SUST Journal of Agricultural and Veterinary Sciences (SJAVS)
Vol. 19 No.(2)
ISSN: 1858 6775

December 2018

SUST
Journal of Agricultural and Veterinary Sciences
Journal homepage:
http:// journals.sustech.edu

Survival of *Bifidobacterium Longum* BB536 in Fermented Peanut Milk Supplemented with Gum Arabic during the Storage

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Article history: Recieved: August 2018
Accepted: September 2018

Abstract:
The study aim to evaluate the survival of the approved probiotic strain with health benefits *Bifidobacterium longum* BB536 and related physicochemical changes during refrigeration storage of roasted fermented peanut based milk supplements with different types of Gum Arabic. Peanut was roasted (130°C for 20 min), soaked in water (12 h), blended (5 min) and filtered using a double layered cheese cloth to obtain the roasted peanut milk. The obtained milk was supplemented with 10% (V/V) Gums Arabic solution. After sterilization of milks it was inoculated with 3% (V/V) active probiotic strain *Bifidobacterium longum* BB536 and incubated under controlled conditions at 37ºC for 24h. The fermented milks were held at refrigerator temperature for two weeks. Different analyses were conducted, including: strain BB 536 survival, pH, TSS, total sugars, acidity, and moisture. The results showed significant ($p<0.05$) decreases in the total count of strain BB 536, pH, TSS, total sugars and moisture. However, significant ($p<0.05$) increases in titrable acidity and moisture were recorded during the storage. The rates of strain BB 536 reductions in first week refrigeration storage were 2.04, 2.75, 2.25 , and 2.06 log CFU /ml in fermented peanut milk, fermented peanut milk supplemented *with Acacia senegal,* fermented peanut milk supplemented with *Acacia seyal*, fermented peanut milk supplemented with gums mix (*Acacia senegal* + *Acacia seyal*), respectively. While on the second week the reductions were 1.19, 1.0, 0.95 and 1.08 log CFU/ml in fermented peanut milk, fermented peanut milk supplemented *with Acacia Senegal*, fermented peanut milk supplemented *with Acacia seyal*, fermented peanut milk supplemented with gum mix, respectively. The final viable count of strain BB536 in fermented peanut milk supplemented with gums mix after two weeks refrigeration storage exceded the minimum number required for the probiotic to exert health benefits upon consumption which was 6 log CFU /ml. Therefore, fermented peanut milk supplemented with gums mix (*Acacia senegal* + *Acacia seyal*) is a good carrier for *Bifidobacterium longum* strain.
Keywords: Gum Arabic, Peanut Milk, Bifidobacterium, Survival, Refrigeration

Introduction:

The Joint FAO/WHO Working Group defines probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host.” (FAO/WHO, 2001).In the development of human probiotics, strains belonging to the genera *Lactobacillus* and *Bifidobacterium* have been most commonly used, even though some probiotic preparations are based on other

LAB or even non-LAB species and yeasts (Holzapfel *et al.*, 1995).

The use of probiotics and prebiotics functional foods has been well-established in recent years. Probiotics and prebiotics have become more accepted as supplements for human consumption. The prebiotics supplementation on human diet has a beneficial effect, which enhancing the gut microflora growth and activity (Kolida *et al*., 2002).

B. longum BB536 is one of the bifidobacteria species mainly found in the human gut and is considered as the main familiar species of bifidobacteria, which existing in the human gut. The attainable benefits from making use of B. *longum* include the antagonistic action toward intestinal harmful microorganisms, improved the utilization of lactose, and organize the serum cholesterol levels. Several scientific studies have shown the benefits offered by *Bifidobacterium longum* BB536 (Kojima *et al*., 1996; Namba *et al*., 2003; Asahara *et al*., 2004).Thus there is considerable interest in incorporating these health promoting *bifidobacteria* into food. Growth and survival of probiotic bacteria had been found to be affected by the chemical and microbiological composition of milk, milk solids content, and availability of nutrients (Shah, 2000). The use of prebiotic with probiotic in dairy products was found to improve the increase and survival of probiotic bacteria in fermented dairy products (Desai *et al*., 2004).

Bifidobacterium longum BB536 has been added to several products and has been fermented with peanuts, but to our knowledge, no information is available about the effect of adding gum Arabic as a prebiotic on the life of the this strain.

A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the large intestine, and thus improves host health (Gibson and Roberfroid, 1995). Prebiotics must be resistant to enzymatic digestion and absorption in the gastrointestinal tract and must be fermented by intestinal bacteria in the colon (Roberfroid, 2007). Among prebiotic gum Arabic is primarily indigestible to both humans and animals. It is not degraded in the small intestine, but fermented in the large intestine by probiotic to short-chain fatty acids, particularly propionic acid. (Badreldin *et al.,* 2008).Gum Arabic is rich in dietary fiber that it is derived from dried exudates of A. Senegal(Nasir *et al.,* 2008).

The aim of this study was to evaluate the *Bifidobacterium longum BB536* survival and related physio-chemical changes during refrigeration storage of different fermented peanut milk based beverages supplemented with gum Arabic.

Material and Method:

Materials: Red-skinned peanut seeds (*Arachis hypogaea*) (v. Ashford) were obtained from Samil Industrial Co in Kober, Khartoum North (Khartoum State, Sudan). The Aflatoxin is less than 10ppb.

Preparation and maintenance of starter culture: *Bifidobacterium longum* BB536 strain was obtained from the store strains of the Department of Food Science and Technology, College of Agricultural Studies, Sudan University of Science and Technology, were used. Cultures were transferred into 10% sterilized (121 ºC for 15 min) skim milk and incubated anaerobically at 37^{o}C for 24 h. The culture was further sub cultured twice in a similar sterilized skim milk for 48 h prior to use for fermentation.

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 130°C for 20 min in an oven ((Baird & Tatlock (London) LTD. Chadwell – Heat. Essex. England). The roasting process was found to improve the nutrient component, facilitate the removal of the crust and decrease the peany flavor of peanut.The roasted peanuts 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 tap water. The roasted kernels were then mixed with sterile water in a ratio of 1:5w/w [peanuts (200g): water (1L)] and transferred to a blender (Panasonic – MX – 101 SP2.Japan) and blended for 5 min at high-speed. The slurry formed was filtered using a double cheese cloth to obtain the peanut milk, which was sterilized in an autoclave at 121^{oC}/ 15min.

Preparation of Gum Arabic solution: Fifteen gram from different three samples of Gum Arabic *Acacia senegal, Acacia seyal* and gum mix of both were weighted, dissolved in small amount of sterile water, completed to150 ml to obtain 10% w/v and sterilized in a water path at 60°C / 30min.

Fermentation medium: The growth media were formulated from peanut milk (control), peanut milk supplemented with (10% v/v),different types of gums Arabic (*Acacia senegal, Acacia seyal* and mixed *Acacia (senegal + seyal*) .Formulated media were sterilized (121ºC for 15 min) and inoculated by a 3% active culture of *B. longum* BB536 then incubated at 37º C for 24h.

The storage of the fermented products: The fermented beverages were held at refrigerator temperature 5-7 ºC for two weeks. Throughout the storage time, the viable counts of *Bifidobacterium longum* BB536, pH, titrable acidity, TSS, moisture and sugar content of the fermented beverages were determined. Analysis of samples was carried out at the initial time (0 days), after one week and two weeks intervals**.**

Enumeration of Bifidobacterium longum BB536: The enumeration of *B. longum* BB536 of different fermented beverages were attained using the plate count technique with MRS medium. The fermented samples were drawn at the initial time and weekly during the storage period. One ml of fermented beverage was used to make serial dilution in 9 ml peptone water, followed by plating on Rogosa agar (MRS) supplement with 0.05% L- cystiene. The plates were incubated anaerobically at 37˚C for 48 h. The growth was calculated as Colony Forming Unit per ml (CFU/ml).

Determination of titratable acidity: The titratable acidity (TA) of the Fermented beverages were determined according to the AOAC method (1990). Ten ml of the sample was drawn into a conical flask. Distilled water was added to the volume in the flask to complete 150 ml. The sample was then vigorously agitated and filtered. Twenty five milliliters of the filtrate were pipetted into a flask, five drops of phenolphthalein were 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 were calculated as below.

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

Were N = Normality of NaoH.

0.9 = Factor of lactic acid.

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 was 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 were directly measured.

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

Total sugars: From the previous clear sample solution for determination of acidity, 50 ml was pipetted into a 250 ml conical flask and 5g citric acid and 50 ml distilled water were added slowly. Then, the mixture was gently boiled for 10 min to complete the inversion of sucrose and left to cool at room temperature. After that, the solution was transferred to 250 ml volumetric flask, neutralized with 20% NaOH solution in the presence of a few drops of phenolphthalein (NO. 6606 J. T Baker, Holland) until the color of the mixture disappeared and the sample was made up to volume before titration.

Procedure: A volume of 10 ml of a mixture of Fehling's A and B solutions was pipetted into 250 ml conical flask. Then, sufficient amount of the clarified sugar solution was added from a burette to reduce Fehling's solution in the conical flask. After that, the solution was boiled until a faint blue color is obtained. Then, a few drops of methylene blue indicator (S-d-FINE-CHEM LIMITED) were added to the Fehling's solution and titrated with sugar solution until brick-red color of cuprous oxide precipitate was observed. Finally, the titer volume was recorded and the amount of inverted sugars was obtained from Lane and Eynon Table. The total sugars were calculated by using the following formulas:

$$\text{Total sugars } \{\% \text{ DM}\} = \frac{\text{invert sugar (mg)} \times \text{dilution factor}}{\text{Titer} \times \text{sample weight (g)} \times (100\% - \text{moisture }\%) \times 1000} \times 100$$

Statistical analysis: The One- way ANOVA was carried out to determine significant differences between normally distributed data of replicated independent storage of samples. Probability levels of less than 0.05 were considered significant ($p < 0.05$). All data were analyzed using vision 17 MINITAB statistical software for Windows.

Results and Discussion:

The survival of Bifidobacterium longum BB536 (log CFU/ ml) during the storage of different fermented beverages: Table 1 shows the survival rate of *Bifidobacterium longum* BB536 over 14 days during refrigeration storage of different fermented beverages to assess the shelf life. There was significant reduction in viable counts between beverages during the storage as compared to the initial storage level.

The rate of reduction in the first week of the refrigerated storage were 2.04, 2.75, 2.25 , and 2.06 log CFU /ml in fermented peanut milk, fermented peanut milk supplemented with *Acacia Senegal* Hashab), fermented peanut milk supplemented with *Acacia seyal* (Taleh) and fermented peanut milk supplemented mix *Acacia (senegal + seyal*), respectively. It is clear that the rates of reduction were differed among different fermented beverages. Moreover *Bifidobacterium longum* BB536 reductions recorded in the second week of the refrigerated storage, which were 1.19, 1.0, 0.95, 2.06 and 1.08 log

CFU/ml of fermented peanut milk, fermented peanut milk supplemented with *Acacia senegal*(hashab), fermented peanut milk supplemented with *Acacia seyal* (Taleh), and fermented peanut milk supplemented with gum mix *Acacia (seyal + seyal*), respectively. The final viable count of *B. longum* BB536 in fermented peanut milk supplemented with mix *Acacia (seyal + seyal*), after two weeks refrigeration storage was above the minimum number required to presence in probiotic food to exert health benefits upon consumption, which is 6 log CFU/ml Viderola and Reinheimer, 2000; IDF, 1992., while the strain counts in the other fermented beverages were below the number required to fulfill probiotic effect.

Table 1: *Bifidobacterium longum* BB 536 log CFU/ ml) survival during the storage of different fermented beverages

Fermented Beverages period	**The viable count of *Bifidobacterium longum* BB536**			
	Peanut milk without GA (Control)	Peanut milk supplemented with gum *Acacia senegal*	Peanut milk supplemented with gum *Acacia seyal*	Peanut milk supplemented with gum mix *Acacia senegal+ seyal*
Initial 0 week	7.88 ± 0.030^{d}a	$9.56 \pm 0.03^{b}{}_{a}$	$8.88 \pm 0.01^{c}{}_{a}$	$9.87 \pm 0.01^{a}{}_{a}$
Week1	$5.84 \pm 0.03^{d}{}_{b}$	$6.81 \pm 0.10^{b}{}_{b}$	$6.63 \pm 0.03^{c}{}_{b}$	$7.81 \pm 0.01^{a}{}_{b}$
Week2	$4.65 \pm 0.04^{d}{}_{c}$	$5.81 \pm 0.03^{b}{}_{c}$	$5.68 \pm 0.04^{c}{}_{c}$	$6.73 \pm 0.03^{a}{}_{c}$

Values are mean ± SD for replicate independent runs.
Values that carried the different superscript letter in the same raw are significantly different at p<0.05.
Values that carried the different subscript letter in the same column are significantly different at p<0.05.
Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* (Hashab) and *Acacia seyal* (Taleh) in 1:1.

In this respected Kabeir *et.al* (2005) stated that the *B. longum* BB536 viability and survival under refrigerated storage of Sudanese fermented Medida beverages was not affected for a period of two weeks. Although Alkalin *et al*. (2004) reported that there was a significant decreased in the viable count of *B.longum* BB46 in yogurt after just one week refrigeration. This signify that the viability and survival of *Bifidobacterium* in fermented products was dependent on the carrier medium type and pH levels of the fermented foodstuffs during the storage.The supplementation of beverages with gum Arabic improved survival as compared to control beverages without Gum Arabic. The possibility of growth ability may change the chemical composition of the fermentation medium to better survival environment. 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; Modeler *et al*., 1990; Laroia and Martin, 1991). The survival of probiotics bacteria in fermented dairy bio-products depends on such varied factors as the strains used, interactions 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 may be influenced by the nitrogen and carbon source activity and availability in the growth media as stated by Chou and Hou, (2000).

Lactobacillus.acidophilus and bifidobac-teria survival may be affected by the fat composition of the fermentation medium, in addition it is found that full fat yogurt reduced the property for *Bifidobact-erium.bifidum* as compered with the low-fat yogurt (Vinderola *et al*., 2000). The growth and survival of probiotics in dairy products affected by the fermentation time, incubation temperature, storage temperature, and the addition of casein. While the level of dissolved oxygen in the product have also been the main factor that influence the survival of probiotic bacteria in dairy products (Klaver *et al*., 1993).

The survivability of Probiotics in fermented products should be fixed in the shelf life of the fermented products (Dinakar and Mistry, 1994),while a vastly reduced in the viable number of the growth of the probiotic bacteria during the shelf life of others fermented products (Stanton *et al.,* 2003) has been reported. The survival of *B. longum* BB536 in fermented beverages refrigerated storages for 2 weeks decreased to a level of < 7log CFU/ml due to pH reduction, which were explained by increases in acidity, (Stanton *et al.,* 2003).

The reduction of pH during the storage of different fermented beverages: The reduction of pH during the refrigeration storage of different fermented beverages was presented in Table 2. There was significant ($p<0.05$) reduction in pH of all types of fermented beverage during the two weeks of refrigeration. The rate of pH reductions in the first week were 0.15, 0.06, 0.43, and 0.69 pH in fermented peanut milk (control), fermented peanut milk supplemented with *Acacia senegal* (Hashab), fermented peanut milk supplemented with *Acacia seyal* (Taleh), and fermented peanut milk supplemented with mix *Acacia (senegal + seyal*), respectively .

Table 2: The pH of the different fermented beverages during refrigerated storage

Fermented Beverages Time(h)	Types of beverages pH% **(%)**			
	Peanut milk without GA (Control)	Peanut milk supplemented with gum *Acacia senegal*	Peanut milk supplemented with gum *Acacia seyal*	Peanut milk supplemented with gum mix*Acacia senegal+ seyal*
Initial storage	5.51 ± 0.02^{c}	5.24 ± 0.03^{c}	5.45 ± 0.04^{c}	5.51 ± 0.01^{d}
After Week 1	5.36 ± 0.08^{b}	5.30 ± 0.01^{b}	5.02 ± 0.01^{b}	4.82 ± 0.03^{b}
After Week 2	4.95 ± 0.04^{c}	5.04 ± 0.01^{c}	4.81 ± 0.04^{c}	4.78 ± 0.03^{c}

Values are mean ± SD for replicate independent runs.
Values that bear different superscript letter in the same column are significantly different at $p<0.05$.
Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* (Hashab) and *Acacia seyal* (Taleh) in 1:1.

The reduction of pH is mainly due to the relatively acidic pH in the large intestine, thus preventing the growth of pathogens. However, fermentation of sugars and accumulation of acid. That is why *Bifidobacterium* maintains a low pH and storage temperature were the most important factors in *Bifidobacterium* mortality (Kabeir. *et al*., 2015: Sakai *et al*.1987).

The pH decreases recorded in the second week of refrigeration were 0.41, 0.26, 0.21and 0.04 in fermented peanut milk, the fermented peanut milk supplemented with Acacia senegal (Hashab), fermented peanut milk supplemented with Acacia seyal (Taleh), and fermented peanut milk supplemented with mix Acacia (senegal + seyal), respectively .

These results can indicate that the use of Gum Arabic may control pH reduction, and can increase the shelf life of fermented beverages. Thus, Bifidobacterium maintains production of lactic and acetic acid, hydrogen peroxide, and the bactericides which are identified as inhibitor factors of the development of pathogenic bacteria. Also lactic acid and acetic acid in fermented dairy product have an antibacterial effect reported by Bullen *et al.* (1976), Therefore, pH is a major factor that restricts growth and stability of probiotic bacteria.

Lankaputhra *et al.* (1996) reported survival of three out of nine bifidobacteria strains in the pH range of 4·3 – 3·7. Kabeir et.al (2005) found the decrease of *B. longum* BB 536 in cold storage of the fermented madida was less than that justified for bifidobacteria. Followed by 2 weeks refrigerated storage the count of *B. longum* BB536 in the fermented *madida* reduced by 0·9 log CFU ml−1. Thus, the shelf life of this strain on the fermented peanut milk supplemented with gum Arabic is better.

Titratable acidity of the fermented beverages during refrigerated storage: Table 3 shows the titratable acidity of different fermented beverages. Titratable acidity of the different fermented beverages increased by extending the storage period for two weeks .The rate of titratable acidity increases were 0.03, 0.05, 0.07, and 0.1in fermented peanut milk, fermented peanut milk supplemented with *Acacia senegal* (Hashab), fermented peanut milk supplemented with *Acacia seyal* (Taleh), and fermented peanut milk supplemented with mix *Acacia (senegal + seyal*), respectively. The increasing rates recorded in the second week were 0.68, 0.55,0.51, and 0.48 in fermented peanut milk, fermented peanut milk supplemented with *Acacia senegal* (hashab), fermented peanut milk supplemented with *Acacia seyal* (taleh), and fermented peanut milk supplemented with mix *Acacia (senegal + seyal*), respectively. The increased acidity could be explained by the accumulation of lactic acid and other organic acids produced during fermentation of the formulated beverages by *B.longum* BB536 (Sefa *et al.,* 2003). Increasing the acidity of foods, both through fermentation or the addition of weak acids, has been used as a preservation method since early times.

Desjardins *et al.* (1990) stated that the short shelf life of numerous fermented milk products during refrigerated and frozen storage was correlated to increased acidity. Adding to the low tolerance of bifidobacteria strain to lactate and acetate acids (Rasic and Kurman 1983). Increased acidity, could cause the decrease of bifidobacteria growth by 1–2 log CFU ml−1 in the fermented milk after cooling storage at 4°C for 15 days (Hughes and Hoover 1995). Rasic and Kurman (1983) clarified that increased acidity affects the growth of Bifidobacteria and can most likely reduce 2 log in milks medium (pH

4·7–4·3) through 1–2 weeks refrigerated storage. Chou and Hou.(2000) reported the decrease in B.longum counts by 3.3 log CFU ml−1 fermented soy milk beverage in two weeks storage at 5°C.

Table 3: Titratable acidity of different fermented beverages during refrigerated storage

Fermented Beverages Time(h)	Types of beverages			
	Titratable acidity (%)*			
	Peanut milk without GA (Control)	Peanut milk supplemented with gum *Acacia Senegal*	Peanut milk supplemented with gum *Acacia seyal*	Peanut milk supplemented with gum mixAcacia *senegal+ seyal*
Initial storage	0.28±0.00^{c}	0.28±0.00^{c}	0.28±0.00^{c}	0.29±0.00^{c}
After Week 1	0.31 ± 0.02^{b}	0.33 ± 0.02^{b}	0.35 ± 0.01^{b}	0.39 ± 0.03^{b}
After Week 2	0.99 ± 0.01^{a}	0.88 ± 0.00^{a}	0.86 ± 0.01^{a}	0.87 ± 0.01^{a}

Values are mean ± SD for replicate independent runs.
Values that bear different superscript letter in the same column are significantly different at $p<0.05$.
Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* (Hashab) and *Acacia seyal* (Taleh) in 1:1.

Changes of total soluble solids (TSS) during refrigerated storage period of different fermented beverages: The presented results in Table 4 showed the TSS of different fermented beverages**.** There was a significant (p<0.05) decrease in the total soluble solids of all types of fermented beverages under refrigerated storage for two weeks. The decrease in the first week of refrigerated storage of formulated fermented peanut milk, fermented peanut milk supplemented with *Acacia senegal* (Hashab), fermented peanut milk supplemented with *Acacia seyal* (Taleh), and fermented peanut milk supplemented with mix *Acacia (senegal + seyal*), respectively, were 1.1, 0.30, 0. 43, and 0.58 %, respectively.

The decrease in the second week was lower as compared to that of the first week recording values of 1.4, 0.51, 0.6 and 0.39 respectively. The decrease of total soluble solids could be due to slight degradation of carbohydrates, protein and enzyme activity utilizing these nutrients and other organic solid component produced during fermentation of the formulated beverages.

Total sugars during refrigerated storage period of different fermented beverages: The results in Table 5 presented the total sugars during the refrigerated storage period of different fermented beverage. There was significant (p<0.05) decrease in the total sugars of different fermented beverages which correlated well with reduction of TSS in Table 4. The percentages of sugar decrease in the first week were 0.01., 0.04,0.01 and 0.02 in fermented blend peanut milk, fermented peanut milk supplemented with *Acacia senegal* (Hashab), fermented peanut milk supplemented with *Acacia seyal* (Taleh), and fermented peanut milk supplemented with mix *Acacia* (*senegal + seyal*), respectively.

Table 4: TSS % of different fermented beverages during refrigerated storage

Fermented Beverages Time(h)	Types of beverages			
	TSS %			
	Peanut milk without GA (Control)	Peanut milk supplemented with gum *Acacia senegal*	Peanut milk supplemented with gum *Acacia seyal*	Peanut milk supplemented with gum mix*Acacia senegal*+ *seyal*
Initial storage	2.29±0.01[a]	3.15±0.14[a]	3.11±0.01[a]	2.62 ±0.03[a]
Week 1	2.20 ± 0.02[b]	3.13 ± 0.01[a]	3.11 ± 0.01[a]	2.60 ± 0.01b
Week 2	0.80 ± 0.01[c]	2.62 ± 0.02[b]	2.51 ± 0.0[b]	2.21 ± 0.01[c]

Values are mean ± SD for replicate independent runs.
Values that bear the different superscript letter in the same raw are significantly different at $p<0.05$.
Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* (Hashab) and *Acacia seyal* (Taleh) in 1:1.

Table 5: Total sugar of different fermented beverages during refrigeration storage

Fermented Beverages Time(h)	Types of beverages			
	Total sugars (%)			
	Peanut milk without GA (Control)	Peanut milk supplemented with gum *Acacia senegal*	Peanut milk supplemented with gum *Acacia seyal*	Peanut milk supplemented with gum mix*Acacia senegal*+ *seyal*
Initial storage 0h	0.31±0.01[a]	1.21 ± 0.00[a]	1.85 ± 0.00[a]	1.02 ± 0.02[a]
Week 1	0.30 ± 0.00[b]	1.17 ± 0.00[b]	1.84 ± 0.00[b]	1.00± 0.0[b]
Week 2	0.13 ± 0.00[c]	0.62 ± 0.00[c]	0.93± 0.00[c]	0.81± 0.00[c]

* Values are mean ± SD for replicate independent runs.
**Values that bear different superscript letter in the same column are significantly different at $p<0.05$.
*Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* (Hashab) and *Acacia seyal* (Taleh) in 1:1.

The percentages of sugar reduction in the second week were higher as compared to that of the first week recording values of 0.17, 0.55,0.91, and 0.19 in the fermented peanut milk, peanut milk (*senegal*)peanut milk (*seyal*) peanut milk mix *Acacia* (*senegal* + *seyal*),respectively.

These results could have an important nutritional significance, because they indicate that the prebiotic addition improves bifidobacterial enzyme activity during the peanut milk fermentation (Cumchuere &Robinson1999; Wang *et al.* 2003; Donkor *et al.* 2007).

The changes in moisture throughout the storage period of different fermented beverages: In food, moisture content is essential for shelf life, it is used to predict microbiological and chemical stability of food products (Gabriel. 2008). As presented in Table 6, moisture of different fermented beverages significantly ($p<0.05$) increased during the storage period for two weeks due to release of water from the breakdown of macro components. The moisture increased during the first week of refrigerated storage of different formulated fermented peanut milks, peanut milk supplemented with *Acacia senegal* (Hashab), peanut milk supplemented with *Acacia seyal*(Taleh), and peanut milk supplemented with a mix (*senegal* + *seyal*), to 1.1, 1.12, 2.27, and 2.38%, respectively. While the increase in the second week of refrigerated storage of peanut milk, peanut milk supplemented with *Acacia senegal* (Hashab), peanut milk supplemented with *Acacia seyal* (Taleh), and peanut milk supplemented with a mix *Acacia* (*senega* + *seyal*), was 1.35, 1.92, 3.24, and 3.1, respectively . That rate of increase was high in the second week of storage as compared to the first week. In the fermentation process, increase in moisture might indicate a high enzymatic activity that breaks down the macro components into simpler ones and to the release of water.

Table 6: Moisture % of different fermented beverages during refrigeration storage

Fermented Beverages Time(h)	Types of beverages **Moisture %**			
	Peanut milk without GA (Control)	Peanut milk supplemented with gum *Acacia senegal*	Peanut milk supplemented with gum *Acacia seyal*	Peanut milk supplemented with gum mix*Acacia senegal*+ *seyal*
Initial storage time 0 h				
Week 1	88.66 ± 0.00[b]	87.18 ± 0.01[b]	88.86 ± 0.00[b]	88.20 ± 0.00[b]
Week 2	89.91 ± 0.00[a]	89.10± 0.00[a]	92.10 ± 0.00[a]	91.30 ± 0.00[a]

Values are mean ± SD for triplicate independent runs.
Values that bear different superscripts letter in the same column are significantly different at $p<0.05$.
Peanut milk (mix) prepared using peanut milk and 1% gums of *Acacia senegal* (Hashab) and *Acacia seyal* (Taleh) in 1:1.

Conclusions:

The results of this study proved the effect of different types of gum Arabic on improving survival of *Bifidobacterium longum* BB536 during refrigerated storage of roasted peanut milk supplements with different types of gum Arabic. The maximum viable count of strain BB536 in different fermented peanut milk supplemented with gum mix was above the minimum growth number required to survival in probiotic foods up to two weeks refrigerated storage in all fermented peanut milk samples. There was a decrease in total viable account of *B. longum* BB536 by extending the storage period due to increase acid production as a result of sugars fermentation. Moreover, the rate of *B. longum* BB536 reductions was higher at the first week refrigeration as compared to strain level of reduction at the second week.

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بقاء بكتريا *Bifidobacterium longum* BB536 فى لبن الفول السودانى المخمر المدعم بالصمغ العربى اثناء التخزين

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المستخلص

هدفت هذه الدراسة لتقييم حياة البكتريا الصديقة *Bifidobacterium longum* BB536 ذات الفوائد الصحية والتغيرات الفيزيوكيميائية ذات الصلة أثناء تخزين لبن الفول السوداني المخمر المدعم باصماغ عربية مختلفة. تم تحميص الفول السوداني بدرجة حرارة (130 درجة مئوية) لمدة 20 دقيقة، وغمرت في الماء (12 ساعة)، تم خلطها (5 دقائق) وتمت التصفية باستخدام طبقتين من قماش لجبن للحصول على لبن الفول السوداني المحمص.اللبن المتحصل عليه تم تعقيمه و تدعيمه ب 0 % (حجم / حجم) من صمغ الهشاب والطلح. تم إضافة % (حجم/حجم) بادئ نشط للبكتريا الصديقة *Bifidobacterium longum* BB536 وحضنت تحت ظروف متحكم عليها (37 درجة مئوية) لمدة 24 ساعة. الألبان المخمرة تم حفظها في الثلاجة لمده أسبوعين. أجريت تحاليل مختلفة شملت حياه البكتريا الصديقة، الرقم الهيدروجينى، الجوامد الصلبة الذائبة، درجة الحموضة، السكريات الكلية و الرطوبة. أظهرت النتائج انخفاض معنوي في عدد البكتريا الصديقة *Bifidobacterium ongum* BB536 ، الرقم الهيدرجيني، الجوامد الصلبة الذائبة والسكريات الكلية. تم تسجيل زيادة معنوية في الحموضة والرطوبة أثناء التخزين وكانت نسب الانخفاض في البكتريا الصديقة *Bifidobacterium longum BB536* في الأسبوع الأول من التخزين في الثلاجة .04 ، 2.75 ، 2.25 ، 2.06 log CFU/ml في لبن الفول المخمر، لبن الفول المخمر المدعم بصمغ الهشاب، لبن الفول المدعم بصمغ الطلح، لبن الفول المدعم بخليط صمغ الهشاب والطلح على التوالي. بينما كان الانخفاض في الأسبوع الثانى .09 ، .0 ، 0.95 ، .8 log CFU/ml في لبن الفول المخمر، لبن الفول المـ مرالمدعم بصمغ الهشاب، لبن الفول المخمر المدعم بصمغ الطلح، لبن الفول المخمر المدعم بخليط صمغ الهشاب والطلح على التوالي. العدد الحي للبكتريا الصديقة *Bifidobacterium longum* BB536 في لبن الفول المخمر المدعم بخليط من صمغ الهشاب والطلح بعد أسبوعين من التخزين في الثلاجة كان أعلى من الحد الأدنى المطلوب وجودة في منتجات البكتريا الصديقة *Bifidobacterium longum BB536* وهي 6 log CFU/ml لإحداث فوائد صحية أثناء الاستهلاك. ولذلك فان لبن الفول السوداني المخمر المدعم بخليط صمغ الهشاب والطلح حامل جيد للبكتريا الصـ يقة *Bifidobacterium longum* BB536 .