of cultivating rice crop; especially those living in China, India, and Japan. polished rice or white rice, which primarily consists of starch ,is produced through a series of mechanised processes including hulling and milling **(Miller *et al.*, 2002)**, and it is the predominant type of rice consumed worldwide **(Villegas *et al.*, 2007)**. Rice is now grown every when and provides food for more than half of the world's population. Although the glycaemia index value of a specific white rice variety depends on the degree of processing, cooking time, and amylase content, the glycaemia index values of white rice are higher on average than those of white grains. For example, the mean glycaemia index values were 64(SD7) for white rice, 55(5) for brown rice, 41(3) for whole wheat, and 25(1) for barley in a various meta-analysis **(Foster *et al.* , 2002)** . In addition, white rice is the primary contributor to dietary glycaemia load for population that consume rice as a staple food **(Village's *et al*., 2007).**

However, dies with a high glycaemia index or glycaemia load were associated with increased risk of developing type 2diabetes **(Villegas *et al.*, 2007).** a significant positive association between white rice consumption and risk of diabetes was

observed among two cohort of Chinese and Japanese women **(Nandi *et al*., 2010)**, although The association in western population with much lower consumption levels of rice than Asia population also generated **(Sun Q *et al*., 2010)**. these studies were heterogeneous with respect to sample size. White rice intake level, and other characteristic that may contribute to inconsistencies in addition. Whether any dose-response relation exists between white rice consumption and risk of diabetes is unknown. Therefore, meta-analysis evaluates white rice intake and incidence of type2 diabetes and quantified dose-response response relations between intake of white rice and risk of type2 diabetes.

2.1.2 Definition of Brown Rice

Rice is whole grain, with the outer hull; white rice is the same grain after further milling and polishing to remove the bran layer and germ. Brown rice has a mild, nutty flavour, and is chewier and more nutritious than white rice. Red rice and black rice, also eaten unfilled, are essentially the same varieties with a differently-pigmented outer layer. Any type of rice, unpolished, may be eaten as brown rice. Brown rice is in many ways superior nutritionally to white rice, but there have been concerns over excessive arsenic levels, though the amount of arsenic in rice varies with the rice plants' exposure to arsenic, with more danger from farmers' use of inorganic arsenic as a pesticide rather than from naturally occurring arsenic **(Sohn, Emily., 2014)**.

2.1.3 Nutritional Value of rice:

A detail 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 which, brown, black, red and purple varieties. of rice - each prevalent in different ports of the world. It also depend 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.

Rice is consumed as milled rice after de hulling process and whitening (removal of per car, bran layer and embryo from brown rice). After whitening ports of rice were removed which was very low starch content but has high percentages of oil, protein, vitamins and minerals **(Sohn, Emily., 2014).** Rice is great source of compiles carbohydrate witch gives us the enough we need. According to FAO, rice provides 20% of the world's dietary supply.

2.2 Peanut

2.2.1 History of Peanut

John Harvey Kellogg was issued a peanut for a "process of producing alimentary products" in 1898 and used peanut although he boiled the peanut vat her than roasting them. Kellogg served peanut butter to the patient at his Battle Creek sanitanum. other makers of modem peanut butter include George , a snack –took maker in st.louis, Missouri, who was making peanut butter with roasted peanut as carry as 1894, and Gorge wash-ingot carver, who is often mistakenly credited as

the inventor due to his extensive work in cleverly peanut crop and disseminators recipes **(CRB, 2002)**.

2.2.2 Usage of peanut

Peanut have much usage. the can be eaten row, used in recipes, made into solvents and oil, medicines , textile materials, and peanut butter, as well as many other uses, popular confections made from peanut include salted peanut, peanut butter (Sandwiches, peanut candy bars, peanut cookies, and cups). Peanut brittle and shelled nuts (plain roasted). Salted peanut are usually cups, peanut brittle, and shelled nuts (plain /roasted). Salted peanut are usually roasted in oil and packed in retail. Size plastic bags or energetically scaled cans. Dry roasted peanut are also marketed in significant quantities. peanut are often a major ingredient in mixed nuts because of their relative cost compared to brazil nuts , cashews, walnuts , and so an . although peanut butter has been a tradition an camping trips because of its protein content and because in resists spoilage for large quantities are also used in the commercial Mann rapture of sandwiches , candy, and bakery product . Boiled peanut are a preparation of row, unshelled green peanut boiled in brine and often eaten as a snack. More recently, fried peanut recipes have emerged-allowing both shell and to be eaten. Peanut are also used in a wide variety of other areas, such as cosmetic, plastic, dyes and paints nitro-glycerine **(Bonnie, 1988)**.

2.2.3 Protective Nutrients of peanut:

Research on peanut have shown that all of these component promote health and reduce the risk of chronic disease people who eat to take in more key nutrient critical health. In more than 15,000 people who consumed and peanut products, it was found that levels of vitamin A, Vitamin E, float, magnesium, Zinc, iron, calcium, and dietary fibres were higher that those who did not consume peanut **(Alger and Mattes, 2003)**.

2.3 Probiotic

2.3.1 Definition of probiotic

The word ‘probiotic’ , derived from the Greed language , means ‘ for life. ‘ and has had many definitions in such as ‘substances produced by protozoa that stimulate the growth of another ‘ or ‘organisms and substance that have a beneficial effect on the host animal by contributing to its intestinal microbial balance‘ were used **(Full,1989)**.

2.3.2 History of probiotics

The idea that some bacteria contained in our food may have beneficial effect in mush older than the term probiotic. At the beginning of the 20th century , the Russian Nobel laureate elide methinks off associated the observed longevity of Bulgarian peasants with their high consumption of live microbes in fermented milk product , as he reported in his book the prolongation of live . In 1930, the Japanese Scientist Minor Shirt Isolated a lactic acid bacterium from the faces of a healthy

infant. five years later, one of the first fermented milk drinks thought to support intestinal health was produced whit 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 product were eventually introduced in Europe **(Metchnikoff, 1907)**.

2.3.3 Application of probiotic culture into food

Probiotic bacteria are applied in many different products worldwide. In addition to food product. Probiotic cultures are also used in pharmaceuticals and animal feed. Most definition of probiotics and based on live bacteria that confer a health benefit for consume thus. it is considered as important that probiotic products contain an effective dose of living cell during their whole shelf life. The application of probiotic in food products depends on factors like water activity, processing and storage temperature, shelf life , oxygen content ph and mechanical stress.

The development of new functional foods which combine the beneficial is a challenging issue. Nevertheless cereal –based products in the world required a lactic fermentation, often in association with years or modes. Cereal is good substrates for the growth of probiotic strains and due to and the presence of non-Digestible component of the cereal matrix may also serve as probiotic

(Goktpe *et al*, 2006).

2.3.4 Characteristics of probiotic microorganisms

Probiotic microorganism has to be alive when administered. **(Killer and Chores, 2008)**. One of the concerns throughout the signify 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 in the intestinal ecosystem probiotic 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 **(Petro, 2009).** However, represent a major challenge because several difficulties arose, such as variability of probiotic specially used in the site (Oral, vaginal, intestinal).and mode of its application.

The probiotic candidate must be a taxonomically defined microbe or combination of microbes (genus, species and strain level). It is commonly admitted that most effect of probiotic of the same genus or species **(Sethi,2009).**

2.3.5 Functional properties of probiotic

In spite of research 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).**

Numerous beneficial functions have been suggested for probiotic bacteria include:

-Vitamin production, availability of minerals and trace element.

-Production of important digestive enzymes.

-Production of b-glycosidase of alleviation of factors in tolerance of lactose.

- Barrier , restoration , antagonistic effects against :
  -Infections diarrheal.
  -Antibiotic-associated diarrheal, irradiation –associated diarrheal.
- Cholesterol _lowering.
- Stimulation and improvement of the immune system.
- Anti-carcinogenic effects in the colon.
- Maintenance of mucosal integrity.
- Reduction of inflammatory allergic reaction.
- Adherence and colonization resistance.
- Ant oxidative activities **(Kullisaar, *et al* ., 2002).**

2.3.6 Criteria of Selection of appropriate probiotic:

Different aspects have to be considered in probiotic selection safety criteria or any successful probiotic have been defined in several reviews **(Lee and Salminen, 1995; Donohue and Salminen,1996.Adams,1999).**

Include the following specification:

1. Strains use is preferably of human origin.
2. They are isolated from healthy human G1 tract.
3. They have a history of being non-pathogenic.

4. They do not deconjugate bile salts (bile salt deconjugate or dehydroxylation would be a negative triat in the small bowel **(Marceau *et al.;* 1995).**
5. They have no history of association with diseases such as infective endocarditic or GI disorders.
6. They do not carry transmissible antibiotic resistance genes.

2.3.7 Species of Bifidobacterium

B. angulatum; B. Animalis; B. Steroids; B. Bifidum; B. boum; B.breve; B.catenulatum; B.gallinarum; B.indicum; B.longum; B.magnum;B.merycicum; B. minimum; B.pesudocatennulatum; B.pseudolongum; B.psychraerophilum; B.pullorum; B. Thermacidophilum; B. Thermophilum ; B.urinalis; B.sp.

2.3.7.1 *Bifidobacterium Infantis 20088*

Bifidobacterium infantis 20088 is a probiotic bacterium that inhabits the intestines of both infants and adults. Thus type of bacteria is considered beneficial because of the acids it product. The acids produced by *Bifidobacterium infantis20088* may help impede the growth or colonization of farm full bacteria within the colon. According to medical news today, *Bifidobacterium infants20088* in "normal, friendly bacteria that play an important role in basic digestion, proper metabolism and overall well-being. *Bifidobacterium infants 20088* works within the digestive system to restore intestinal balance and maintain normal digestive health ." According to a study by **(Sanders, 2007)** .

Bifidobacterium infants20088 may be beneficial to those from symptoms of Irritable Bowel Syndrome (IBS) including bloating, gas, diarrheal, 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. thus breakdown not only makes the food more susceptible to digestive enzymes but it also allows the food particles travel more easily through the digestive tract the large intestine has a high number of microbes present that help complete process of food digestive .microbes are tiny, living organisms usually too small to be seen with the naked eye and are al also commonly referred to as microorganisms. these microbes or microorganisms include bacteria, viruses, fungi, able, and protozoa. *Bifidobacterium Infants20088,* which is a bacterium, falls into the microbes or microorganism category.

Bifidobacterium infants20088 is considered a "good "or beneficial. 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 content , creating conditions less desirable for harmful bacteria '' For these reasons *BIfidobacterium infants20088* may help provide relief to individuals suffering from IBS symptom **(Sanders, 2007)**

CHAPTER THREE

MATEIALS AND METHOED

3.1 Raw Materials

The peanut seeds were purchased from a local crops market (Kordophan State). Care was taken to ensure that good quality and mould free seeds were selected. The brown rice was purchased from Bahary markazi market (Khartoum State, Sudan). Fresh milk control was obtained from department of animal production, Collage of Agriculture Studies, Sudan University of Science and Technology (Khartoum Sudan).

3.2 Preparations of peanut and brown rice milk

Peanut was roasted (100C^{o} for 20min). Soaked in water (8h), blended (5min), and filtered to prepare the roasted peanut milk. Brown rice were Soaked in water (12h), blended (5min), and filtered to obtain the brown rice milk.

3.3 Preparation of fermentation inoculums

Bifidibacterium infantis 20088 was obtained from the stock culture collection of microbiology laboratory (Department of Food Science Technology, Collage of Agriculture Studies, SUST, Sudan). The strain stock was maintained at -20 ˚C in glycerol solution. A working culture was prepared by activation of the strain in

MRS broth, incubation under anaerobic condition at 37 ˚C for 2h. The obtained broth activated again under the same condition 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 two days.

3.4 Fermentation of different milk with *Bifdobacterium Infants 20088*

The brown rice and peanut milk were sterilized, after cooling 2g of malted brown rice flour, was added. The different sterilized milk were cooled to 37^{o} and inoculated with 2% *Bifidobacterium infantis 20088* culture and incubated for 48h at 37^{o}.

3.5 Enumeration of *Bifdobacterium Infants 20088*

MRS was used to enumerate *B.Infants 20088* of fermented beverages using the plate count technique .Samples were drawn at initial and every 6 h intervals during fermentation .1ml of fermentation broth was diluted in 0.15% peptone water, followed plating on rogues agar (MRS). The plates were an aerobically incubated at 37 ˚C for 48h. The growth was calculated as colony forming unit (CFU/ml).

3.6 pH and total soluble solids measurements

The pH level during fermentation was determined using a pH meter (Jewry model 351).While the total Soluble solid was determined using Erma model refract meter.

3.7 Determination of moisture content

Moisture was determined using thermal drying method, Two gram of each sample were heated under careful specified conditions (104 ◦c for 6 hours), and the loss of the weight was taken as a measure of the moisture content.

3.8 Statistical analysis

One- way ANOVA way used to examine significant differences between normally distributed data. Turkey's-test usually be to perform multiple comparisons between means within each specific growth medium. Probability level of less than 0.05 was considered significant ($p<0.05$). All data were analyzed using MINITAB statistical software.

CHAPTER FOUR

RESULTS DICUSSION

4.1 Growth of probiotic Bifidobacterium infantis 20088 during fermentation

The result presented in Table1 showed that Growth *Bifidobacterium Infantis* 20088, in different beverage during fermentation process. There were significant ($p< 0.05$) increases in viable count of the strain 20088 fermentation of different milk as comparative to its entail levels at the beginning of fermentation. At maximum growth (36h) the viable count were 7.91, 8.91. 9.91 And 9.72 log cfu/ml in fermented brown rice milk, peanut milk, mixed and reconstituted skim milk, respectively.

Table 1: Growth of probiotics Bifidobacterium INFANT20088 during fermentation of different beverages*

Time (h)	Brown rice bevera\ge	Peanut	Mixed	Skim milk
Initial (0h)	2.54± 0.09 ^{H}	2.74± 0.06 ^{I}	2.85± 0.02^{E}	2.87± 0.04 ^{H}
6	2.80± 0.02^{G}	3.72± 0.03 ^{H}	4.71± 0.05 ^{E}	3.93 0.01 ^{G}
12	3.59± 0.16^{F}	5.78± 0.03 ^{G}	5.94± 0.02 ^{D}	5.89± 0.02 ^{F}
18	4.72± 0.02 ^{E}	6.61± 0.05^{F}	7.85± 0.02 ^{C}	7.69± 0.05 ^{E}
24	5.87± 0.03 ^{D}	7.87± 0.03 ^{C}	8.69± 0.0 $^{B\,C}$	8.51± 0.05 ^{C}
30	6.91± 0.02 ^{B}	8.68± 0.03 ^{B}	9.56± 0.03^{AB}	8.97± 0.03 ^{B}
36	7.96± 0.01 ^{A}	8.91± 0.01 ^{A}	9.91± 0.02 ^{A}	9.72± 0.02 ^{A}
42	7.91± 0.02^{A}	7.72± 0.02 ^{D}	9.54± 0.09^{AB}	8.92± 0.02 ^{B}
48	6.66± 0.04^{B}	6.97± 0.03 ^{E}	8.38± 0.70 ^{C}	7.80± 0.02 ^{D}

*****Values are mean of replicate independent runs,
*****Values that carry the same superscript letter in the same column are not significantly different.

4.2 Reduction of pH during fermentation

Referring to the result in Table2, the reductions of pH during fermentation of different milk with *Bifidobacteirum Infantis* 20088 were significant ($p < 0.05$) due to the acid production by growth of strain 20088. These results were in agreement with those reported by (Kabeir, 2015) who reported that there was reduction in pH during fermentation of rice milk with *Bifidobacterium*.

Table 2: Reduction of pH during fermentation of different beverages with Bifidobacterium infantis 20088

Time (h)	Brown rice	Peanut	Mixed	Skim milk
Initial (0h)	5.68± 0.03^{A}	5.39± 0.01 ^{A}	5.48 ± 0.26 ^{A}	5.68± 0.11 ^{A}
6	5.61± 0.06 ^{A}	5.29± 0.02 ^{A}	5.21± 0.01 ^{A}	5.63± 0.04 ^{AB}
12	5.47± 0.04^{A}	5.23 +0.04 ^{A}	5.16± 0.06 ^{A}	5.52 ± 0.11^{AB}
18	5.33± 0.04^{AB}	5.11 0.01^{A}	5.00 ± 0.00 ^{A}	5.37± 0.05 ^{BC}
24	4.94± 0.08^{B}	4.30± 0.28^{B}	4.37 ± 0.04 ^{A}	5.25± 0.07 ^{C}
30	4.25± 0.35^{C}	4.00 ± 0.00^{BC}	3.80 ± 0.14 ^{A}	4.98± 0.03 ^{D}
36	3.79± 0.041^{C}	3.76± 0.01^{C}	3.68 ± 46.9 ^{A}	4.79± 0.02 ^{E}
42	3.84± 0.02 ^{C}	3.74 ± 0.05^{C}	5.19 ± 2.18^{A}	4.26± 0.06 ^{E}
48	3.91± 0.01^{C}	3.85 ± 0.06^{C}	3.70 ± 0.14 ^{A}	4.36± 0.06 ^{D}

*****Values are mean of replicate independent runs.

*****Values that carry the same superscript letter in the same column are not significantly different.

4.3 Total Soluble Solid (TSS) during fermentation with strain 20088

The result presented Table3 Showed The increasing of PH during fermentation of different Beverage with *Bifidobacteirum* of the beverage with Infantis. The reduction of pH during fermentation of The different Beverage with Bifidobacterium were significant. There were continuous reduction of TSS at The different beverage , This reduction may be due the Growth of *Bifidobactirem.* These result were in agreement with those reported by **(Salma, 2015)** who reported that there were reduction of PH in peanut and milted Beverage during fermentation.

Table 3: TSS of different beverages during fermentation with probiotics *Bifidobacterium infantis 20088*

Time (h)	Brown rice	Peanut	Mixed	Skim milk
Initial (0h)	15.30± 0.42 ^{A}	14.20± 0.28 ^{A}	14.60± 0.28^{A}	14.05 ± 1.34^{A}
6	14.50±0.00^{AB}	13.75±0.07^{AB}	13.40± 0.85 ^{A}	13.00 ± 2.26^{A}
12	14.25±0.07^{ABC}	13.30± 0.42^{AB}	12.40± 0.57^{A}	58.0 ± 61.9 ^{A}
18	14.10± 0.14^{BC}	12.95± 0.07^{AB}	11.20 ±1.70^{A}	13.50 ± 0.76^{A}
24	14.00± 0.00^{BC}	12.35± 0.35^{BC}	10.00 ± 2.83^{A}	9.80 ± 0.85 ^{A}
30	13.65±0.21^{BC}	11.25± 0.35^{CD}	9.70 ± 2.83 ^{A}	9.25 ± 4.17^{A}
36	13.35±0.21 ^{C}	9.90± 0.14 ^{DE}	9.55 ± 2.76^{A}	9.55 ± 4.88^{A}
42	11.55± 0.64 ^{D}	9.20 ± 0.28^{E}	8.80 ± 2.55 ^{A}	8.75 ± 5.30 ^{A}
48	9.10 ± 0.14 ^{E}	9.20 ± 0.85 ^{E}	8.45 ± 2.19^{A}	8.00 ± 4.24 ^{A}

*****Values are mean of replicate independent runs

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*****Values that carry the same superscript letter in the same column are not significantly different.

4.4 Moisture levels during fermentation process with the strain 20088

The increasing of moisture during fermentation of the different Beverage with Bifidobacterium were significant. There were continuous increasing of moisture at the different Beverage, This increasing might indicate high enzymatic activity that break down the macro component into simple and to the relate of water. These results were in agreement with those reported by **(Kabeir, 2015)** who reported that there was increasing TSS of peanut and milted beverage during fermentation.

Table 4: The development in moisture during fermentation of different beverages with *Bifidobacterium infantis 20088*

Time (h)	Brown rice	Peanut	Mixed	Skim milk
Initial (0h)	72.24± 0.34 ^{E}	83.18± 0.25 ^{D}	83.38± 0.03 ^{D}	93.26± 0.23^{A}
6	72.56±0.02^{DE}	83.57±0.10^{CD}	83.78±0.11^{CD}	93.47±0.382^{AB}
12	73.36±0.09^{CDE}	83.83±0.04^{CD}	83.88±0.08^{CD}	93.88±0.11^{AB}
18	73.80± 0.06^{CD}	83.73±0.57^{CD}	85.15±0.28 ^{BC}	94.09±0.36^{AB}
24	74.23± 0.44^{BC}	84.54± 0.04^{BC}	85.20± 0.76^{BC}	94.38±0.86^{AB}
30	75.42± 0.11^{AB}	84.71±0.06^{ABC}	85.43±0.78^{ABC}	94.39±0.56^{AB}
36	76.08± 0.54 ^{A}	85.12±0.18^{AB}	86.06±0.40^{AB}	94.81± 0.21^{AB}
42	76.21± 0.47^{A}	85.27±0.58 ^{AB}	86.78± 0.31^{AB}	94.87± 0.36^{AB}
48	76.43± 0.60^{A}	85.75± 0.14^{A}	86.86± 0.18 ^{A}	95.11 ± 0.33^{B}

*****Values are mean of replicate independent runs,

*****Values that carry the same superscript letter in the same column are not significantly different. .

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Frome the result of this study we can conclude that it was possible to grow probiotic *Bifidobacterium infantis20088* in none milk bases beverage such as (brown rice milk and peanut milk and mixed milk). At maximum growth (36h) of each types of fermented milk, levels of the viable strain 2008 existed were more than 6 logCFU/ml. This number is the least level required to presence in probiotics foods. Therefore, these numbers of strain 2008 in different fermented milk is quite enough for using these products as functional fermented foods.

5.2 Recommendations

1. Fncovncy the use of Bifidobacerium infatins in fermentation of Peanut milk and Brown rice milk.

2. More studies to be carried out on new carrier for probiotics from none dairy.

3. Further studies on shelf life of the strain and nutritional are required

References

Alger, CM. And Matte, BD. (2003) Peanut adults. J Am Coll Nutr, 22(2): 133-41.

Bonnie. L (1988) "The Peanut controversy _ Peanut oil and risk of atherosclerosis" –Nutrition Action Health letter Bellis Mary.

Compbell-Platt, G. (1994). Fermented Foods _Nwokalo, E.1996. Peanut (Arachis lypogaea L.) In: Nwokaolo, E, Mart, J.S. (Eds). Legnmes and oilseeds in nutrition Chapman and Hall, London Pp.49.

Fetro-Powell K, HOH SH, Broand-Miller C. International Table of Glycemic Index and Glycemic load Values: 2002. Amylclin Nut 2002; 76:5-56 .

Fuller, R. (1989). Probiotic in man and animal, Journal of Applied Bacteriology,66, 365-378.

Goktepe, I, and Juneja, V. K. ,Ahemedna, M.(2006). Probiotic in Food Safety and Human Health.Taylor and Francis Group Boca Raton, NewYourk.

Holzapfel, W. H. And Geisen, R., Schilinger, U(1995). Biological Preservation of Food With reference to Protective Cultures , bacteria and Food –grade enzymes .International Journal of Food Microbiology 24: 343_362.

Ibrabeem. E. SALMA,(2015). Survived of Bifidobacterium longum BB536 and physiochemical changes during refrigeration Storage of Fermented Roasted Peanut Milk partially Substituted with Millet thin porridge, MS.C in Food Science ang Technology. Department of Feed Science and technology Sudan Unversity.

Kabeir, M.B., Abd Aziz, S., Muhammed, M. , Yazid, A.M. 2005. Growth of Bifidobacterium Longum BB356 in Medida (Fermented cereal porridge)

Kligler .B. and Cohrssen .A.(2008). Probiotic American Family Plysician.; 78(9): 1073-1078.

Kutlisaar, T.,Zimer, M, Mikelsaar, Vihalemn .T.,Annuk, H., Kairance, C., Kill,. A.(2002).Two antioxidative Lactobacilli Strain as Promising Probiotic .Int.J. Food Microbial., 72:21-224.

Lee, Y, -K., Salminen , S.(1995). The coming of age of Probiotic Trends Food Sci .Technol, 6:241-245.

Marten P., Gerhardt, M.F., Myara, A., Bonvier, E, Trivin, F. Rambaud, J.C. (1995). Metabolism of bile salts by alimeritary bacteria during transit in the human small intestine –Microb, Ecol. Health Dis .8:151-157.

Mayo, B., Van Sinderen, D. 2012. Bifida bacteria: Genomics and Molecular Aspect. Caister Academic Press ISBN 978-1-904455-4.

Metchnikoff, E. 1907. The Prolongation of Life: optimistic Studies Butter worth-Heinemann, London.

Miller G, Prakash A, Decker E. Whole –grain Foods in health and disease Awerican Association of cereal chemists 2002.

Naidu, A.S., Bidlac, W.R., Clemens, R.A.1999. Probiotic Spectra of Lactic acid bacteria. Crit . Rve. Food Sci. Nutrition .,

Nanri A, Mizoue T, Noda M, Takahashi Y, Kato M, lnoue M, et al. Rice intake and Type2 diabetes injanese man and women: the Japan public Health center based prospective study, Amjclin Nutr 2010;92:1468-77.

Petrof, E. O. 2009. Probiotic and gastro intestinal disrase: Clinical evidencoand.

Sanders, M. E. 2007. Probiotic, Strains Matte r. Functional Foods and Nutretional Magazing -36-41.

Sethi, R., and Anjn S.2009. "Can Quality oriented Firms Develop Innovative New Probiotic? "Journal of Product Innovation Management,26(2) 206-21.

Sohn, Emily (2014-10-12).”Contamination: the toxic Side of rice ” .Retrieved (2015-3-27).

Sun Q. Spiegelman D, Van Dam RM, Holmes MD, Malik VS,Willett Wc, et al. White rice, brown rice, and risk of Type 2 diabetes in US men and women. Arch Intern med Zolo, 170:961-9.

The History of Peanut, Huffington Post. January 23, 2012.

Villegas R, Lius, Gao YT, Tang G, LiH, Zheng W, et al . Prospective Study of dietary Carbohydrates, glycwmic inder, glycemic load, and incidence of Type 2 diabetes welliusin middle –aged Chinese women, Arch Interm med 2007;167:2310-6.