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EVALUATION OF PHYSICOCHEMICAL PROPERTIES AND GROWTH OF BIFIDOBACTERIUM LONGUM BB536 DURING FERMENTATION OF BARLEY BEVERAGES

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This study was carried out to evaluate growth and related physicochemical changes during fermentation of malted barley with Bifidobacterium longum BB536. Two Yamani barley varieties (Bukur, Balady) and another Sudanese one (Local 46) were evaluated in formulation of beverage. All barley grains were cleaned, soaked, germinated at temperature of 30±2 ºC for 120 hours. And then were dried at 55 ºC for 12 hours. 10% malted barley was blended for 5 mins at medium speed. Then the slurry was filtered by double layer cheese cloths, the obtain the barley beverages. On the other hand, 10% skim milk was used as a control. The barley and skim milk beverages were sterilized at 121 °C for 15 mins, then inoculated with 3% active culture of B. longum BB536, and fermented by incubation at 37 °C for 24 h. During the process, B. longum BB536 count was Increased significantly ($P<0.05$) by extended fermentation period. The highest level of growth took place at (18 h), B. longum BB536 might of growth in skim milk, Bukur, Balady and Local 46 were 8.13, 8.21, 8.08 and 8.14 log CFU/ml, respectively. At 24 h fermentation, the highest value count of skim milk, Bukur, Balady and Local 46 exceed the minimum number normal range of probiotic food which is at least 6 log CFU/ml fermented products. On the other hand, the pH and Total Soluble Solids (TSS) significant decreased ($P<0.05$) due to acid production during fermentation process. While the moisture content was significant increased ($P<0.05$) due to breakdown macro-component and release of water.

Keywords: *Bifidobacterium longum*, Physiochemical, Fermentation, Barley

INTRODUCTION

Cereals for use in beverage production are usually sprouted and dried in the process known as malting (Pyler and Thomas, 2000). This modifies the grains physically, chemically and biologically (Palmer, 2006). Desired changes such as hydrolysis of starch and protein in sugars and amino acids, respectively occurring in cereals used for the production of beverages and other cereal-based food have been widely studied (Uvere and Orji, 2002). The fermentation processes of these cereal foods involve lactic acid fermentation, which is performed by a number of complex populations of environmental microorganisms, which lends me a taste of stress and longevity storage. Species of *Lactobacillus* and *Bifidobacterium*, which are native to the human intestines, and are often chosen for use although some other species have also been used (Holzfel and Schlanger, 2002). The viability of these bacteria when swallowed and enough to survive through the gut is critical

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to give any health benefits to the host. Today most probiotics available are dairy based, but cereals can prove to be a healthier option for developing new non-diary probiotic foods since they can overcome some of the disadvantages associated with fermented dairy products like lactose intolerance, allergy and the impact in cholesterol levels (Prado *et al.*, 2008). Consumers have become more aware of health because of the wide availability of scientific evidence on various aspects related to food quality and safety. During the past decade, some studies have opened up a new area of research that addresses health-enhancing features of functional foods (Gobbetti *et al.*, 2010). Probiotics are viable, non-pathogenic microorganisms (bacteria and yeasts) that when ingested they are able to reach the intestines in sufficient number to grant the health benefits of the host (Rijkers *et al.*, 2011). Therefore, the objective of this study was evaluate growth of *Bifidobacterium longum* BB536 and related physicochemical changes during fermentation of malted barley based beverage.

MATERIAL AND METHODS

Malting of Barley

Varieties of barley used in this study were obtained from Yemen (Bukur and Balady and from Sudan (local 46. Malting of barley was carried out at Food Microbiology Laboratory, Collage of Agricultural studies, Sudan University of Science and Technology (SUST). Cleaned barley were washed and soaked in distilled water at ratio of (1: 3 w/v), using glass beaker at 30 ºC for 24 h. then water was renewed every 12 h. the barley grains were spread on aluminum foil and incubated for four days at 30 °C with interval spraying with under every 2 h. At the end of germination period, the grains were dried in an oven at 55 °C for 12 h, and the roots of the germinated barley were removed manuly (Badau, 2004).

Preparation of Barley Beverage Fermented

Barley beverage was prepared by the same method that reported by (kabeir *et al.*, 2009) with slight modification. The malted barley flours (10%) was mixed with water in a ratio of (1:4 w/v) and then transferred to a blender for 5 mins at medium speed. The slurry, which was formed because of blending, was filtered by using a double layer cheese cloth. The barley beverages were sterilized at 121 °C for 15 mins.

Preparation of Fermentation Inoculums

Bifidobacterium longum BB536 was obtained from the stock culture of Microbiology Laboratory (Department of Food Science Technology, Collage of Agriculture Studies, SUST). The strain was maintained at -20 °C in 20% glycerol solution. Stock culture was prepared by activation of the strain in skim milk, incubation anaerobically at 37 °C for 24 h. The obtained culture was reactivated again under the same conditions to prepare enough stock for the experiment. The working culture was prepared by twice success transformation in 10% sterilize skim milk (121 °C for 15 mins) and incubation at 37 °C for 24 h.

Growth Medium and Fermentation Conditions

The barley beverage Bukur, Balady, Local 46 and 10% skim milk (control) were inculcated with 3% active *Bifidobacterium longum* BB536 culture After that the mixture was incubated anaerobically at 37 ºC for 48 h to obtain the fermented beverage.

Enumeration of Viable Cell

De Man Rogosa Sharpe (MRS) agar medium was used to enumerate *B. longum* BB536 of different fermented beverages using the plate count technique. Fermented Samples were drawn at initial (0 h) and every 6 h intervals during fermentation. One ml of fermentation broth was spend diluted in peptone water, followed by plating on Rogosa agar (MRS) supplement with 0.05% L-cystiene. The plates were incubated an aerobically at 37 °C for 24 h. The growth was calculated as Colony Forming Unit per ml (CFU/ml).

Physico-Chemical Composition

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/CÚ 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 digital Refractometer with degree Brixº scale 0-100 according to AOAC (1990).

Determination of Titratable Acidity

The Titratable Acidity (TA) of the different fermented beverages was determined according to AOAC method (1990). Ten ml of sample were weighted into a conical flask.

Distilled water was added until the volume in the flask was 150 ml. The sample was then vigorously agitated and filtered. Twenty-five milliliters of the filtrate were pipette into a porcelain dish, five drops of phenolphthalein added, and the sample was titrated against 0.1 N NaOH till a faint pink color that lasted for at least 30 seconds was obtained. Acidity of different beverage samples was calculated from the following equation:

$$Titratable\ acidity = \frac{0.09 \times N\ of\ NaOH \times Titre\ Value\ of\ NaOH \times 100}{Weight\ of\ Sample}$$

where,

N = Normality of NaOH.

Determination of Moisture Content

Moisture content was determined according to the method of (AOAC, 1990). Five grams of each fermented beverage was weighted by sensitive balance, transferred to an oven at 105ºC for 6 h. Afterwards, the dish with dried samples were transferred to desiccators and allowed to cool at room temperature before re-weighing to calculate moisture.

Statistical Analysis

One way ANOVA and two sample-paired test were performed to examine significant differences between normally distributed data of triplicates independent runs. Probability level of less than 0.05 was considered significant ($p<0.05$). All data were analyzed using MINITAB statistical software for windows (MINITAB, 2006) version 18.

RESULTS AND DISCUSSION

Growth of *Bifidobacterium longum* BB536 During Fermentation of Different Barley Beverages

The results in Table 1 showed that there was significant increase ($p<0.05$) in *Bifidobacterium longum* BB536 value count during fermentation of different barley beverages. The maximum growth of *B. longum* BB536 was attained at 18 h in all types of fermented beverages, the maximum growth was obtained of samples skim milk, Bukur, Balady and Local 46 are 8.13, 8.19, 8.08 and 8.17 log CFU/ml, respectively. The rate of *B. longum* BB536 increased in different fermented beverages was 2.40, 2.50, 3.06 and 2.38% in fermented skim milk, Bukur, Balady and Local 46 respectively, in other words The high growth of *B. longum* BB536 in due to the special content of barley fermented beverage which is saccharides specifically monosaccharide's and disaccharides (Charalampopoulos *et al.*, 2002 and Rathore *et al.*, 2012). After that 18 h fermentation, the strain level declined in all types of fermented barley beverages. However, the reduction in growth after 18 h fermentation is mainly referred to the accumulation of acids or reduction of availability of nutrient required for the growth as stated by (Kabeir *et al.*, 2005). Similar maximam growths for other *Bifidobacterium* strains in peanut milk and skim milk suplemented with fructooligosaccharides a have amounted to about 8.39 CFU/ml after 24 h incubation as reported by Kebier *et al.* (2014). Also Wang *et al.* (2002) and Laine *et al.* (2003) reported that the growths of *Bifidobacterium* strains in soymilk and oat based medium have amounted to about 7 log CFU/ml after 24 h incubation. Other study by Arora *et al.* (2010) revealed that the number count 7.58×108 cfu/ml of *Lacidophilus* in fermented food mixture formulated from germinated barley flour. The international standard FIL/IDF describe that the probiotic products should be contained minimum of 10^6 viable probiotic bacteria per gram of product at the time of consumption for health and functional claiming (Samona and Robinson, 1991; and Roy, 2005). The viable cell levels in the final fermented beverages (10^8-10^9 cfu/ml) were above the minimum dose (10^6 cfu/ml) to maintain the intestinal population and to ensure that the consumer will derive health benefits (Ukwuru and Ohaegbu, 2018).

Changes in pH Levels During Fermentation of Different Barley Beverages

During fermentation process with strain *Bifidobacterium longum* BB536 there was significant ($P<0.05$) decrease in pH levels in all types of beverages by extended fermentation

Table 1: The Growth of Bifidobacterium Longum BB536 During Fermentation of Different Barley Beverages

Time (h)	Fermented Beverages			
	Re-constituted Skim Milk	Barley Varieties		
		Bukur	Balady	Local 46
Initial (0 h)	5.73±0.03^{e}	5.71±0.49^{d}	5.02±0.005^{e}	5.79±0.02^{e}
6	7.35±0.56^{c}	7.18±0.05^{b}	7.28±0.03^{c}	7.29±0.01^{d}
12	7.98±0.19^{b}	8.19±0.02^{a}	7.94±0.07^{b}	8.14±0.04^{b}
18	8.13±0.01^{a}	8.21±0.04^{a}	8.08±0.05^{a}	8.17±0.06^{a}
24	6.17±0.05^{d}	7.09±0.02^{c}	7.11±.07^{d}	7.67±0.54^{c}

Note: Mean ± SD; Values that bear different superscript letter in the same column are significantly different at $p<0.05$.

period to 24 h (Table 2). The initial pH of samples was 6.55, 5.52, 5.57 and 5.72 pH in fermented skim milk, Bukur, Balady and Local 46 respectively. The pH decrease at maximum growth (18h) of *Bifidobacterium longum* BB536 to 4.10, 3.57, 3.55 and 3.60 pH in fermented skim milk, Bukur, Balady and Local 46 respectively. The decreases in pH are due to increased acids production during fermentation process as a result of fermenting sugar by *Bifidobacterium longum* BB536, and production of acetic and lactic acids. Study by Hosseini *et al.* (2012) reported that the optimum pH for the growth of *Bifidobacterium* ranged between 6.5 and 7.0 pH. The pH of malted-roasted beverages was generally higher than that obtained for malt beverages from barley and oats (pH 4.01). The results agree with the results recorded by RozadaSánchez *et al.* (2008) on *Bifidobacterium spp*. they studied that production of a potentially probiotic malt-based beverage 14 hours of fermentation was the pH ranges between 4.30 and 4.10 pH. also, Buruleanu (2009) reported that pH value of the carrot juices was decreased from an initial value by 6.45 recorder value below 4.3 after 48 hours fermentation with *Bifidobacterium sp.* (Angelov *et al.*, 2005) revalued that the pH of a fermented beverage must be between 4 and 4.5 pH, due to fermentation with *Bifidobacterium* spp. for a period of 12-14 h.

Table 2: Changes in pH Levels During Fermentation of Different Barley Beverages

Time (h)	Fermented Beverages			
	Re-constituted Skim Milk	Barley Varieties		
		Bukur	Balady	Local 46
0	6.55±0.005^{a}	5.52±0.015^{a}	5.57±0.005^{a}	5.72±0.005^{a}
6	6.30±0.005^{b}	4.60±0.005^{b}	4.61±0.005^{b}	5.12±0.005^{b}
12	5.81±0.005^{c}	4.21±0.010^{c}	4.17±0.011^{c}	4.81±0.005^{c}
18	4.18±0.005^{d}	3.57±0.005^{d}	3.55±0.010^{d}	3.60±0.005^{d}
24	4.10±0.005^{e}	3.36±0.005^{e}	3.41±0.005^{e}	3.47±0.005^{d}

Note: Mean ± SD; Values that bear different superscript letter in the same column are significantly different at p<0.05.

Total Soluble Solid (TSS) Levels During Fermentation of Different Barley Beverages

Table 3 showed the changes in TSS during fermentation of different formulated beverages with *B. longum* BB536. There were significant (P<0.05) decrease in TSS levels in all types of fermented beverages by extended fermentation period to 24 h. The rates of TSS decreases at maximum growth were 2, 0.3, 1.07 and 1.47 in fermented of skim milk, Bukur, Balady and Local 46 respectively. The reduction in TSS could be due to enzymatic activity of the strain during fermentation process (Kabeir *et al*., 2005). A similar decrease in TSS during traditional microbial processing of Malwa beverage by fermentation was decaled (Muyanj *et al.*, 2010).

Table 3: Total Soluble Solid (TSS) Levels During Fermentation of Different Barley Beverages

Time (h)	Fermented Beverages			
	Re-constituted Skim Milk	Barley Varieties		
		Bukur	Balady	Local 46
0	7.9±0.10^{a}	4.3±0.10^{a}	4.06±0.05^{d}	5.5±0.10^{b}
6	6.06±0.11^{b}	3.9±0.100^{c}	5.46±0.05^{b}	6.1±0.10^{a}
12	5.9±0.10^{c}	4.06±0.05^{b}	6.13±0.05^{a}	4.03±0.05^{d}
18	5.9±0.10^{c}	4.00±0.10^{b}	5.13±0.05^{c}	4.03±0.20^{d}
24	5.56±0.20^{d}	3.43±0.11^{d}	5.16±0.15^{c}	4.06±0.05^{c}

Note: Mean ± SD; Values that bear different superscript letter in the same column are significantly different at p<0.05.

Total Acidity

Table 4 showed the Titratable Acidity of different fermented beverages. There were significant (p<0.05) increases in titrtable acidity by extended fermented period to 24 h. At maximum growth of strain BB536 (18 h), the rates of titratable acidity increase were 0.27, 0.23, 0.14 and 0.17% in fermented skim milk, Bukur, Balady and Local46 respectively. The

Table 4: Titratable Acidity (TTA) Levels During Fermentation of Different Barley Beverages

Time (h)	Fermented Beverages			
	Re-constituted Skim Milk	Barley Varieties		
		Bukur	Balady	Local 46
0	0.13±0.05^{e}	0.10±0.00^{e}	0.16±0.06^{d}	0.20±0.1^{e}
6	0.20±0.00a^{d}	0.13±0.0577^{d}	0.23±0.06^{c}	0.23±0.05^{d}
12	0.27±0.057^{c}	0.23.0.050^{c}	0.30±0.00^{b}	0.30±0.05^{c}
18	0.40±0.00^{b}	0.33±0.057^{a}	0.30±0.00^{b}	0.37±0.05^{b}
24	0.60±0.00^{a}	0.30±0.00^{b}	0.43±0.06^{a}	0.43±0.06^{a}

Note: Mean ± SD; Values that bear different superscript letter in the same column are significantly different at p<0.05.

Table 5: Moisture Content of Different Barley Fermented Beverages

Time (h)	Fermented Barley Beverages			
	Re-constituted Skim Milk	Bukur	Balady	Local 46
Initial	86.20±0.02[a]	86.22±0.005[a]	86.08±0.01[a]	86.21±0.05[a]
Maximum growth (18 h)	86.22±0.10[a]	86.28±0.01[b]	86.14±0.01[b]	86.45±0.29[b]

Note: Mean ± SD; Values that bear different superscript letter in the same column are significantly different at $p<0.05$.

increase in titrable acidity content in fermentation might be due to the production of organic acids by *Bifidobacterium longum* (Sefa Dedeh *et al.*, 2003). The increase in acidity causes decrease in the number of *Bifidobacterium spp*. growth when a pH 4.0-3.6 is reached. *Bifidobacterium* is less acid tolerant and its growth is retarded at highest pH of 5.0-4.5 (Shah, 2007).

Moisture Content of Different Barley Fermented Beverages

Table 5 showed the moisture content of barley fermented beverages with *B. longum* BB536 at initial (0 h) and maximum growth time (18 h). The result revealed significant ($p\leq0.05$) increases in moisture content of beverages between at maximum growth as compared to initial growth excepted for skim milk. The amount of moisture increases in fermented beverage included skim milk, Bukur, Balady and Local 46 beverages were 0.2, 0.8, 0.60 and 0.24%, respectively. This increase in moisture might indicate high enzymatic activity that break down the macro component into simple and release of water (Ibraheem *et al.*, 2015).

CONCLUSION

The results obtained in this study indicated significant ($p<0.05$) increases in viable numbers of *Bifidobacterium longum* BB 536 during fermentation of barley beverages. The maximum numbers fulfill the requirement of probiotic food. However, there were significant ($p<0.05$) decreases in pH and TSS. While moisture and acidity Titrabile were increased. The developed medium such as, malted barley beverage based is cheap and could contribute positively to deliver *Bifidobacterium longum* BB536, besides confer additional nutritional value to the diet.

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