



## Original Research Article

# Roasted Peanut Milk Partially Substituted with Millet Thin Porridge as a Carrier for *Bifidobacterium longum* BB536

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## ABSTRACT

This study was carried out to develop probiotic fermented beverage based on roasted peanut milk. Peanut was roasted (100 °C for 20 min) to improve nutrient component, facilitate the removal of the crust and decrease the peany flavor of peanut. Roasted peanut and yellow millet were soaked in water (12 h), blended (5 min) and filtered using a double layered cheese cloth to prepare the roasted peanut milk and millet beverage. Yellow millet beverage was boiled (70°C for 3 min), malted millet flour (1:5 (w/w) was added, cooled (37°C), maintain 14 min to prepared millet thin porridge. Different formulation based on roasted peanut milk partially substituted with 15% (A), 30% (B), and 45% (C) with millet thin porridge was prepared. Formulations were sterilized (121°C for 15 min), inoculated (3% active culture of *B. longum* BB536), and incubated (37°C) for 24 h. *B. longum* BB536 viable count significantly ( $P < 0.05$ ) increased by extended fermentation period. At maximum growth (18 h), there was 3.15, 2.9, 2.89, 2.76, 2.43, and 2.1 log CFU/ml increase in fermented peanut milk, millet thin porridge, cow milk, blend (B), blend (A) and blend (C), respectively. At 24 h fermentation, the number of the strain in all fermented beverages still above the number required to presence in probiotic food which is at least 6 log CFU/ml fermented products; except blend C (5.77 CFU/ml) didn't fulfill probiotic requirements in food. The pH significantly ( $P < 0.05$ ) decreased due to increase acids production from fermentation of sugar. Thus both sugars and total soluble solids (TSS) decreased by fermentation. Therefore, peanut milk and millet thin porridge and their blends could be suitable carriers to strain *BB536* for probiotic requirements.

## Keywords

Peanut,  
Millet,  
*Bifidobacterium*,  
Growth,  
pH,  
TSS,  
Sugars,  
Porridge

## Introduction

Cereal and legumes are mostly used to develop fermented beverages. Fermented foods can even have beneficial health

effects, when microorganisms used possess probiotic activity. Legumes (*Arachis hypogaea* L.) groundnut has a potential role

in combating malnutrition are a major source of edible oil and protein meal and therefore considered to be highly valuable in human and animal nutrition (Nwokalo, 1996). It's rich in protein, energy and other nutrient. Peanut- based formulated food can be developed to for a therapeutic purposely and to aid in famine relief. There for the present low level in peanut consumption, especially in the developing countries, should be increased. It is, therefore, necessary to direct research into the possibility of peanut processing into other useful and edible products. Fermentation of groundnut milk may serve as one such effort that can increase the protein availability and consumption (Roberts-Sunny *et al.*, 2004). On the other hand, millet is the sixth most important grain in the world. Millet is equal or superior to grain of wheat, corn sorghum and rice in protein and oil content, it contains similar amount of calcium (Ca) and phosphorus (P), more iron (Fe) than the cereals grains (Marwa, 2005). Millets have an alkaline pH and are the only grains that keep their alkaline properties even after being cooked. As another plus, millet is a gluten free grain and thus, is ideal for people with wheat/gluten allergies or intolerance (Baltensperger and Cai, 2004).

Fermentation is one of the oldest known uses of biotechnology. All over the world, fermented foods continue to constitute an important part of our diet and together with beverages are estimated to present some 20-40% of our food supply world-wide (Campbell-Platt, 1994). Particularly in developing countries, where refrigeration is not always an option, the fermentation process is widely used. Fermentation prolongs the shelf-life of foods in addition to improving the nutritional value and reducing the risk for food borne illness (Campbell-Platt, 1994). The word probiotics derived from Greek and means "for life"

(Metchnikoff, 1907). One of the more detailed current definitions of probiotics is; "a microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract". Mainly specific strains of lactobacilli, Bifidobacterium, enterococci and yeast are today used commercially as probiotics (Naidu *et al.*, 1999; Holzapfel and Schillinger, 1995; Saxelin *et al.*, 2005). Bifidobacterium are considered as important probiotics and used in the food industry to relieve and treat many intestinal disorders. *Bifidobacterium* exert a range of beneficial health effects, including the regulation of intestinal microbial homeostasis, the inhibition of pathogens and harmful bacteria that colonize and/ or infect the gut mucosa, the modulation of local and systemic immune responses, the repression of procarcinogenic enzymatic activities within the microbiota, the production of vitamins, and the bioconversion of a number of dietary compounds into bioactive molecules (Mayo and van Sinderen, 2010).

*Bifidobacterium longum* may be considered the most common species of Bifidobacterium, being found both in infant and adult feces (Bivati *et al.*; 1984). Potential benefits from consumption of *B. longum* include: antagonistic action toward intestinal pathogens, improved lactose utilization, anticarcinogenic action and control of serum cholesterol levels. Many scientific studies showed the benefits offered by *Bifidobacterium longum* BB536 (Kojima *et al.*, 1996; Namba *et al.*, 2003). Thus there is considerable interest in incorporating these heaths promoting bifidobacterium into food. On the other hand, dairy products are the main carriers of probiotic bacteria to human, as these products provide a suitable environment for

probiotic bacteria that support their growth and viability. However, with an increase in the consumer vegetarianism throughout the developed countries, there is also a demand for alternative carrier for beverage. The development of new nondairy probiotic food products is very much challenging, as it has to meet the consumer's expectancy for healthy benefits (Stanton *et al.*, 2003). Nevertheless, there were no many studies regarding application of probiotic bifidobacterium into fermented Sudanese foods. In previous investigation, (Kabeir *et al.*, 2005) successfully incorporated *B. longum* BB536 into Sudanese cereal beverage Medida.

In this respect, the use of peanut milk and millet blend will complement nutrients same time can be a successful non-dairy carriers for Bifidobacterium strain. There for the objective of this study is to evaluate the growth of *B. longum* BB536 and its related physio-chemical changes during fermentation of different formulated beverages.

## **Materials and Methods**

### **Raw Materials**

The red-skinned peanut seeds (*Arachis hypogaea*) (*V. Natal*) were purchased from a local crops market in Bahri (Kartoum State, Sudan). Care was taken to ensure that good quality and mould-free seeds were selected.

The yellow millet (*Panicum miliaceum*) (*V. Proso*) was purchased from Alzraiga village (Eldwaim, White Nile State, Sudan).

Fresh cow milk control was obtained from Department of Animal Science, Collage of Agriculture Studies, Sudan University of Science and Technology (Khartoum, Sudan).

### **Preparation of peanut milk**

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

### **Malting of millet**

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

The flour was packed in a plastic container and kept at refrigeration temperature until used.

### **Preparation of millet thin porridge**

Yellow millet thin porridge was prepared according to procedure by Kabeir *et al*(2005), with some modifications. 200g cleaned yellow millet was weighted, washed and soaked in 400 ml distilled water in 2l beaker, and placed at room temperature for 7 h. Water was drained and millet was blended with 800ml clean water at medium speed for 5 minutes.

The slurry formed was filtered using a double layered cheese cloth and boiled in hot plate at 70°C for 3 min magnetic stirrer was used for mixing. Malted millet flour was added in ratio 1:5 w/w after cooling at 37 °C and maintain for 14 min to prepared millet milk with low viscosity and flowing characteristics in addition TSS was high recording values of 6%.

### **Preparation of fermentation inoculums**

*B.longum BB536* was obtained from the stock culture of microbiology laboratory (Department of Food Science and Technology, Collage of Agriculture Studies, Sudan University of Science and Technology).

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 at 37 °C for 24h. The obtained culture was re-activated 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.

### **Growth medium and fermentation conditions**

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

### **Enumeration of viable cell**

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

### **Determination of reducing sugars**

Ten gram of sample was weighted in volumetric flask. The volume of the solution was completed to 100 ml in conical flask. Burrete (50 ml) was filled with the prepared sugar solution. Ten milliliters of sugar solution was transferred into a conical flask containing 10 ml Fehling's solution representing 5 ml of Fehling A (6.928 gm CuSo<sub>4</sub>.5 H<sub>2</sub>O per 100ml distilled water) and 5 ml Fehling B (34.6 sodium potassium titrate and 10 gm NaOH per 100 ml distilled water) mixed well and then heated moderately to boiling on an electrical hot plate heater. The liquid was kept boiling for about 2 minutes then 3 drops of methylene

blue indicator (1%) was added. The titration was then completed by the addition of sugar solution drop by drop until the color of the indicator disappeared and red brick color appeared, then reducing sugar was calculated following Schneider (1979) method.

#### **Determination of titratable acidity**

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

#### **Determination of total soluble solids (TSS)**

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

#### **Determination of pH value**

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

#### **Statistical analysis**

One-way ANOVA was performed to examine significant differences between normally distributed data of replicated independent runs. Probability level of less than 0.05 was considered significant ( $p < 0.05$ ). All data were analyzed using version 16 MINITAB statistical software for windows (2006).

#### **Results and Discussion**

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

Comparative growth of *Bifidobacterium longum* BB536 cultured in different beverages (cow milk, peanut milk, millet thin porridge and different blends) is shown in table 1.

There were significant ( $p < 0.05$ ) increases in *B. longum* BB536 viable count by extended fermentation period in all type of formulated beverages, as compared to strain level at beginning of fermentation. The maximum growth of *B. longum* BB536 was attained at 18h in all type of fermented beverages, except in fresh cow milk it was attained at 12 h fermentation. After the maximum growth, the strain declined in all types of fermented beverages (Table 1).

The rate of *B. longum* BB536 increases in different fermented beverages were 3.15, 2.9, 2.89, 2.76, 2.43 and 2.1 CFU/ml in fermented peanut milk, millet thin porridge, cow milk, blend (B), blend (A), and blend (C), respectively. These variations in growth could be attributed to variances in availability of nutrients required for growth in different fermented beverages. Peanut contains almost the essential nutrient for strain growth. Combination of peanut with

millet could complement the nutrient component of growth medium. However, the growth of strain *B. longum* BB536 was affected by supplementation with millet milk (Table 1), that could be due to increase viscosity of beverages by supplementation with millet milk. However at 24hour of fermentation there was reduction in number of *B. longum* BB536 in all fermented beverages due to the accumulation of acids or reduction of availability of nutrient required for the growth as stated by Kabeir *et al.*, (2005). In spite of declining in viable count of *B. longum* BB536 in all types of fermented beverages at 24h fermentation, the count still above the number required to presence in probiotic food which is at least 6 log cfu/ml fermented product (Vinderola and Reinheimer, 2000). Nevertheless, table 1 shows that the viable count of the strain in blend C (5.77 cfu/ml) is not fulfill propiotic requirement in food.

### **pH changes**

During fermentation process with strain BB536 there were significant ( $P<0.05$ ) decrease in pH levels in all types of beverages (Table 2). The decreases are due to increased acids production during fermentation process as a result of fermented sugar by *Bifidobacterium* BB536, which is produce acetic to lactic acid in ratio of 1.5:1 as reported by De Vries *et al.* (1967). Moreover, the accumulated acids produced by *bifidobacterium* strain, reported to have antibacterial activity such as prevention of the proliferation of pathogens (Bullen *et al.*, 1976). The rate of pH decreases at maximum growth of strain BB536 were 0.67, 0.64, 0.60, 0.60, 0.57 and 0.37 pH in fermented blend (B), peanut milk, blend (C), blend (A), millet thin porridge and then the cow milk respectively. These variances in pH reduction are not expected (Table 1). On other hand levels of pH attained at maximum growth (18h) and end of

fermentation at (24h) was above 5, and this is more suitable for consumption, further expected to have potential as carrier for the strain survival during the storage period.

### **Total Soluble Solids**

Table 3 shows the changes in TSS during fermentation of different formulated beverages with *B. longum* BB536. There were significant ( $P<0.05$ ) decrease in TSS levels in all types of fermented beverages. The rates of TSS decreases at maximum growth (18 h) of strain BB536 were 0.3, 0.4, 1.3, 0.1 and 0.3 % in fermented peanut milk, millet thin porridge followed by the three different blends, respectively. While at 24 h fermentation the amount of TSS reductions were 1.75, 0.75, 0.7, 0.45 and 0.35% in the fermented millet thin porridge, peanut milk, blend (C), blend (A) and blend (B), respectively. A strain activity and suitability of growth medium plays a vital role in rate of TSS reduction during fermentations.

### **Reduction of sugars**

There were significant ( $P<0.05$ ) decrease in sugars levels of all fermented beverages (Table 4). *Bifidobacterium* strain ferment sugars and produce organic acids mainly acetic, lactic, probionic, butyric and other organic acids (Sefa-Dedeh *et al.*, 2003).

The rates of sugar reduction at maximum growth of strain BB536 were 0.15, 0.04, 0.03, 0.02, and 0.01 mg/100 ml in fermented peanut milk, blend (C), blend (B), millet thin porridge and then blend (A) respectively. Moreover, after 24 h of fermentation, the maximum reductions in sugar were 0.19, 0.11, 0.1, 0.08 and 0.03 mg/100 ml in fermented, blend (C), blend (A), millet thin porridge, blend (B), and then fermented peanut milk, respectively. These variances in invert sugar reduction refer to the strain activity in different fermented beverages.

**Table.1** The viable count of *Bifidobacterium longum* BB536 (log CFU/ml) during fermentation period of different beverages\*

Time(h)	<i>Bifidobacterium longum</i> BB536 growth in beverages**					
	Cow milk	Peanut milk	Millet thin porridge	A	B	C
0	4.8 ± 0.12 <sup>a</sup>	5.68 ± 0.10 <sup>d</sup>	4.89 ± 0.06 <sup>d</sup>	5.51 ± 0.05 <sup>e</sup>	4.84 ± 0.08 <sup>d</sup>	4.53 ± 0.07 <sup>d</sup>
6	5.84 ± 0.15 <sup>b</sup>	6.96 ± 0.04 <sup>c</sup>	5.61 ± 0.18 <sup>c</sup>	5.89 ± 0.02 <sup>d</sup>	5.78 ± 0.07 <sup>c</sup>	4.92 ± 0.04 <sup>c</sup>
12	7.69 ± 0.14 <sup>c</sup>	7.85 ± 0.056 <sup>a</sup>	6.89 ± 0.06 <sup>a</sup>	7.66 ± 0.11 <sup>b</sup>	6.95 ± 0.04 <sup>b</sup>	5.77 ± 0.09 <sup>b</sup>
18	6.86 ± 0.11 <sup>d</sup>	8.83 ± 0.07 <sup>b</sup>	7.79 ± 0.06 <sup>b</sup>	7.94 ± 0.05 <sup>a</sup>	7.60 ± 0.08 <sup>a</sup>	6.63 ± 0.04 <sup>a</sup>
24	6.03 ± 0.01 <sup>d</sup>	7.60 ± 0.08 <sup>b</sup>	6.68 ± 0.11 <sup>b</sup>	6.85 ± 0.02 <sup>c</sup>	6.77 ± 0.09 <sup>b</sup>	5.77 ± 0.01 <sup>b</sup>

\* Values are mean ± SD for replicate independent runs.

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

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

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

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

**Table.2** pH changes during the growth of *Bifidobacterium longum* BB536 in beverages\*

Time (h)	pH**					
	Peanut milk	Millet thin porridge	Cow milk	A	B	C
0	6.88 ± 0.02 <sup>a</sup>	6.72 ± 0.09 <sup>a</sup>	6.24 ± 0.49 <sup>a</sup>	6.80 ± 0.04 <sup>a</sup>	6.84 ± 0.04 <sup>a</sup>	6.71 ± 0.07 <sup>a</sup>
6	6.35 ± 0.05 <sup>b</sup>	6.46 ± 0.11 <sup>ab</sup>	6.42 ± 0.54 <sup>a</sup>	6.37 ± 0.02 <sup>bc</sup>	6.49 ± 0.01 <sup>b</sup>	6.58 ± 0.01 <sup>b</sup>
12	6.34 ± 0.04 <sup>b</sup>	6.28 ± 0.15 <sup>b</sup>	6.05 ± 0.64 <sup>a</sup>	6.52 ± 0.05 <sup>b</sup>	6.37 ± 0.05 <sup>b</sup>	6.11 ± 0.01 <sup>c</sup>
18	6.24 ± 0.02 <sup>b</sup>	6.15 ± 0.04 <sup>b</sup>	6.00 ± 0.22 <sup>a</sup>	6.20 ± 0.03 <sup>c</sup>	6.17 ± 0.07 <sup>c</sup>	6.11 ± 0.01 <sup>c</sup>
24	5.15 ± 0.04 <sup>c</sup>	5.06 ± 0.06 <sup>c</sup>	5.83 ± 0.11 <sup>a</sup>	5.90 ± 0.12 <sup>d</sup>	5.69 ± 0.04 <sup>d</sup>	5.58 ± 0.01 <sup>d</sup>

\*Values are mean ± SD for replicate independent runs.

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

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

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

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

**Table.3** TSS (%) changes during the growth of the strain *Bifidobacterium longum* BB536 in different beverages \*

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

\*Values are mean ± SD for replicate independent runs.

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

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

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

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

**Table.4** Reducing of sugars (mg /100ml) % during the growth of *Bifidobacterium longum* BB536 in different beverages\*

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

\*Values are mean ± SD for replicate independent runs.

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

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

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

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

**Table.5** Titratable acidity (%) during the growth of the strain *Bifidobacterium longum* BB536 in different beverages\*

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

\*Values are mean ± SD for replicate independent runs.

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

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

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

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

### Titratable acidity changes

Table 5 shows the titratable acidity of different fermented beverages. There, were significant (p<0.05) increases in titratable acidity by extended fermented period to 24h. At maximum growth of strain *BB536* (18h), the rates of increase were 0.2, 0.09, 0.08,0.08 and 0.07% in fermented peanut milk, blend (A), blend (B), millet thin porridge and blend (C), respectively. The increased in acidity is explained by accumulation of lactic acid and other organic acids produced during fermentation of the formulated beverages (Sefa –Dedeh *et al.*, 2003).

Fermentation of the peanut milk, millet thin porridge and their different blends with *Bifidobacterium longum BB536* was successful. Sufficient numbers of *Bifidobacterium longum BB536* were obtained in different types of fermented beverages. The viable number of the strain at maximum growth (18 h) during fermentation was above 6 Log CFU/ml in all fermented beverages, thus they fulfilled probiotic requirements. Moreover, this study

facilitated the development of new fermented non-dairy carrier for strain *BB536* with acceptable physical properties from cheap and locally available source in Sudan.

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