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

Prebiotication with Gum Arabic on Growth of *Bifidobacterium longum* BB536 during Fermentation of Peanut Milk

Hala Mahmoud Mohamed Elgazouly[1*], Yousif Mohamed Ahmed Idris[1] and Baraka Mohamed Kabeir Baraka[1]

1. Deapartment of Food Science and technology, College of Agricultural Studies, Sudan University of Science and Technology, P.O. Box: 71, Khartoum North, Sudan.
*Corresponding author: email: halamahmoud7@hotmail.com
Article history: Recieved: August 2018
Accepted: September 2018

Abstract

This study was carried out to explore the synbiotic of supplemented Gum Arabic and *Bifidobacterium longum* BB536 for developing functional peanut milk. Peanut was roasted at 130°C for 20 min and soaked in water for 12 h, blended 5 min and filtered using a double layered cheese cloth to prepare the roasted peanut milk. Ten grams of two types of gums Arabic (*Acacia senegal* and *Acacia seyal*) were weighted, dissolved in 90 ml of distilled water and heated (60°C for 30 min). Peanut milk beverages were supplemented with Gum Arabic solutions (100ml/ 900ml) *Acacia senegal*, *Acacia seyal* and mixed (*A.senegal* and *A. seyal*) and then were inoculated with *Bifidobacterium longum* BB536. The inoculation was carried out under controlled conditions at 37 ºC. The initial pH of the peanut milk was adjusted to 6.7 before mixing with gum Arabic solutions. Total bacterial count, pH, titrable acidity, TSS, total sugars, moisture, and *Bifidobacterium longum* BB536 viable count in peanut milk beverage were determined. There was an increase in total viable count and titrable acidity, decrease in pH, TSS and total sugars for all treatments. The maximum counts were 5.36, 6.79, and 6.95 log CFU/ml in fermented peanut milk, fermented peanut milk supplemented with *Acacia senegal* gum, fermented peanut supplemented with *Acacia seyal* , fermented peanut milk supplemented with gum mix,respectively. The maximum counts were attained at 18 h fermentation in all fermented milks exept in peanut supplemented with *Acacia seyal* gum (6.3 log CFU/ml) was attained at 24 h fermentation. The high levels of strain BB 536 in all fermented milk exceeded the minimum numbers required to presence in probiotics functional foods which are at least 6.0 log CFU/ml except fermented peanut milk without gum (5.36 log CFU/ml). Therefore, fermentation of peanut milk with *Bifidobacterium longum* BB536 and Gums Arabic could exert prebiotication effect and make suitable carrier for development of functional peanut milk.

Keywords: Gum Arabic, Peanut milk, *Bifidobacterium longum* BB536, Prebiotication

Introduction:

Prebiotics such as fructooligosaccharide, galactooligosaccharide, xylooligosaccharide, beta-glucans and inulin are a non-digestible food ingredients that induce growth or activity of probiotic bacteria (Birkett and

Francis 2010; Nauta *e t al*., 2010 and Paineau *et al.*, 2010). Among prebiotics gum Arabic (GA) is one of the prebiotics when mixed with probiotic synbiotic product to enhance the growth and survival of probiotic bacteria in fermented dairy products (Desai et *al.,* 2004). Gum Arabic (GA) is a mixture of carbohydrates such as arabinose, rhamnose, galactose and glucuronic acid. The proportion of these carbohydrates varies according to the origins and sources of GA (Montengro *et al*., 2012). Probiotic bacteria include some species of Bifidobacteria and other types of bacteria such as lactobacillus. It can grow on gut of humans and animals and produce beneficial metabolic materials such as organic acids, peptides, bacteriocins and short chain fatty acids (Caramia and Silvi 2011). *Bifidobacteria* have been used in many food products such as dairy products, meat products, fermented vegetables and pickles.

Gale, (1948), defined fermentation as the process leading to anaerobic breakdown of carbohydrates,other major compounds such as organic acids, proteins, and fats. In a broader view, fermentation is an energy yielding process (Kosikiowski, 1982).

Peanut (*Arachis hypogea* L.) milk is the milk substitute, which provides over 30 essential nutrients and phytonutrient. It is rich in niacin, folate, fiber, vitamin E, magnesium and phosphorus (Griel *et al,* 2004). There is considerable interest in incorporating the 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 aim of this study was to evaluate the symbiotic effect of gum Arabic and *Bifidobacterium longum BB536 i*n peanut milk.

Materials and Methods:

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 10 ppb the permissible level.

Preparation and maintenance of starter culture: *Bifidobacterium longum* BB536 strain was obtained from the stored culture of the Department of Food Science and Technology, College of Agricultural Studies, Sudan University of Science and Technology. 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 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 water. The roasted soaked kernels were then mixed with water in a ratio of 1:5w/w [peanuts (200g): water (1L)], 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 cloth to obtain the peanut milk, which was sterilized in autoclave at 121°C for 15min.

Preparation of Gum Arabic: 15g from different samples of Gum Arabic powder (*Acacia senegal, Acacia seyal* and mixed *Acacia senegal and seyal*) were weighted and dissolved in small amount of sterile water and completed to150 ml to obtain 10% w/v water. These three solutions were sterilized in a water bath at 60°C for 30min.

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

Enumeration of Bifidobacterium longum BB536: The enumeration of *B. longum* BB536 of different fermented beverage were attained using the plate count technique with MRS medium. The fermented samples were drawn for analysis at the initial time and weekly during the storage period. One ml of fermented beverage was used to make serial dilution in 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 different fermented beverages was determined according to the AOAC method (1990). Ten ml of the sample was drawn into a conical flask. Distilled water was added to bring 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 were added as lactic acid, 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 titratable acidity:

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 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.

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 Brixº 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 was added slowly. Then, the mixture was gently boiled for 10 min to complete the inversion of sucrose and left to cool at room temperature. The solution was then 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 the 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 the precipitate cuprous oxide was observed. Finally, the titer volume was recorded and the amount of inverted sugars was obtained from Lane and Eynon Table. The total sugars, reducing and non-reducing sugars were calculated by using the following formulas:

Calculation:

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

Statistical Analysis: The One- way ANOVA was carried out to determine the 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.

Results and Discussion:

Bifidobacterium longum BB536 growth in Peanut Milk supplemented with different Types of Gum Arabic: Comparison of *Bifidobacterium longum* BB536 growth in different beverages (peanut milk, peanut milk supplemented with *Acacia senegal*, peanut milk supplemented with *Acacia seyal* and peanut milk with a mix of *Acacia enegal seyal*), and is shown in table 1 . All beverages supplemented with Gum Arabic showed a high growth of *Bifidobacterium longum* BB536 compared to the control. *Bifidobacterium longum* BB536 viable count significantly ($p<0.05$) increased by extending the fermentation period in all types of fermented beverages, as compared to strain in the initial level in the starting of the fermentation. Different maximum growth of strain *BB536* was detected. The maximum growth for control, peanut milk supplemented with *Acacia senegal* and peanut milk supplemented with mix *Acacia (senegal+ seyal*) was attained at 18h of incubation, while the maximum growth of strain BB536 supplemented with *Acacia seyal* was attained at 24h of fermentation .The growth rate of *B. longum* BB536 in different fermented beverages were 5.36, 6.79, 6.3 and 6.95 log CFU/ml for control, peanut milk supplemented with *Acacia senegal,* peanut milk supplemented with *Acacia seyal* and peanut milk mix *Acacia (senegal+ seyal*) respectively, as compared to strain at initial growth of fermentation. These variations in the growth rate of strain BB536 could be attributed to the types of Gum Arabic used as supplements. Peanut contains almost the essential nutrient needed for bacterial growth. The growth of strain BB536 improved by supplementation with different GA as compared to the peanut milk without GA (Table1). Gum Arabic contain about 78-88% solid materials and essential amino acids (Montengro *et al*., 2012). After the maximum point, there was a reduction in the viable number of strain BB536 that could be due to lack of availability of nutrient required for the growth as stated by Kabeir et al., (2005). Also increase of acidity and cold storage of beverages decreases the viability of *bifidobacteria* cells (Tamime *et al.*, 2005). Inspite of decline in viable count of strain BB536 in all types of fermented beverages at fermentation time, it s still above the number required to presence in probiotic food which is at least 6 log cfu/ml fermented product at the time of consumption (Viderola and Reinheimer, 2000 IDF, 1992; Lourens-Hattingh *et al.,* 2001).

The highest growth of the strain BB536 was detected in peanut supplemented with mixed *Acacia (senegal+ seyal*). The mix of Gums Arabic show better promoting effect as compared to the control in the growth and survival of probiotic bacteria. It was found to be affected by the chemical and microbiological composition of the medium and availability of nutrients (Shah, 2000b). These indicate the importance of using prebiotic gum Arabic for the fermentation with bifidobacteria. The mixture of gum Arabic with peanut milk is considered as an excellent medium for *B. longum* BB 536 growth.

Table 1: Viable count of *Bifidobacterium longum* BB536 Log (CFU/ml) during fermentation period of different beverages*

Beverages Fermented Time (h)	Total growth of *B. longum* BB536 (Log CFU/ml)			
	Peanut milk without GA (Control)	Peanut milk supplemented with gum *Acacia senegal gum*	Peanut milk supplemented with gum*Acacia seyal gum*	Peanut milk supplemented with gum mix*Acacia* (*senegal+ seyal)*
0	$2.52\pm 0.07^{h}_{c}$	$2.77\pm .07^{h}_{b}$	$2.63 \pm 0.06^{h}_{bc}$	$2.90 \pm 0.01^{h}_{a}$
6 hrs	$3.68\pm 0.02^{f}_{c}$	$4.73\pm 0.03^{f}_{b}$	$4.52\pm 0.04^{f}_{c}$	$5.83\pm 0.01^{f}_{a}$
12 hrs	6.81 ± 0.03bd	8.72±.017cb	7.97 ±0.01cc	8.92±0.01ca
18 hrs	$7.88 \pm 0.03^{a}_{d}$	$9.56\pm 0.03^{a}_{b}$	$8.88\pm 0.01^{c}_{c}$	$9.86\pm 0.01^{a}_{a}$
24 hrs	$7.83\pm 0.02^{a}_{d}$	$8.74\pm 0.02^{b}_{b}$	$8.93 \pm 0.02^{b}_{c}$	$9.75\pm 0.02^{b}_{a}$
30 hrs	$6.66 \pm 0.04^{c}_{d}$	$7.51\pm 0.03^{d}_{b}$	$7.83\pm 0.02^{d}_{c}$	$8.71\pm 0.02^{d}_{a}$
36 hrs	$5.87\pm 0.03^{d}_{d}$	$7.66\pm 0.02^{d}_{b}$	$7.54\pm 0.04^{d}_{c}$	$8.73\pm 0.03^{d}_{a}$
42 hrs	$4.71\pm 0.04^{e}_{d}$	$6.87\pm 0.02^{e}_{b}$	$5.92\pm 0.02^{e}_{c}$	$7.93\pm 0.01^{e}_{a}$

Values are mean ± SD for replicate independent runs.
Values that carried the different superscript letter in the same row are significantly different at $p<0.05$.
Values that carried the same suberscript letter in the same column aren't significantly different at $p<0.05$.
Peanut milk (mix) prepared using peanut milk and 1% gums 0f *Acacia senegal* (Hashab) and *Acacia seyal* (Taleh) in 1:1.

pH and Titratable acidity during fermentation of peanut milk with Bifidobacterium longum BB536: The result in Table 2 showed the pH values during the fermentation of different peanut milk samples. There was significant ($P<0.05$) decrease in pH for the all types of beverages with extending the fermentation period. The decrease in pH is due to increased acid production as a result of fermentation of sugars by *B.longum BB536* which produces acetic and lactic acid this is in agreement with Elghali *et al.,* (2014) who stated that, highest increase in acid production was associated with the maximum increase in the viable population of bacteria. Moreover, the accumulated acids produced by strain *BB536* reported to have antibacterial activity such as prevention of the growth of pathogens (Bullen *et al*., 1976; Goderska *et al.* 2002; Samona *et al.* 1996).

The rate of pH decreases at maximum growth of strain BB536 were 1.16, 1. 01 ,1 .21 and 0.9 in fermented peanut milk, peanut milk supplemented with *Acacia senegal* (Hashab) peanut milk supplemented with *Acacia seyal* (Taleh) peanut milk supplemented with a mix *Acacia (senegal + seyal)* respectively. Peanut milk supplemented with gum Arabic may offer some better properties to the fermentation medium, indicated by lowering the pH .The decrease of pH values in beverages supplemented with Gum Arabic could be due to the variety of carbohydrates, which the strain BB536 can ferment (Osman *etal.*,1993).

Table 3 showed the titratable acidity of different peanut fermented milk samples. Titratable acidity increased by extending the fermentation period. High acidity in supplemented beverages with GA was due to high growth of the strain BB536 and carbohydrates breaks down. The increase in acidity is correlated well with pH reduction mainly due to growth of *Bifidobacterium longum BB536* The rate of pH decreases at maximum growth of strain BB536 were 1.16, 1. 01 ,1 .21 and 0.9 in fermented peanut milk, peanut milk supplemented with *Acacia senegal* (Hashab)peanut milk supplemented with *Acacia seyal* (Taleh) peanut milk supplemented with a mix *Acacia (senegal + seyal)* respectively. The maximum growth rate of *B. longum* BB536 in different fermented beverages were 5.36, 6.79, 6.3 and 6.95 log CFU/ml for control, peanut milk supplemented with *Acacia senegal,* peanut milk supplemented with *Acacia seyal* and peanut milk mix *Acacia (senegal+ seyal*) respectively, they produce acids, which causes an increase in acidity and a decrease in pH (Abou-Dobara.*etal,*2016). The fermentation profile for increase in cell numbers and production of acids resulted in a reduction in the growth rate after maximum growth attained, at the end of these fermentations suggests that the growth stopped because of lack of carbohydrate substrate. The level of organic acids at the end of the fermentation might be responsible for the reduction in cell numbers (Gupta *et al.* 2010). Studies carried out under controlled pH conditions have indicated that the accumulation of acids during the fermentation is responsible for decrease in growth rate (Desjardins *et al.,* 1990).

Table 2: pH changes during the growth of *Bifidobacterium longum BB536* in fermented beverages*

Fermented Beverages Time (h)	pH			
	Peanut milk without GA control	Peanut milk supplemented with *Acacia senegal*	Peanut milk supplemented with *Acacia seyal*	Peanut milk supplemented with mixAcacia *senegal+ seyal*
Initial growth time 0 h	6.67± 0.04^{a}	6.25 ± 0.05^{a}	6.35±0.02^{a}	6.41 ± 0.04^{a}
6h	6.46 ±0.03^{a}	6.11± 0.01 ^{ab}	6.21± 0.01^{b}	6.39 ± 0.02^{b}
12h	6.21± 0.02^{b}	6.03 ± 0.07 ^{bc}	6.15 ± 0.01^{bc}	6.20 ± 0.02^{c}
18 h	5.51 ± 0.02^{c}	5.24 ± 0.03^{c}	5.45 ± 0.04^{c}	5.51 ± 0.01^{d}
24h	5.26 ± 0.01^{d}	5.0 ± 0.01^{d}	5.15± 0.02^{d}	5.08 ± 0.02^{e}
30h	4.69 ± 0.03^{e}	4.55 ± 0.02^{e}	4.87 ± 0.10^{e}	4.98 ± 0.03^{f}
36h	4.51 ± 0.02^{f}	4.24 ± 0.01^{h}	4.45 ± 0.02^{f}	4.51 ± 0.01^{h}
42 h	4.10±0.01^{h}	4.07 ±0.00^{i}	4.25 ±0.01^{h}	4.48±0.01^{i}

Values are mean ± SD for replicate independent runs.
Values that carried the different superscript letter in the same row are significantly different at $p<0.05$.
Values that carried the same superscript letter in the same column aren’t 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 3: Titratable acidity (%) during the initial and maximum growth of *Bifidobacterium longum BB536* in different beverages *

	Types of beverages			
	Titratable acidity (%)			
	Peanut milk without GA control	Peanut milk supplemented with *Acacia senegal*	Peanut milk supplemented with *Acacia seyal*	Peanut milk supplemented with mixAcacia *senegal+ seyal*
0 h	0.17 ± 0.00^{c}	0.19 ± 0.00^{e}	0.19 ± 0.00^{c}	0.18 ± 0.00^{f}
6h	0.19 ± 0.00^{c}	0.21 ± 0.02^{d}	0.21 ± 0.02^{d}	0.20 ± 0.01^{e}
12h	0.26 ± 0.02^{b}	0.23 ± 0.03^{cd}	0.22 ± 0.02^{c}	0.21 ± 0.01^{d}
18h Maximum growth time	0.28 ± 0.00^{b}	0.28 ± 0.00^{c}	0.24 ± 0.00^{d}	0.29 ± 0.00^{cd}
24h	0.29 ± 0.00^{b}	0.30 ± 0.01^{b}	0.26 ± 0.01^{c}	0.30 ± 0.00^{c}
30h	0.30 ± 0.02^{ab}	0.31 ± 0.02^{b}	0.29 ± 0.03^{b}	0.33 ± 0.01^{b}
36 h	0.31 ± 0.00^{ab}	0.33 ± 0.00^{ab}	0.31 ± 0.00^{ab}	0.34 ± 0.00^{a}
42 h	0.33 ± 0.00^{a}	0.34 ± 0.01^{a}	0.32 ± 0.01^{a}	0.34 ± 0.00^{a}

Values are mean ± SD for replicate independent runs.
Values that carried the different superscript letter in the same row are significantly different at p<0.05.
Values that carried the same superscript letter in the same column aren't 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.

There is a relation between microbial growth and acidity production the strong of the relationship as demonstrated by percent correlation (60 – 96) dependent on types of Gum Arabic supplement.

Total Soluble Solids (TSS) changes during fermentation of different beverages with Bifidobacterium longum BB536: Table 4 shows changes in total soluble solids (TSS) during fermentation of different formulated beverages with *Bifidobacterium longum* BB536.
There were significant (P<0.05) decrease in TSS levels in all types of fermented beverages by extending the fermentation period. The rates of TSS decrease at maximum growth were 0.08, 0.33, 0.03, and 0.27 in fermented peanut milk, peanut milk supplemented with *Acacia senegal* (hashab) peanut milk supplemented with *Acacia seyal* (taleh) and peanut milk supplemented with mix *Acacia (senegal + seyal).* A similar decrease in TSS during traditional fermentation of malwa was reported (Muyanj et al., 2010). Gum Arabic contains about 78-88% of solid substances and amino acids (Montengro *et al.,* 2012). The GA supplementation in peanut milk led to a high concentration of solid substances during the fermentation time in the beverages. The main metabolic products of carbohydrate fermentation by probiotic activity are organic acids substantiated by a drop in pH of the surrounding environment. This result agreed with the study of McMaster *et al*. (2005), who noted a great loss in viability of Bifidobacterium due to increased acidity, which lowers the survivability in fermented milk than in control

without fermentation (Ouwehand *etal,*2002)

Table 4: TSS (%) changes during the growth of the strain *Bifidobacterium longum*BB536 in different beverages *

Fermented beverages time (h)	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*
0h	2.37±0.05^{a}	2.82± 0.02^{abc}	2.96±0.01^{b}	2.89± 0.01^{a}
6h	2.23±0.03^{b}	2.68± 0.01^{abc}	2.77±0.02^{c}	2.43± 0.02^{d}
12h	2.07±0.06^{c}	3.03± 0.07^{ab}	3.06±0.12^{ab}	2.31± 0.01^{e}
18h maximum growth	2.29±0.01^{b}	3.15±0.14^{a}	3.11±0.01^{a}	2.62 ±0.03^{b}
24h	1.81±0.01^{d}	2.98±0.02^{ab}	2.99±0.03^{ab}	2.52±0.01^{c}
30h	1.63±0.03^{e}	2.47± 0.03^{bc}	2.33±0.03^{d}	2.22±0.03^{f}
36h	1.39±0.01^{f}	2.36±0.06^{c}	2.22± 0.02^{d}	1.12±0.03^{g}
42h	1.17±0.02^{g}	1.32±0.05^{d}	1.99±0.01^{e}	0.80±0.12^{h}

Values are mean ± SD for triplicate independent runs.
Values that carried the different superscript letter in the same row are significantly different at p<0.05.
Values that carried the same superscript letter in the same column aren't significantly different at p<0.05.
Peanut milk (mix) prepared using peanut milk and 1% gums 0f *Acacia senegal* (Hashab) and *Acacia seyal* (Taleh) in 1:1.

Total sugar during fermentation of different beverages with Bifidobacterium longum BB536: The result in Table 5 showed significant (P<0.05) decrease in sugar levels of all fermented beverages with an extended fermentation period. The strain BB536 ferment Gum Arabic and produces sugar and organic acids, mainly acetic, lactic, propunic, butyric and other organic acids(Sefa-Dedeh et al.,2003).

Table 5: Total sugars (%) during the initial and maximum growth of *Bifidobacterium longum BB536* in different beverages*

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 mix gum *Acacia senegal+ seyal*
Initial growth 0h	0.41±0.00^{a}	0.29 ± 0.09^{e}	0.38 ± 0.00^{de}	0.39 ± 0.00^{de}
6h	0.39±0.01^{a}	0.32±0.01^{e}	0.63 ± 0.03^{e}	0.51±0.02^{c}
12h	0.31±0.01^{c}	0.89±0.01^{c}	0.98±0.00^{d}	0.88±0.01^{b}
18h Maximum growth	0.35±0.02^{b}	1.21 ± 0.00^{a}	1.85 ± 0.00^{a}	1.02 ± 0.02^{a}
24h	0.28±0.01^{d}	1.19±0.01^{a}	1.81±0.01^{a}	1.01±0.01^{a}
30h	0.22±0.01^{e}	1.03±0.05^{b}	1.43±0.09^{b}	0.95±0.09^{ab}
36 h	0.21±0.00^{e}	0.95±0.04^{c}	1.22±0.03^{c}	0.93±0.04^{ab}
42h	0.12±0.01^{f}	0.65±0.01^{d}	0.95±0.08^{d}	0.85±0.015^{b}

Values are mean ± SD for triplicate independent runs.
Values that carried the difference superscript letter in the same row are significantly different at p<0.05.
Values that carried the same superscript letter in the same column aren't 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 rates of sugar decreased at maximum growth of strain *Bifidobacterium longum* BB536 and they were 0.06, 0.92, 1.47, and 0.63 in fermented peanut milk, the peanut milk supplemented with *Acacia Senegal* (Hashab), peanut milk supplemented with *Acacia seyal* taleh, and peanut milk mix , respectively. Gum Arabic is a branched-chain, complex polysaccharide, (Badreldin *et al.,* 2008; Abdul-Hadi *et al*., 2010), and these variations in total sugar refer to the strain activity, which break down complex polysaccharide during the fermentation time in different fermented beverages containing gums Arabic, and correlated well with the decrease in TSS.

Changes in moisture during the fermentation of different fermented beverages: Results in Table 6 showed the moisture content of different fermented beverages. There were a light increases in moisture of different fermented beverages by extending the fermentation period. The amount of moisture in fermented peanut milk, peanut milk supplemented with *Acacia senegal* (Hashab) peanut milk supplemented with *Acacia seyal* (Taleh) peanut milk supplemented with a mix (senegal + seyal), was 0.58, 0.73 ,0.71, and 0.98 % respectively. Gum Arabic is utilized in food products as an emulsifier and stabilizer material (Montengro *et al*., 2012). The stabilizer reduces water in fermented medium production. Therefore, during the fermentation process, increase in moisture might indicate a high enzymatic activity that breaks down the macro component into simpler ones and to the release of water.

Table 6: Moisture %of the different fermented beverages during growth and 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 mix gum *Acacia senegal+ seyal*
Initial growth time 0 h	87.30 ±0.01^{b}	86.26±0.01^{b}	86.59 ±0.00^{b}	86.12±0.02^{b}
Maximum growth time 18 h	87.88 ±0.06^{a}	86.99±0.06^{a}	87.30 ±0.01^{a}	87.10± 0.01^{a}

Values are mean ± SD for triplicate independent runs.
Values that carried the different superscript letter in the same row are significantly different at p<0.05.
Values that carried the same superscript letter in the same column aren't 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.

Conclusion:

The results obtained in this study show a certain stimulatory effect of the addition of gum Arabic on the *Bifidobacterium* longum BB536 growth in peanut milk. The addition of GA had a positive effect resulting in the largest number of bifidobacterial cells in fermented peanut milk, and a faster pH value decreases.

The supplementation of different types of Gums Arabic has shown a very good effect on growth of *B. longum* BB536. The growth of strain BB536 in formulated peanut milk with gum Arabic improved in comparison to the control without gum Arabic. The developed media could contribute positively to deliver B. *longum* BB536 besides conferring additional nutritional value to the diet. Therefore, gums Arabic has exerted a prebiotic effect at 1% in peanut milk, since its stimulation and promotion of growth of *Bifidobacterium longum* BB536 is proved.

References:

Abdul-Hadi, A.H., Mahmoud, A.E. and AbdelWahab, H.M. (2010). Effect of gum Arabic on coagulation system of albino rats. *Int. J. Pharm. Tech. Res*., **2**: 1762-1766.

Abou-Dobara M. Ismail., Ismail M.M. and Refat. N M. (2016). Chemical Composition, Sensory Evaluation and Starter Activity in Cow, Soy, Peanut and Rice Milk. *American Journal of Applied and Industrial Chemistry*. **2** (4): 21-28.

AOAC. (1990). Association of Official Analytical Chemists. Official Methods for Analysis (15th ed). Washington, D.C., USA.

Badreldin, H.A., Amal, Z. and Gerald, B. (2008). Biological Effects of Gum Arabic: A review of some recent research. *Food Chem. Toxicol.*, **47:** 1-8.

Birkett, A.M. and Francis, C.C. (2010). Short-chain fructo-oligosaccharide: A low molecular weight fructan. In: *Handbook of Prebiotics and Probiotics Ingredients: Health Benefits and Food Applications*. Cho, S.S. and Finocchiaro, E.T. (Eds). CRC Press, USA, pp: 13-42.

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.

Caramia, G. and Silvi, S. (2011). Probiotics: from the ancient wisdom to the actual therapeutical and nutraceutical perspective. In: *Probiotic Bacteria and Enteric Infections*. Malago, J.J.; Koninkx, J. F. J. G. and Marinsek-Logar, R. (Eds). Springer, New York USA. 3-37.

Desai, A.R., Powell, I.B., and Shah, N.P. (2004). Survival and Activity of probiotic Lactobacilli in skim milk containing prebiotics. *Journal of Food Science*, **69**: S57-S60.

Desjardins, M.L., Roy, D. and Toupin, C., (1990). Uncoupling of growth and acids production in *Bifidibacterium* spp. *J. Dairy Sci*. **73**:1478–1484.

Elghali, S. Mustafa, SH. Amid, M. Abd Manap, M.Y. Ismail, A and Abas, F. (2014). Variations on soymilk components during fermentation by Lactobacillus and Bifidobacterium strains. *Journal of Food, Agriculture & Environment*, **12** (2): 1-5.

FAO/WHO. (1979). Energy and protein requirements. *Bulletin of the World Health Organization* **57**(1): 65-79.

Gale, E. F. (1948). Chemical activates of bacteria. Academic press Inc. N.Y.

Goderska, K., Czarnecka, M. and Czarnecki, Z. (2002) Survival rate of chosen lactobacillus bacteria type in media of different pH. *Food Sci Technol,* **5***:* 1–9.

Griel, A.E., Eissenstat, B., Juturu, V., Hsieh, G. and Kris-Etherton, P.M. (2004). Improved diet quality with peanut consumption. *J Am Coll Nutr*., **23**(6): 660-8.

Gupta,S., Abu-Ghannam.N.and Scannell. A. G.M. (2010) .Growth and kinetics of Lactobacillus plantarum in the fermentation of edible Irish brown seaweeds. *Food Bioprod Process*, / j.fbp .10- 01.

IDF. (1992). General standard of identity for fermented milks. International Dairy Federation, 163.

Kabeir, B.M., Abd Aziz, S., Muhamed, M.and Yazid. A.M. (2005). Growth of Bifidobacterium longum BB536 in Media (femented cereal porridge) and their survival during storage. *Let. Appl. Microbial*, **l41**: 12-131.

Kabeir, B. M., Ibraheem, S. E., Mohammed, L. H and Bhagiel, B. T. (2015).Roasted peanut milk partially substituted with millet thin porridge as a carrier for Bifidobacterium longum BaB536," *Int. J Current Microbiology and Applied Science*, **4**: 299-308.

Kosikowsiski, F.V. (1982). *Cheese and Fermented Milk Products*, 2nd. ED., F. E. Kosikowiski and Associates Brooktonadae. N. Y.

Lourens-Hattingh, A. and Viljoen, B.C. (2001). Yogurt as probiotic carrier food. *International Dairy Journal*; **11**: 1-17.

McMaster, LD, Kokott SA, Reid SJ, Abratt VR (2005). Use of traditional African fermented beverages as delivery vehicles for Bifidobacterium lactis DSM 10140. *Int. J. Food Microbiol.* **102**: 231- 237.

MINITAB (2006). Statistical software, Release 17 for Windows, Minitab Inc, USA.

Montenegro, M.A., Boiero, M.L., Valle, L. and Borsarelli, C.D.(2012). Gum Arabic: more than an edible emulsifier. In: *Products and Applications of Biopolymers*. Verbeek, J. (Ed.). InTech, Croatia, pp: 3-26.

Muyanja, C. Birungi, S., Ahimbisibwe, M., Semanda, J. and Namugumya B. S., (2010). Traditional Processing, Microbial and Physicochemical Changes during Fermentation of Malwa. *African Journal of Food, Agriculture, Nutrition and Development*, **10**(10): 2124-2138.

Nauta, A., A.M. Bakker-Zierikzee and M.H.C Schoterman (2010). Galacto-oligosaccharides. In: *Handbook of Prebiotics and Probiotics Ingredients: Health Benefits and Food Applications*. Cho, S.S. and Finocchiaro, E.T. (Eds.). CRC Press, USA, pp: 75-94.

Osman, M.E., Menzies, A.R. P.A. Williams, Phillips, G.O. and Baldwin, T.C.(1993). The molecular characterization of the polysaccharide gum from *Acacia Senegal*. *Carbohydrate Research*, **246**: 303-318.

Osman, M.E.; Williams, P. A.; Menzies, A. R. and Phillips, G. O. (1993) Characterization of Commercial Samples of Gum Arabic. *Journal of Agricultural and Food Chemistry*, **41**(1): 71-77.

Ouwehand, A.C., Salminen, S and Isolauri, E. (2002). Probiotics :an overview of beneficial effects.

Antonie Van Leeuwenhoek, 82:279-289.

Ouwehand, A.C., Salminen, S and Isolauri, E. (2002). Probiotics: an overview of beneficial effects. *Antonie Van Leeuwenhoek*, **82**:279-289.

Paineau, D., Respondek, F. and Bouhnik,Y. (2010). Inulin and oligosaccharides: A special focus on human studies. In: *Handbook of Prebiotics and Probiotics Ingredients: Health Benefits and Food Applications*. Cho, S.S. and Finocchiaro, E.T. (Eds). CRC Press, USA, pp: 43-74.

Salunkhe, D.K and Kadam, S.S. (1989). Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology and Utilization. 1st edn., CRC Press. Inc., Boca Raton.Floridea.323

Samona, A., Robinson, R.K. and Marakis, S. (1996) Acid production by bifidobacteria and youghurt bacteria during fermentation and storage of milk. *Food Microbiol,* **13**: 275–280.

Sefa-Dedeh, S., Cornelius, B., Amoa-Awua, W., Sakyi-Dawson, EO, and Afoakwa, EO. (2003). The Microflora of Fermented Nixtamalized Corn. *Int. J. Fd. Microbiol*, **2755**: 1-6.

Shah, N.P. (2000a). Probiotic bacteria: Selective enumeration and survival in dairy food. *Journal of Dairy Science*; **83**: 894-907.

Shah, N.P. (2000b). Effect of milk-derived bioactive: an overview. *British Journal of Nutrition*, **84**: S3-S10.

Tamime, A. Y., and Robinson, R. K. (1985). *Yoghurt: Science and Technology*. Pergamon Press Ltd., Oxford, UK.

Tamime, A.Y., M. Saarela, A.K. Sondergaard, V.V. Mistry and N.P. Shah. (2005). Production and maintenance of viability of probiotic microorganisms in dairy products. In: *Probiotic Dairy Products*. Tamime, A. (Ed.). Oxford, UK, pp: 39-56.

Viderola, C.G and Reinhelmer, J. (2000). Survival of probiotic microflora in Argentinian yoghurts during refrigerated storage. *Food Research International*, **33**: 97-102.

Wang Y.-C., Yu R.-C., Yang H.-Y., Chou C.-C. (2003). Sugar and acid contents in soymilk fermented with lactic acid bacteria alone or simultaneously with bifidobacteria. *Food Microbiology*, **20**: 333–338.

مدى استفادة بكتريا *Bifidobacterium longum* BB536 من التاثير التحفيزى للصمغ العربى اثناء تخمير لبن الفول السودانى

هاله محمود محمد الجزولى [1] ، يوسف محمد احمد ادريس [1] و بركة محمد كبير بركة [1]

- كلية الدراسات الزراعيا – جامعة السودان للعلوم والتكنولوجيا

المستخلص :

أجريت هذه الدراسة لاكتشاف استفادة البكتريا الصديقة *Bifidobacterium longum* BB536 من تدعيم الصمغ العربي حليب الفول السوداني. تم تحميص الفول السوداني عند درجة حرارة 130 م لمدة 20 دقيقة ونقعه في الماء لمدة 12 ساعة ، تم خلطه لمدة 5 دقائق . تم الترشيح باستخدام طبقتين من قماش الجبن لتحضير حليب الفول السوداني المحمصر . تم وزن 10 جرام من نوعين من الصمغ العربي (الهشاب والطلح) كل على حده وعينة من خليط (الهشاب والطلح ، ثم أذيبت في 90 مل من الماء المقطر . سد ت محاليل الصمغ العربي في درجة حرارة (60 م لمدة 30 دقيقة). دعمت مشروبات لبن الفول السوداني بمحاليل الصمغ العربي (100 مل/ 900 مل) ثم لة ت بالبكترايا الصديقة *Bifidobacterium longum* BB536 تم التحضير تحت ظروف متحكم يها (37 م). الرقم الهيدروجيني الأولي لحليب الفول السوداني (7.ı) قبل الخلط مع محاليل الصمغ العربي. أجريت تحا ل مختلفة شملت لعد البكتيري الكلي، الرقم الهيدروجيني، درجة الحموضة، الجوامد الصلبة الذائبة، السكريات الكلية والرطوبة. كان هنالك زيادة في درجة الحموضة، وانخفاض في الرقم الهيدروجيني و الجوامد الصلبة الذائبة والسكريات الكلية. ذ ت هنالك زيادة معنوية في العد الحي للبكتريا صديقة مع استمرار فترة التخزين . كان أقصى نمو 5.36،6.79 ، 6.96 6 log CFU/ml في لبن الفول المخمر و لبن الفول المدعم بصمغ الهشاب و أقصى عدد تم الحصول عليه عند 18 ساعة تخمير فى كل الالبان المخمرة ما عدا لبن الفول المدعم بصمغ الطلح(og CFU/ml 6.3) حيث تم الحصـ ل على اقصى عدد عند 24 ساعة تخمير . هذا النمو العالى للبكتريا الصديقة*Bifidobacterium longum* BB536 يزيد عن الحد الادنى (log CFU/ml 6) المطلوب وجوده للبكتريا فى الاغذية الوظيفية ما عدا لبن الفول المخمر غير المدعم بالصمغ العربى(log 5). CFU/ml لذلك يمكن أن يكون تخمير حليب الفول السوداني بالبكتريا الصديقة *Bifidobacterium longum* BB536 المدعم بالصمغ العربي مناسباً لتطوير الأغذية الوظيفية.