International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

http://ijpsat.ijsht-journals.org
Vol. 24 No. 2 January 2021, pp. 255-263

Propotication with Bifidobacterium Longum BB536 into Different Sudanese Local Rice Types

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Abstract – Probotication (addition of probiotic) with *Bifidobacterium longum* BB536 to beverages formulated from three different Sudanese rice types denoted locally (Crown, Narica and Pabenjeda) was assessed in this study. 10% rice (w/v) was cooked at 60 ºC using a hot plate for 7 min; after cooling 1g of fetarita sorghum malt was added for liquification followed by addition of10% whey (v/v) and 1% hashab gum (w/v). The mix was sterilized at 121 ºC for 15 min., cooled to 37 ºC, 3 % active strain BB.536 starter culture was added and incubated at 37 ºC for 36 hrs (maximum strain BB536 viable count). Different analyses were carried out for final fermented rice beverages including physiochemical properties (TSS, pH, Acidity), growth and survival of strain BB 536. During fermentation the maximum growth of strain BB 536 was accompanied with significant ($p<0.05$) increases in strain BB 536 viable count and acidy. However, there were also significant ($p<0.05$) reductions in pH and TSS. The level of strain BB536 growth in all fermented beverages was improved by supplementation with whey and further improved more by supplementation with whey and hashab gum. The rates of strain BB536 increases in all fermented rice beverages were in range of 5.24 - 6.99 log CFU/ml. The maximum viable number of strain BB 536 in all fermented rice beverages was attained at 36 h incubation (except in fermented Narica rice beverage at 30 h) and ranged between 9.83 - 9.87 log CFU/m. These numbers exceed the amount required to presence in probiotic food which at least 6 log CFU/ ml. Moreover, at three weeks refrigeration storage the viable counts of strain BB 536 still fulfil probiotic requirement. Therefore, probiotication of rice beverages with strain BB.536 was successful to develop probiotic functional food.

Keywords – Probitication, *Bifidobactrium*, Sudanese, Rice, Physicochemical.

I. INTRODUCTION

Probiotics defined as 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 [1], [2], [3]. *Bifidobacterium longum* is the most common species of *bifidobacteria*being found in both infant and adult [4]. Many scientific studies showed the benefits offered by *Bifidobacterium longum*BB536 [5], [6]. Thus there is considerable interest in incorporating these heaths promoting *bifidobacterium* into food.

Prebiotic is mainly indigestible food item that usefully affect the host by selectively stimulating the growth and activity of one or a partial number of bacteria in the large intestine, and thus improves the host health [7]. The non-digestible prebiotics such as inulin, fructooligosaccharide (FOS), sorbitol and arabinan selectively stimulate the growth and /or activity of indigenous probiotic *bifidobacteria* and *Lactobacillus* in the intestinal tract [7], [8], [9].

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It is prepared by inclusion of other ingredients such as probiotics and prebiotics to levels that enable the consumer to derive optimal health benefits. The presence of probiotics and prebiotics in the same product is termed as synbiotic [10]. The approach has been reported to enhance the efficiency through supporting viability and enhancing enzymatic activity of probiotics [11], synbiotic products enhance the growth and survival of probiotic bacteria in fermented dairy products [12]. Among prebiotics gum arabic (GA) is one of the prebiotics found to support growth of probiotics [13]. Gum arabic or *acacia gum* is the exudate from the *Acacia Senegal* and *Acaciaseyal* trees, belonging to *Leguminosae* family,it is a complex, branched heteropolysaccharidesolublein water, neutral or slightly acidic salt of a complex polysaccharide containing calcium, magnesium and potassium salt. It is acomplex edible mixture of saccharides and glycoproteincomposed of 1, 3-linked β-d-galactopyranosyl units. L-arabinose, L-rhamnose, anglucuronic which gives it most useful property [14].

The functional food is the food that can positively impact the health as it contains health promoting and biologically active components in adequate amounts [15]. [16] stated that functional food improves the general and physical conditions of the human organism and/or decrease the risk of occurrence of disease, it either be dairy based or cereal based. Among cereal, rice (*Oryzasativa L.*) is one of the most important cereal crops and is the staple food of over half the world's population, it is also one of the largest consumed agricultural crop in the world, only next to maize. Rice is consumed as milled rice after dehulling process or removal of bran layer and embryo from brown rice [17], thus need to be supplemented to improve its health and nutritional properties. Whey is defined as water which drains from the crud during cheese making processes. It contains nearly all the lactose, 20 % of milk protein (albumin and glublin) and soluble minerals in original milk [18], [19], [20]. Probotication started in dairy food and then expanded to include cereal based food [21] instead of dairy base. In Sudan probotication was successful in Medida the Sudanese thin porridge formulated from rice. However, no report on use of local rice types in probiotic food formulation. Therefore, in this study the suitability of different Sudanese rice types as a successful carrier for probiotic was assessed.

II. MATERIALS AND METHODS

2.1 Rice varieties

Long grain rice locally denoted (Crown) planted in Sudan was collected from supermarket in Khartoum State, Papenjeda rice is a wild rice grown in white Nile State was collected from the local market in (Kosti Town), and Narica rice planted in Algazira scheme was obtained from the Rice Department, Ministry of Agriculture, (Khartoum – Sudan). Care was taken to ensure that good quality of rice grains was selected.

2.2 Preparation of whey

Whey is water which drains from the crud during cheese making processes. Milk was heated in water bath followed by addition of rennin enzyme dissolved in water to the milk to cause coagulation and formation of curds and left for 30 min .Then the curld was cutand whey drained off by sieving through a piece of cheesecloth. The collected whey was packed in cleaned bottles then preserved in a freezer.

2.3 Preparation of additive gum arabic

Hashab gum was obtained from north Kordofan State from batches prepared for export. The gum arabic was ground to fine powder using a commercial grinder. The powder was packed in a plastic container and kept at refrigeration temperature until used.

2.4 Preparation of sorghum malt

Fitareta sorghum grain was obtained from Agricultural Research Corporation (ARC)- Wad Medani town, Aljazeira State, Sudan. Sorghum malted following the procedure reported by [22]. Germinated sorghum were dried in an oven at 50°C for 48 hrs, after that the roots of the germinated sorghum were removed and the malted sorghum 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.

2.5 *Bifidobacterium longum BB536* Culture

Bifidobacterium Longum BB536 culture was prepared by activation of the strain by transferring the strain into 10% sterilized (121ºC for 15 min) skim milk and anaerobic incubation at 37ºC for 24 hrs. The culture was further sub cultured twice in a similar sterilized skim milk for 24 hrs to use for fermentation**.**

2.6 Fermentation of the three different rice varieties supplemented with sorghum malt, whey and gum arabic

The three different varieties of rice grains were grounded separately to fine flour using a commercial grinder. The flour was packed in a plastic container and kept at refrigeration temperature until used. 10% of different types of rice flour (w/v) were prepared in a separate volumetric flask. The mixture was cooked in hot plate at 60 ºC for 7 minutes. After cooling1% sorghum malt was added for liquificaton. 10% ml of whey (V/V) and 1% gum arabic(w/v) were supplemented. The final mixture was autoclaved at 121 ºC for 15 minutes, cooled to 37ºC, 3% active culture of strain BB536 was added followed by incubation at 37ºC for 36 hours.

2.7 Enumeration of *Bifidobacterium longum BB536*

The media used was MRS (de Man Rogosa Sharpe Medium agar, Merck, Germany) agar supplemented with 0.05% L-cysteine. It was prepared following manufacture instructions. 1 ml of each fermented rice variety was serially diluted in 9 ml peptone water. 0.1 ml of each dilution was aseptically plated on MRS agar plates. The plates were then incubated anaerobically at 37°C for 48 hours and colony-forming units (CFU) per ml were counted.

2.8 Determination of pH

The pH was determined using a pH-meter (model HI8521 microprocessor bench pH / MV / CO meter, Romania) **.**Two standard buffer solution of pH 4.00 and 7.00 were used for calibration at room temperature. The pH meter was allowed to stabilize for one minute and then the reading was directly measured.

2.9 Determination of titratable acidity

The titratable acidity (TA) was determined according to [23] method. Ten ml of sample were pipetted 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 pipetted into a porcelain dish, five drops of phenolphthalein added, and the sample was titrated against 0. IN NaOH till a faint pink color that lasted for at least 30 seconds was obtained, then acidity was calculated.

2.10 Determination of total soluble solids (TSS)

A total soluble solid (TSS) of fermented rice beverages was determined at room temperature using digital refracotmeter with degree Brixº scale 0-100 according to [23].

2.11 The storage of the fermented three different rice varities products

After 36 hours of fermentation (the maximum growth of *Bifidobacterium longum* BB536) fermented rice beverages were held at refrigeration temperature for a period of 3 weeks. During the storage period, the viable counts of *Bifidobacterium longum* BB536 were caounted after one week, two weeks and three weeks intervals.

2.12 Statistical analysis

One- way ANOVA and two sample paired test were performed to examine significant differences between normally distributed data of triplicated independent analysis. Probability level of less than 0.05 was considered significant ($p<0.05$). All data were analyzed using vision 17 MINITAB statistical software for windows [24].

III. RESULTS

3.1 The growth of *Bifidobacterium longum BB536* (log CFU/ml) during fermentation of three rice beverages supplemented with whey and hashab gum

Comparative growth of strain BB536 cultured in rice (Crown, Narica and Pabenjeda) beverages supplemented with whey and then whey and hashab gum displayed in Tables (1), (2) and (3). There were significant ($p<0.05$) increases in strain BB536 viable count by extended fermentation period in all fermented beverages as compared to initial level at the beginning of fermentation (Tables 1, 2 and (3). The rate of growth of strain BB536 in all fermented beverages was improved by supplementation with whey and further improved more by supplementation with whey and gum as shown in Tables 1, 2 and 3. The maximum growth of strain BB536 was attained at 36 h in all fermented beverages except in fermented Narica beverage supplemented with whey which was attained at 30 h (Tables 1, 2 and 3). These maximum viable counts of strain BB536 in all fermented beverages fulfill the requirement of probiotic food which is at least 6 log CFU/ml fermented product. After the maximum growth of strain BB536 (36 h except for fermented Narica beverage after 30 h), there were reductions in the viable number by extend fermentation to 48 h.

3.2 Physiochemical properties of different *Bifidobacterium longum* BB536 fermented rice beverage

Table (4) represent the pH values, titerable acidity (TA) and total soluble Solids (TSS) of strain BB536 fermented Crown, Narica and Pabenjeda rice beverages at initial (0 h) and maximum growth (36 h). There was significant ($p<0.05$) decreases in pH and TSS in all fermented rice beverages (Table 4). While titerable acidity (TA) significantly ($p<0.05$) increased at maximum strain BB536 growth.

3.3 Survival of *Bifidobacterium longum BB536* log (CFU/ ml) supplemented with whey and gum arabic during the storage of fermented rice beverages

Table (5) showed the survival of strain BB536 during refrigeration storage of fermented rice beverages. There were significant ($p<0.05$) reduction in strain BB536 viable count in all fermented rice beverages during extension of storage period to three weeks. The rate of reduction in the first week refrigeration were 2.0, 1.32, and 2.94 log CFU /ml in fermented Crown, Narica and Pabenjeda rice beverages, respectively. While the decrease in the second week refrigeration were 2.29, 2.09 and 2.97 log CFU/ ml in the fermented Crown, Narica and Pabenjeda rice beverages, respectively. At the end of refrigeration, the total strain reductions were 3.01, 3.30 and 4.00 log CFU/ ml in the fermented Crown, Narica and Pabenjeda rice beverages, respectively.

IV. DISCUSSION

The rate of strain BB536 viable count increases during fermentation of different rice beverages were high. The increases in all fermented rice beverages were in range of 5.24 - 6.99 log CFU/ml. The better growth of probiotic bacteria was based on availability of nutrients as stated by [25]. Thus, supplementation with whey improved strain BB536 growth as compared with the control. Addition of whey was documented to enhance *bifidobacteria* growth [26], [27]. However, the maximum growth development was attained by supplementation with whey and hashab gum Arabic together. These findings indicate the importance of addition of prebiotic to nourish and activate the growth of strain BB536 in fermented rice beverages in presence of whey.

Substances include fructooligosaccahrides (FOS), aseinomacropeptides (CMP), whey protein concentrate (WPC), tryptone, yeast extracts, certain amino acids, nucleotide precursors and an iron source were also documented as most importance in the viability of probiotic bacteria in fermented formula [26]. [28] stated that the viability of probiotic bacteria in fermented formula due to the fact that probiotic in particular, bifidobacteria increases the rate of growth if the food is fortified with different growth factors (consumed by probiotics as nutrients) and/or growth promoters (which improve viability of probiotics but not as a direct nutrient) such as casein, whey protein hydrolysates, L-cysteine, yeast extract, glucose, vitamins, minerals and antioxidant. These supplements have significant positive effects on the survival of probiotic microorganisms. Supplementation with gum Arabic as a prebiotic also had a significant increase in viable count of *Bifidobacterium* strain as stated by [29]. Prebiotics are non–viable and non-digestible (or minimally digestible) food ingredients which are metabolized selectively by beneficial intestinal bacteria and enhance their growth and/or activity. They are mostly sugar-like compounds (oligosaccharides) comprising between two and ten monomers that largely resist digestion by pancreatic and brush border enzymes.Also can have suitable effect on retention of probiotics viability (especially *bifidobacteria*) in food products as well as in gastrointestinal tract [30], [28], [31].

The reduction in strain BB536 viable count after 36 h fermentation (maximum growth) was due to lack of available nutrients required for the growth [22] and increase of acidity [32]. In spite of the noticeable declining in strain BB536 viable count of different fermented rice beverages, their maximum number still above the requirement to presence in probiotic food which is at least 6 log CFU/ml fermented Crown and Pabenjeda rice beverages. pH and titrable acidity of probiotic products considerably affect cell survival of probiotic microorganisms [33]. Low pH is of the most important factor that restricts the growth and stability of probiotic bacteria in fermented products and this because hydrogen ions damage probiotic cells via disrupting mass transfer through the cell membranes and acidic starvation of the cells [34].

During growth strain BB536 utilized sugar (TSS) as energy source thus it decreased by fermentation. The rates of TSS decreases at maximum growth of strain BB536 were 1.79, 1.89 and 2.11% in fermented Crown, Narica, and Pabenjeda rice beverages, respectively. Enzymatic activity of the strain plays a vital role in rate of TSS fermentations as stated by [35]. The reduction in sugar often accompanied by decrease in pH and increase of acid as a result of fermentation of sugars by strain BB536 and production of mainly acetic and lactic acids. The segmental reduction in pH is in agreement with [36] findings. They stated that, the highest increase in acid production was associated with maximum increase in the viable population of bacteria. Moreover, the accumulated acid produced by *Bifidobacterium longum* BB536 reported to have antibacterial activity such as prevention of growth of pathogens

[37], [38], [39]. The variances in reduction rate of pH could be attributed to the types of carbohydrates (sugars) which can be ferment [40]. The increase in acidity is correlated well with pH reduction as stated by [41].

It was clear that after three weeks refrigeration storage there is a reduction in viable count of strain, this might be due to increases of acidity in addition to the cold storage of the fermented product decreases enzymatic activity of *bifidobacteria* cell [32]. After three weeks refrigeration, the viable count of strain BB536 in fermented Crown and Narica rice beverages fulfill the number needed to presence in probiotic food(6 log CFU /ml) to exert health benefits upon consumption [42]. These result agreed with [22] on survival of *B. longum* BB.strain 536 in Sudanese fermented Medida during the refrigeration storage, and disagreed with [43] on strain survival in refrigerated yogurt. These results confirm the finding by [44] who reported a decline in total viable count of *Lactobacillus reuteri* and *Biofidobacterium* in whey based probiotic beverage stored at 4 ± 1°C. This indicated that the survival of *Bifidobacteria*in fermented products was dependent on the carrier media type or substrate in addition to pH levels of the fermented product during the storage [13]. Addition of whey and hashab gum arabic to rice beverages change the composition of the fermentation medium to better for strain BB.536 survival environment. [45] stated that the survival of probiotic bacteria in fermented dairy bio- products depends on strain used, interaction between species present, culture conditions, chemical composition of the fermentation medium e.g. carbohydrate source, final acidity, solid content, availability of nutrients, growth promoters and inhibitors, concentration of sugars, dissolved oxygen, level of inoculation, incubation temperature, fermentation time and storage condition. 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 [46], [47], [48].

The growth and survival of probiotic in dairy² products affected by the fermentation time, incubation temperature, storage temperature and the addition of casein in addition to the level of dissolved oxygen [42]. Therefore the validity and survivability of probiotic in fermented products should be fixed in the product label as stated by [13].

Table 1: The growth of *Bifidobacterium longum* BB536 log (CFU/ml) during fermentation of Crown rice beverage

Fermented Crown rice beverage with different supplements			
Fermentation period (h)	**Control**	**Whey**	**Whey + hashab gum**
0	3.73±0.03^{g}	3.80 ±0.02^{i}	3.92±0.01^{i}
6	3.90 ±0.02^{f}	3.97±0.01^{h}	4.64±0.04^{h}
12	5.87 ± 0.02^{e}	5.92±0.01^{g}	5.97±0.01^{g}
18	6.70 ± 0.04^{d}	6.74±0.01^{f}	6.88±0.01^{f}
24	8.95 ± 0.01^{a}	7.96±0.03^{d}	8.77±0.02^{d}
30	8.96 ± 0.00^{a}	9.67±0.01^{b}	9.75±0.01^{b}
36	8.97 ± 0.01^{a}	9.84±0.01^{a}	9.87±0.01^{a}
42	7.89 ± 0.02^{b}	8.74±0.02^{c}	8.81±0.02^{c}
48	7.73 ± 0.03^{c}	7.86±0.01^{e}	7.92±0.02^{e}

Values are mean ± SD for triplicate independent analysis value.
Values that bear different superscript letter in the same column are significantly difference at $p<0.05$.

Table 2The growth of Bifidobacterium longum BB536 log (CFU/ml) during fermentation of Narica rice beverage

Fermented Narica rice beverage with different supplements			
Fermentation period (h)	**Control**	**Whey**	**Whey + hashab gum**
0	2.54 ±0.03^{h}	2.74 ±0.01^{i}	2.88 ±0.01^{f}
6	3.88±0.01^{g}	3.92 ±0.01^{h}	3.97 ±0.01^{e}
12	4.86±0.02^{f}	4.97 ±0.01^{g}	5.68 ±0.01^{d}
18	5.85± 0.02^{e}	5.93 ±0.01^{f}	5.97 ±0.01^{d}
24	6.83±0.01^{d}	7.86 ±0.02^{d}	7.92 ±0.01^{c}
30	8.75 ±0.02^{b}	8.92 ±0.01^{a}	7.97 ±0.01^{c}
36	8.90±0.02^{a}	8.73 ±0.02^{c}	9.86 ±0.02^{a}
42	7.70±0.02^{c}	8.80 ±0.02^{b}	9.51 ±0.5^{a}

48	6.88±0.01[d]	7.65± 0.03[e]	8.74 ±0.03[b]

Values are mean ± SD for triplicate independent trails.
Values that bear different superscript letter in the same column are significantly difference at $p<0.05$.

Table 3: The growth of Bifidobacterium longum BB536 log (CFU/ml) during fermentation of Pabenjeda rice beverage

	Fermented Narica rice beverage with different supplements		
ermentation period (h)	**Control**	**Whey**	**Whey + hashab gum**
0	2.64 ±0.04[i]	2.67 ± 0.02[i]	2.78 ±0.02[f]
6	2.95 ±0.01[h]	3.88 ±0.01[h]	3.93 ±0.00[e]
12	3.92 ±0.01[g]	4.92 ±0.01[g]	5.69 ±0.03[d]
18	4.80 ±0.02[f]	5.88 ±0.02[f]	6.74 ±0.02[c]
24	5.95 ±0.02[e]	7.84 ±0.03[d]	7.92 ±0.01[b]
30	7.74 ±0.01[d]	8.87 ±0.02[b]	9.63 ±0.04[a]
36	8.97 ±0.01[a]	9.66 ±0.03[a]	9.83 ±0.01[a]
42	8.62 ±0.02[b]	8.75 ±0.02[c]	8.13 ±0.62[b]
48	7. 92±0.01[c]	7.59±0.05[e]	7.77 ±0.03[b]

Values are mean ± SD for triplicate independent analysis value.
Values that bear different superscript letter in the same column are significantly difference at $p<0.05$.

Table 4: Physicochemical properties (pH, Titratable acidity and Total Soluble Solids) of different fermented rice beverages

	Physicochemical properties at initial and maximum growth of strain BB536					
Types of fermented rice	**pH**		**Acidity**		**TSS**	
	Initial	**Maximum**	**Initial**	**Maximum**	**Initial**	**Maximum**
Crown	6.65±0.02[a]	4.85±0.00[b]	0.19±0.02[c]	0.42±0.02[a]	2.76±0.05[c]	0.97±0.00[a]
Narica	6.37±0.03[b]	4.95±0.01[c]	0.17±0.05[b]	0.38±0.02[a]	2.81±0.12[a]	0.92±0.04[a]
Pabenjeda	6.83±0.05[ab]	4.92±0.12[b]	0.21±0.11[b]	0.51±0.00[a]	2.96±0.01[ab]	0.85±0.1[a]

Values are mean ±SD for triplicate trails.
Value that bear different superscript letters in the same row of each specific physicochemical property is significantly difference at $p<0.05$.

Table 3The survival of Bifidobacterium longum BB536 (log CFU/ ml) during refrigeration of fermented rice beverage supplemented with whey and hashab gum

Storage period (week) of strain BB536 fermented rice beverages					
Types of fermented rice	**Initial**	**One**	**Two**	**Three**	**Reduction Rate**
Crown	9.87 ± 0.02[a]	7.87±0.02[b]	7.58 ± 0.03[c]	6.86 ± 0.02[d]	3.01

Narica	9.86 ± 0.01^{a}	8.54 ± 0.06^{b}	7.77 ± 0.04^{c}	6.56 ± 0.08^{d}	3.30
Pabenjeda	9.83 ± 0.01^{a}	6.89 ± 0.03^{b}	6.86 ± 0.01^{c}	5.83 ± 0.02^{d}	4.00

Values are mean ± SD for triplicate independent trails.
Value that bear different superscript letter in the same row of each specific fermented rice beverage is significantly difference at $p<0.05$.

REFERENCES

[1] [Naidu, A.S., Bidlac, W.R. and Clemens, R.A., Probiotic spectra of lactic acid bacteria. Critical Reviews in Food Science and Nutrition, 1999. 39: p.13 - 126.

[2] Holzapfel, W.H., Geisen, R., Schillinger, U., Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. International Journal of Food Microbiology, 1995. 24: p. 343 - 362.

[3] Saxelin ,M., Pessi , T. and Salminen, S., Fecal recovery following oral- administration of Lactobacillus strain GG (ATCC-53103) in gelatin capsules to healthy volunteers. International Journal of Food Microbiology, 1995. 25: p. 199-203.

[4] Ventura , M. , Canchaya, C., Tauch , A., Chandra , G ., fitzgeralda, G.F., Cater, K.F. and Van Sideren, D., Genomics of Actinobacteria : Tracing the evolutionary history of an ancient phylum , Microbial . Mol Biol Rev., 2007. 71: p. 495 - 548.

[5] Kojima, T., Yaeshima , T., Ishibashi, N., Shimamura, S., Hayasawa, H., Inhibitory effects of human-drived bifidobacterium on pathogenic Escherichia coli serotype O-111. Bioscience Microflora, 1996. 15: p. 17 - 22.

[6] Namba, K.,Yaeshima , T., Ishibashi, N., Hayasawa, H., Yamazaki , S., Inhibitory of Bifidobacterium longum on inter hemorrhagic Escherichia coli O157: H7. Bioscience Microflora, 2003. 22: p. 85 - 91.

[7] Gibson, G.R.,Robertfroid, M.B., Dietary modulation of human colonic microbiota ; Introducing the concept of prebiotic J.Nutr ., 1995. 125 (6): p. 1401 - 12.

[8] De Vaux, A., Morrison, M., Hutkins, R.W., Displacement of Escherichia coli O157:H7 from rumen medium containing prebiotic sugars. Applied Environmental Microbiology, 2002. 68: p. 519 - 524.

[9] Al-Tamimi, M.A., Palframan, R.J., Cooper, J.M., Gibson, G.R., Rastall, R.A., In vitro fermentation of sugar beet arabinan and arabino-oligosaccharides by the human gut microflora. Journal of Applied Microbiology, 2006. 100(2): p. 407 - 414.

[10] Schrezenmeir, J., and De Vrese, M., Probiotics, prebiotics, and synbiotics – approaching a definition. Am J Clin Nutr., 2001. 73: p. 361S-364S.

[11] Collins, M.D. and Gibson, G.R., Probiotics, prebiotics, and synbiotics: approaches for modulation the microbial ecology of the gut. The American Journal of Clinical Nutrition, 1999. 69(5): p. 1052s–1057s.

[12] Desai, A.R., Powell, I.B. and shah, N.P., Survival and activity of probiotc in skim milk containing prebiotics. Journal of Food Science, 2004. 69: p. 57 - 60.

[13] Mahmoud, H.M., Assessment of Two Types of Gum Arabic as Probiotic. Ph.D.Thesis , Sudan university of science and technology, 2018. .

[14] Abdul –Hadi ,A.H.,Mahmoud .A.E and AbdelWahab .H.M., Effect of gum Arabic on coagulation system of albino rats. Int .J.Pharm.Tech.Res., 2010. 2: p. 1762-1766.

[15] Prado, F.C. , Parada ,J.L., Pandey, A., Soccol, C.R., Trends in non-dairy probiotic beverages.Food Research International, 2008. 41 : p. 111-123.

[16] Siro, I.N., Kápolna, E., Kápolna, B.T., Lugasi, A., Functional food. Product development, marketing and consumer acceptance-A review. Appetite, 2008. 51(3): p. 456-467.

[17] Blandino A., Al-Aseeri, M.E., Pandiella, S.S., Cantero, D., Webb, C., Cereal-based fermented foods and beverages. Food Research International, 2003. 36(6): p. 527-543.

[18] Davis, J.G., Cheese basic technology Vol .(1) .J. &A.Churchill Ltd. London,UK, 1965.

[19] Shreyansh J., Revant, G., and Shreya, J., Development of Low Cost Nutritional Beverage from Whey. Journal of Environmental Science, Toxicology and Food Technology, 2013. 5: p. 73 - 88.

[20] Marshall, K.R.,Harper .W.J., Whey protein concentrates. In. Dairy Fed. 388/B: 21-32., 1988.

[21] Blandino, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero, D., & Webb, C., Cereal-based fermented foods and beverages. Food research international, 2003. 36(6): p. 527-543.

[22] Kabeir ,B.M.,Abd Aziz ,S., Muhamed ,M.and Yazid ,A.M., Growth of Bifidobacterium longum BB536 in media (fermented cereal porridge) and their survival during storage. Let.AppI. Microbial., 2005. 141: P. 12 - 131.

[23] AOAC. Official Methods of Analysis. Arlington VA: Association of Official Analytical Chemists, Inc., 1990.

[24] MINITAB. User's Guide 2: MINITAB statistical software. MINITAB INC, USA, 2013.

[25] Shah, N.P., Effect of milk – derived bioactive: an over view .British Journal of Nutrition, 2000. 84: P. 3 -10.

[26] Stephenie, W., Kabeir, B., Shuhaimi , M., Rosfarizan, M., Yazid , A., Growth optimization of a probiotic candidate, Bifidobacterium pseudocatenulatum G4, in milk medium using response surface methodology. Biotechnol. bioproc. eng., 2007 . 12 : p. 106 -113.

[27] Janer, C., Pelaez , C., Requena , T., Caseinomacropeptide and whey protein concentrate enhance Bifidobacterium lactisgrowth in milk. Food chem., 2004 . 86: p. 263-267.

[28] Mohammadi ,R., Mortazavian, A.M., Khosrokhavar, R., da Cruz, A.G., Probiotic ice cream: viability of probiotic bacteria and sensory properties. Annals of microbiology, 2011. 61(3): p. 411-24.

[29] Nobakhti, A.R., Ehsani, M.R., Mousavi, S.M., Mortazavian, A.M., Influence of lactulose and Hi-maize addition on viability of probiotic microorganisms in freshly made synbiotic fermented milk drink. Milchwissenschaft, 2009. 64(2): p. 191 - 3.

[30] Gibson, G. R., Rabiu, B., Catherine, E., Rycroft, E., Rastall, R.A., Trans-Galactooligosaccharides as prebiotics. In: Shortt, C., J.O. Brien (eds) Handbook of Functional Dairy Products, CRC Press LLC USA, pp. 91-109, 2004.

[31] Rycroft, C.E., Jones, M,R,, Gibson, G,R,, Rastall, R,A., A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. Journal of applied microbiology, 2001. 91(5): p. 878-87.

[32] [32] Tamime, A. Y., Saarela, M. A. K. S., Sondergaard, A. K., Mistry, V. V., & Shah, N. P., Production and maintenance of viability of probiotic microorganisms in dairy products. Probiotic dairy products, 2005. 3: p. 39 - 63.

[33] Mortazavian, A. M., Khosrokhavar, R., Rastegar, H., & Mortazaei, G. R., Effects of dry matter standardization order on biochemical and microbiological characteristics of freshly made probiotic Doogh (Iranian fermented milk drink). Italian Journal of Food Science, 2010. 22(1):

[34] Mortazavian, A. M. and Sohrabvandi, S., Probiotics and food probiotic paroducts; based on dairy probiotic products. Tehran: Eta Publication, 2006.

[35] Elhassan, E.S., Formulation of fermented peanut milk millet Blend as a carrier for Bifidobacterium longum BB536 M.Sc. Thesis, Sudan University of Science and Technology, 2014.

[36] Elghali,S.,Mustafa,S.H.,Amid,M.,AbdManap,M.Y.,Ismail,A.,Abas.F., Variation on soya milk components during fermentation by Lactobacillus and a Bifidobacterium strains .Journal of food, Agriculture &Environment, 2014. 12 (2): p. 1-5.

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

[38] Goderska , K., Czarnecka, M. and Czarnecki, Z. Survival rate of chosen lactobacillus bacteria type in media of different pH. Food Sci.Technol., 2002. 5: p. 1 - 9.

[39] Samona, A., Robinson, R.K and Marakis, S., Acid production by bifidobacteria and youghurt fermentation and storage of milk. Food Microbiol., 1996. 13: p. 275 - 280.

[40] Osman , M.E., Menzies , A.R., Williams ,P.A.; Phillips ,G.O., Baldwin , T.c. The molecular characterization of the polysaccharide gum for Acacia Senegal. Carbohydrate Research, 1993 . 246: p. 303 - 318.

[41] Abou - Dobara, M., Ismail, M. M., Refat, .N M. Chemical Composition , Sensory evaluation and starter activity in cow ,soy, peanut and rice milk. American Journal of Applied and Industrial Chemistry, 2016. 2(4): p. 21-28.

[42] Vederola, C.G. and Reinhelmer, J., Survival of probiotic microflora in Argentinian yogurt during refrigerated storage. Food Research International, 2000. 33: p. 97 - 102.

[43] Akalin, A.S., Fendery ,S.A.K., and Bulut ,N., Viability and activity of bifidobacteria in yoghurt containing fructose oligo saccharine during refrigerated storage. International J.Food Sci.Technol., 2004 . 39:p. 613 - 621.

[44] Hernandez-Mendoza, A., Robles, V. J., Angulo, J. O., De La Cruz, J., & Garcia, H. S., Preparation of a whey-based probiotic product with Lactobacillus reuteri and Bifidobacterium bifidum. Food Technology and Biotechnology, 2007. 45(1): p. 27 - 31.

[45] Chou,C.C and Hou ,J.W., Growth of bifidobacteria in soy milk and their survival in the fermented drink during storage. Int J. Food Microbial., 2000. 6: p. 113-121.

[46] Tamime , A.Y., Robinson ,R.K., Yogurt : science and Technology .Pergamon Press Ltd .,Oxford ,UK, 1985.

[47] Modler, H.W., Mckeller ,R.C. and Yaguchi ,M., Bifidobacteria and bifidogenic factors .Canadian Institute of Food Science, 1990. 23: p. 29 - 41.

[48] Laroia, S., and Martin ,J.H.. Methods for enumerating and propagating bifidobacteria. Cultured dairy products Journal , 1991. 26: p. 32 - 33.