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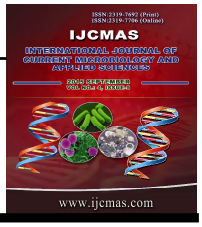

Original Research Article

Survival of *Bifidobacterium longum* BB536 and Physicochemical Changes during Refrigeration Storage of Fermented Roasted Peanuts Milk Partially Substituted with Millet Thin Porridge

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Keywords

Bifidobacterium, Peanut, Millet, Physicochemical, Survival, Refrigeration

A B S T R A C T

This study was carried out to evaluate survival and related physicochemical changes during refrigeration storage of roasted peanut based fermented with *Bifidobacterium longum BB536*. Peanut was roasted (100°C for 20 min). 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 24h) for 18 h, and stored under refrigeration for two weeks. The results obtained on *B. longum BB536* survival during refrigeration revealed significant ($p<0.05$) reduction in *B. longum BB536* viable count in all fermented beverages. The rates of reduction in the first week of the refrigeration storage were 2.39, 2.08, 1.84, 1.7, 1.43and 0.77 CFU /ml in fermented millet thin porridge, peanut milk, blend (C), blend (B), blend (A) and cow milk, respectively. Hopefully, the final viable count of *B. longum BB536* in fermented peanut milk, cow milk and blend (A) was above the minimum number required to presence in probiotic which is at least 6 log CFU/ml. The reductions in *B. longum BB536* in the second week refrigeration was significantly ($p<0.05$) deference in all fermented beverages, except in fermented cow milk and fulfilled probiotic requirement in foods. Therefore, fermented peanut milk and blend A is suitable carrier to *B. longum BB536* for at least one week storage.

Introduction

The word probioticis derived from Greek and means “for life” (Metchnikoff, 1907). One of the more detailed current definitions of probiotics is; “a microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the

intestinal tract". Mainly specific strains of lactobacilli, *Bifidobacterium*, enterococci and yeast are today used commercially as probiotics (Naidu *et al.,* 1999; Holzapfel *et al.,* 1995; Saxelin *et al.,* 2005). *Bifidobacterium* are considered as important probiotics and used in the food industry to relieve and treat many intestinal disorders. *Bifidobacterium* exert a range of beneficial health effects, including the regulation of intestinal microbial homeostasis, the inhibition of pathogens and harmful bacteria that colonize and/ or infect the gut mucosa, the modulation of local and systemic immune responses, the repression of procarcinogenic enzymatic activities within the microbiota, the production of vitamins, and the bioconversion of a number of dietary compounds into bioactive molecules (Mayo and van Sinderen, 2010). *Bifidobacteruim longum* may be considered the most common species of *Bifidobacterium*, being found both in infant and adult feces (Bivati *et al.,* 1984). Potential benefits from consumption of *B. longum* include: antagonistic action toward intestinal pathogens, improved lactose utilization, anticarcinogenic action and control of serum cholesterol levels. Many scientific studies showed the benefits offered by *Bifidobacterium longum BB536* (Kojima *et al.,* 1996; Namba *et al.,* 2003). Thus there is considerable interest in incorporating these heaths promoting *Bifidobacterium* into food.

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).

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 media.

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 El-Gazzar, 2005). Millets have an alkaline pH and are the only grains that keep their alkaline properties even after being cooked. As another plus, millet is a gluten free grain and thus, is ideal for people with wheat/gluten allergies or intolerance (Baltensperger and Cai, 2004).

In this respect, the use of peanut milk and millet blend will complement nutrients same time can be a successful non-dairy carriers for *Bifidobacterium* strain. There for the objective of this study are to evaluate the survival of *B. longum BB536* during refrigeration storage of different fermented 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 18 h (Kabeir *et al.*, 2015).

The storage of the fermented products

Fermented beverages were held at refrigerator for a period of 2 weeks. Samples were collected at initial (0 days), after 1 week and after 2 weeks for analysis.

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 $CuSo_4.5H_2O$ per 100ml distilled water) and 5 ml Fehling B (34.6 sodium potassium titrate and 10 gm NaOH per 100 ml distilled water) mixed well and then heated moderately to boiling on an electrical hot plate heater. The liquid was kept boiling for about 2 minutes then 3 drops of methylene blue indicator (1%) was added. The titration

was then completed by the addition of sugar solution drop by drop until the color of the indicator disappeared and red brick color appeared, then reducing sugar was calculated following Schneider *et al.* (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 refractmeter with degree Brix^{o} 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 vision 16 MINITAB statistical software for windows (2006).

Results and Discussion

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

Table 1 shows the viable counts of *B. longum* BB536 during refrigeration storage of different formulated beverages. There were significant ($p<0.05$) reduction in *B. longum* BB536 viable count in all fermented beverages. The rate of reduction in the first week of the refrigeration storage were 2.39, 2.08, 1.84, 1.7, 1.43 and 0.77 CFU /ml in fermented millet thin porridge, peanut milk, blend (C), blend (B), blend (A) and cow milk, respectively. Hopefully, the final viable count of *B. longum* BB536 in fermented peanut milk, cow milk and blend (A) was above than the minimum number required to presence in probiotic to exert health benefits upon consumption, which was 6 log CFU/ml. While Alkalin *et al.* (2004) noted a significant reduction on *B. longum BB536* in yogurt after one week at refrigeration.

Furthermore, the reductions in the second week of refrigeration storage were 1.21, 0.99, 0.98, 0.89, 0.86 and 0.16 in the fermented blend (B), peanut milk, blend (A), blend (C), millet thin porridge and cow milk, respectively (Table 1). However, Kabeir *et al.* (2005) reported that survivability of *B. longum BB536* under

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

Reducing sugars

Table 2 shows the sugars content of the different fermented beverages during refrigeration storage.

There was significant ($p<0.05$) reduction in reducing sugars of different beverages. The rate of decreasing in all fermented beverages except in blend (A) and blend (B).The amount of sugars decrease in the first week were 0.06, 0.04, 0.03, 0.03 and 0.02 mg/100ml in fermented peanut milk, fermented blend (A), fermented blend (B), fermented millet thin porridge and blend (C), respectively (Table 2). The amount of sugars reductions in the second weak were 0.08, 002, and 0.02 mg/100ml in the fermented blend (C), millet thin porridge and peanut milk, respectively. While slight increase in reducing sugars was recorded in fermented blend A (0.03 mg/100ml) and the fermented blend B (0.05 mg/100ml).

Changes in pH

Table 3 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 refrigeration for two weeks. The rate of pH reductions in the first week were 0.53, 0.41, 0.28, 0.24, 0.31and 0.19 pH in fermented cow milk, peanut milk, millet thin porridge, blend (C), blend (A) and the blend (B), respectively. While the reductions recorded in the second week at refrigeration storage were 0.64, 0.57, 0.22, 0.2, 0.11and 0.03 in fermented blend (A), cow milk, blend (B), millet thin porridge, blend (C) and peanut milk, respectively (Table 3). The reduction of pH is mainly due to the fermentation of sugars and accumulation of acid. That is why *Bifidobacterium* maintain a relatively acid pH in large intestine, thus preventing the proliferation of pathogens. It produces lactic acid, acetic acid, hydrogen peroxide, and bactericides. They are known to inhibit the development of pathogenic bacteria it was also reported that lactic acid and acetic acid in fermented dairy product have antibacterial effect (Bullen *et al.*, 1976). In this respect low pH and storage temperature were the most important determinations in *Bifidobacterium* mortality (Sakai *et al.*, 1987).

Table.1 The survival of *Bifidobacterium longum* BB536 log (CFU/ ml) during the storage of different fermented beverages*

Type of fermented beverages **	Refrigeration period (two weeks)		
	At initial storage	After 1 week	After 2 week
Peanut milk	8.83 ± 0.07[a]	6.75 ± 0.09[b]	5.76 ± 0.03[c]
Millet thin porridge	7.79 ± 0.06[a]	5.58 ± 0.14[b]	4.72 ± 0.09[c]
Cow milk	7.69 ± 0.72[a]	6.92 ± 0.46[a]	6.76 ±0.58[a]
A	7.94 ± 0.05[a]	6.51 ± 0.05[b]	5.53 ± 0.04[c]
B	7.60 ± 0.08[a]	5.90 ± 0.05[b]	4.69 ± 0.12[c]
C	6.63 ± 0.04[a]	4.79 ± 0.07[b]	3.9 ± 0.03[c]

* Values are mean ± SD for replicate independent runs.
**Values that bear different superscript letter in the same raw are significantly different at p<0.05.
A=Blend 1 was prepared using 85% peanut milk and 15% millet 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 Reducing of sugar (mg /100ml) % of the different fermented beverages during refrigeration storage*

Type of fermented beverages **	Refrigeration period (two weeks)		
	At initial storage	After 1 week	After 2 week
peanut milk	0.099 ± 0.00[a]	0.044 ± 0.01[b]	0.018 ± 0.00[c]
Millet thin porridge	0.206 ± 0.01[a]	0.175 ± 0.00[b]	0.158 ± 0.01[b]
A	0.139 ± 0.01[a]	0.101 ± 0.00[a]	0.130 ± 0.05[a]
B	0.127 ± 0.01[ab]	0.110 ± 0.01[b]	0.154 ± 0.01[a]
C	0.201 ± 0.00[a]	0.167 ± 0.01[a]	0.094 ± 0.10[a]

* Values are mean ± SD for replicate independent runs.
**Values that bear different superscript letter in the same raw are significantly different at p<0.05.
A=Blend 1 was prepared using 85% peanut milk and 15% millet 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 pH of the different fermented beverages during refrigeration storage*

Type of fermented beverages **	Refrigeration period (two weeks)		
	At initial storage	After 1 week	After 2 week
peanut milk	6.24 ± 0.02[a]	5.83 ± 0.07[b]	5.8 ± 0.02[b]
Millet thin porridge	6.15 ± 0.04[a]	5.87 ± 0.01[b]	5.67 ± 0.00[c]
Cow milk	6.05±0.65[a]	5.52±0.79[a]	4.95±0.57[a]
A	6.20 ± 0.03[a]	5.89 ± 0.01[b]	5.25± 0.11[c]
B	6.17 ± 0.07[a]	5.98 ±0.00[b]	5.67 ± 0.00[c]
C	6.11 ± 0.01[a]	5.87 ± 0.01[b]	5.76 ± 0.00[c]

* Values are mean ± SD for replicate independent runs.
**Values that bear different superscript letter in the same raw are significantly different at p<0.05.
A=Blend 1 was prepared using 85% peanut milk and 15% millet 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 TSS of the different fermented beverages during refrigeration storage*

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

* Values are mean ± SD for replicate independent runs.
**Values that bear different superscript letter in the same raw are significantly different at p<0.05.
A=Blend 1 was prepared using 85% peanut milk and 15% millet 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 Moisture % of the different fermented beverages during refrigeration storage*

Type of fermented beverages **	Refrigeration period (two weeks)		
	At initial storage	After 1 week	After 2 week
peanut milk	89.27 ± 0.49^{a}	89.96 ± 0.05^{a}	90.02 ± 0.00^{a}
Millet thin porridge	93.73 ± 0.13^{a}	93.94 ± 0.08^{a}	94.00 ± 0.01^{a}
A	89.959 ± 0.20^{a}	90.365 ± 0.53^{a}	90.465 ± 0.53^{a}
B	91.39 ± 0.58^{a}	92.40 ± 0.57^{a}	92.48 ± 0.53^{a}
C	93.28 ± 0.88^{a}	94.03 ± 0.36^{a}	94.03 ± 0.36^{a}

* Values are mean ± SD for replicate independent runs.
**Values that bear different superscript letter in the same raw are significantly different at p<0.05.
A=Blend 1 was prepared using 85% peanut milk and 15% millet 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.6 Titratable acidity of the different fermented beverages during refrigeration storage*

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

* Values are mean ± SD for replicate independent runs.
**Values that bear different superscript letter in the same raw are significantly different at p<0.05.
A=Blend 1 was prepared using 85% peanut milk and 15% millet 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.

Changes in TSS

Table 4 shows TSS of different fermented beverages**.** There were significant (p<0.05) TSS decreases in all types of fermented beverages under refrigerated storage for two weeks. The amounts of reductions in the first week were 1.0, 0.4, 0.15, 0.15and

0.00% in blend (A), blend (C), millet thin porridge, blend (B), and peanut milk, respectively. The amounts of reduction in the second week of refrigerated storage of millet thin porridge, peanut milk, blend (C) and blend (B) were 2.7, 0.55, 0.4, and 0.35% respectively. While there was TSS increase in blend A (Table 4).

Changes in moisture content

Table 5 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 increases of moisture in fermented blend (B), peanut milk, blend (C), blend (A) and millet thin porridge were 1.01, 0.75, 0.69, 0.41 and 0.21% respectively. Over all levels, the moisture content of fermented beverages stored under refrigeration temperature ($4c^{o}$) was increased as compared to their initial value. This increase in moisture might indicate high enzymatic activity that break down the macro component into simple and to the release of water.

Changes in titratable acidity

Table 6 shows the titratable acidity of different fermented beverages throughout storage period. Titratable acidity increased by extended refrigeration storage for two weeks. The rates of titratable acidity were 0.08, 0.04, 0.04, 0.03 and 0.02% in fermented blend (C), the peanut milk, blend (B), millet thin porridge, and the blend (A), respectively. While the rate recorded at second week were 0.12, 0.1, 0.08, 0.03 and 0.01% in fermented blend (C), the peanut milk, millet thin porridge, blend (A) and the blend (B), respectively. The amount of the titratable acidity was significant ($p<0.05$) increased gradually till the end of storage except in blend (A) and blend (C).

In conclusion, sufficient numbers of *Bifedobacterium longum BB536* were maintained in different types of fermented roasted peanut, millet thin porridge and their blends during refrigeration storage. The viable number of the strain during the first week storage was above 6 Log CFU/ml in fermented beverages (peanut milk, blend (A) and cow milk). Therefore, this study can facilitate the development of non-dairy cheap carrier for *Bifedobacterium longum BB536* as compared to fresh dairy milk.

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References

Alkalin, A.S., Fendery, S.A.K., bulut, N., 2004. Viability and activity of bifidobacteria in Yoghurt containing fructose oligo saccharine during refrigerated storage. *Int. J. Food Sci. Tecknol.*, 39: 613–621.

AOAC, 1990. Official methods for analysis, 15th edn. Association of Official Analytical Chemists. Washington, D.C., USA

Baltensperger, D., Cai, Y.Z. 2004. Minor millets. In: C.W. Wrigley, H. Corke and C.E. Walker, (Eds.), Encyclopedia of grain science, Vol. 2. Oxford, U.K. Pp. 261–268.

Bivati, B., Castagnoli, P., Trovatelli, L.D. 1984. Species of the genus *Bifedobacterum* in the feaces of human adults. *Microbilogical*, 9: 39–45.

Bullen, C.L., Tearle, P.V. and Willis, A.T., 1976. Bifidobacteria in the intestinal tract of infants: as in-vivo study. *J. Med. Microbial.*, 9: 325–333.

Campbell-Platt, G. 1994. Fermented foods –

a world perspective. *Food Res. Int.*, 27: 253–257.

Chou, C.C., Hou, J.W. 2000. Growth of bifidobacteria in soymilk and their survival in the fermented drink during storage. *Int. J. Food Microbial.*, 6: 113–121.

Holzapfel, W.H., Geisen, R., Schillinger, U., 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *Int. J. Food Microbiol.*, 24: 343–362.

Kabeir, B.M., Abd Aziz, S., Muhammad, M., Yazid, A.M. 2005. Growth of *Bifidobacterium longum* BB536 in Medida (fermented cereal porridge) and their survival during storage. *Lett. Appl. Microbiol.*, 41: 12–131.

Kojima, T., Yaeshima, T., Ishibashi, N., Shimamura, S., Hayasawa, H. 1996. Inhibitory effects of human-drived bifidobacterium on pathogenic *Escherichia coli* serotype O-111. *Biosci. Microflora*, 15: 17–22.

Marwa, El-Gazzar, M. 2005. Biochemical and technological studies on millet. M.Sc. Thesis, Agric. Sci. (Biochemistry), Faculty of Agriculture, Cairo University.

Mayo, B., van Sinderen, D. 2010. Bifida bacteria: Genomics and Molecular Aspects. Caister Academic Press. ISBN 978-1-904455-4.

Metchnikoff, E. 1907. The prolongation of life: Optimistic studies. Butterworth-Heinemann, London.

Naidu, A.S., Bidlac, W.R., Clemens, R.A. 1999. Probiotic spectra of lactic acid bacteria. *Crit. Rev. Food Sci. Nutr.*, 38: 13–126.

Namba, K., Yaeshima, T., Ishibashi, N., Hayasawa, H., Yamazaki, S. 2003. Inhibitory of *Bifidobacterium longum* on inter hemorrhagic *Escherichia coli* O157: H7. *Biosci. Micro flora*, 22: 85–91.

Nwokalo, E. 1996. Peanut (*Arachis lypogaea* L.) In: Nwokolo, E., Mart, J.S. (Eds.). Legumes and oilseeds in nutrition. Chapman and Hall, London. Pp. 49.

Roberts-Sunny, E.O., Ofunda, E.T., Iwakun, B.T. 2004. An evaluation of some quality parameters of laboratory-prepared fermented groundnut milk. *J. Eur. Food Res. Technol.*, 218: 452–455.

Sakai, K., Mishima, C., Tachiki, T., Kumagai, H., Tochikura, T. 1987. Mortality of bifidobacteria in boiled yoghurt. *J. Ferment. Tech.*, 65: 215–220.

Salunkhe, D.K., Kadam, S.S. 1989. Handbook of world food legumes: nutritional chemistry, processing technology and utilization, 1st edn. CRC Press. Inc., Boca Raton, Floridea.

Saxelin, M., Tynkkynen, S., Mattila-Sandholm, T., de Vos, W.M. 2005. Probiotic and other functional microbes: from markets to mechanisms. *Curr. Opin. Biotechnol.*, 16: 204–211.

Schneider, W.J., Beisiegel, brown, M.S. 1979. *J. Biol chem.*, 257: 264 – 273.

Stanton, C., Desmond, C., Coakley, M., Collins, J.K., Fitzgerald, G., Ross, R.P. 2003. Challenges facing development of probiotic-containing functional foods. In: Farnworth, E. (Ed). Handbook of fermented functional foods. CRC Press, Boca Raton. Pp. 27–58.

Tamime, A.Y., Robinson, R.K. 1985. Yoghurt: Science and technology. Pergamon Press Ltd., Oxford, UK.