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A safe None-alcoholic Banana Beverage Fermented with *Bifidobacterium longum* BB536

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Abstract

This study was carried out to develop safe probiotic fermented banana beverages. Different types of banana were peeled, 10% pulp was blended, cooked, cooled to room temperature, and malted rice flour was added and mixed well to obtain the beverage. Moreover, 10% skim milk was prepared to obtain the re-constituted skim milk. Beverages were sterilized (121ºC for 15 minutes), cooled (37ºC), and inoculated with 3% active *Bifidobacterium longum* BB 536 culture followed by incubation at 37 º C for 48 hours to obtain the fermented beverage. Fermented samples were drawn for analysis of *Bifidobacterium* viable cells, pH, TSS, moisture content, and microbiological safety. *Bifidobacterium longum* BB536 significantly ($p < 0.5$) increased by fermentation continuation in all beverages as compare to strain essential levels at the beginning of fermentation. The viable number of strain BB 536 at maximum growth was more than 6 log CFU/ml (the number required to fulfill probiotics product) in all fermented beverages; except ripe banana beverage and that may be due to its high starch content as compared with medium over ripe and over ripe banana. On the other hand, TSS significantly (0<-0.5) decreased due to metabolic activity of strain BB 536 and break down of some macro-components. During growth acids produced and that result in significant (0<-0.5) decreased of pH. While moisture significantly (0<-0.5) decreased by increase in strain biomass. Yeast and Mould, *Fecal Streptococcus, Staphylococcus Aurous,* and *E. coli*, were not found in all strain BB 536 fermented beverages. Moreover, salmonella was not detected in any of the fermented beverages. The absence of these pathogenic bacteria is due to the bifidogenic effect of strain BB 536. Therefore, formulation of safe strain BB 536 fermented banana beverage is successful.

Keywords: Banana, *Bifidobacterium*, growth, pH, TSS, moisture, Safety.

Introduction

Banana *(Musa sp.)* is one of the most important fruit crops in the world trade (FAO, 2007). In Sudan, banana is the most popular fruit; it is the cheapest, most available throughout the year, plentiful and most nourishing among fruits. It contains nearly all the essential nutrients,

including minerals and vitamins such as A, B_1, B_2 and C (Sugiura and Benedict, 1918). It is grown in almost every state, with annual production of 540 thousand metric tons (AOAD, 2008).

Nowadays, with consumers' health awareness and the need to intake foods low in fats, cholesterol and salt banana is a good choice. The low lipid and high energy contents make banana useful in low- fat diets. In addition the banana has a special place in feeding of obese patients and it is recommended for persons suffering from peptic ulcers (FAO, 1989).

Among popular industries in Sudan the fermentation process is considered as one of the most important methods of food preservation (Dirar, 1993). The fermented food may have an enhanced nutritional value, digestibility, better flavor, improved appearance, reduced cooking time, and good texture (Eka, 1980; Chavan *et al.,* 1988). On the other hand the high viscosity of cereal based beverage may hinder the growth of bacteria during fermentation. The addition of amylase enzyme or malted cereal grains including malted rice was found to be successful in reducing the viscosity or liquefy the beverage (flowing characteristics). Normally, conventional starter cultures are used for food fermentation. Nevertheless, utilization of friendly beneficial bacteria such as probiotics in food fermentation is widely encouraged (Kabeir *et al.,* 2005). Probiotics are live microorganisms that when ingested in adequate amount confer a health benefit to host (FAO/WHO, 2001).

Among probiotics, *Bifidobacterium longum BB536* is successfully approved strain it has been found mainly in human feces and it may be considered as the most common species *Bifidobacterium*, being found both in infant and adult. Potential benefits from consumption of *B. longum* include: antagonistic action toward intestinal pathogens, improved lactose utilization, anticarcinogenic action and control of serum cholesterol levels. Scientific studies showed the benefits offered by *Bifidobacterium longum BB536* (Kojima *et. al.,* 1996; Namba *et al*., 2003). Thus there is a considerable interest in incorporating this health's promoting *Bifidobacterium* strains into food.

Therefore, the objective of this study is to evaluate the growth of probiotic (*Bifid bacterium*) in banana beverage during the fermentation process and assess safety of fermented products.

Materials and methods

***Raw materials*:** Banana fruits at different stages of ripening during marketing, were purchased from Central Fruits and Vegetables markets in Khartoum North (Khartoum State, Sudan).Care was taken to collect ripe, medium over ripe , and over ripe banana. Skim milk and malted rice were obtained from Department of Food Science and Technology (College of Agricultural Studies, Sudan University of Science and Technology, Sudan).

Preparation of beverages: Banana were peeled, 10% pulp was blended using warring blender, cooked in hot plate for

10 min, cooled to room temperature and malted rice was added and mixed well to reduce viscosity and to obtain beverage with flowing characteristics. Moreover, 10% skim milk was prepared to obtain the re-constituted skim milk.

***Fermentation Inoculums*:** *B. longum* BB536 was obtained from the stock culture of microbiology laboratory (Department of Food Science and Technology, College of Agricultural studies, SUST, Sudan). The strain was maintained at – 20 ^{0}C in 20% glycerol solution.

Stock culture was prepared by activation of the strain in skim milk; incubated anaerobically at 37 ^{0}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 successive transformation in 10% sterilized skim milk (121 ^{0}C for 15 min) and incubation at 37 ^{0}C for 24 h.

***Fermentation process*:** The prepared banana beverage and re-constituted skim milk were sterilized by using autoclave (121ºC for 15 minutes). The sterilized mixture was cooled to 37 ºC and then inculcated with 3% *Bifdobacterum longum* BB 536 culture. After inoculation the mixture was incubated anaerobically at 37 ºC for 48 h to obtain the fermented beverage. Fermented sample were drawn at initial and six hours intervals for analysis.

Enumeration of viable cells: MRS medium are used to enumerate *B. longum* BB536 fermented as beverages using the plate count technique. Fermented samples were diluted in peptone water, followed by plating on MRS agar supplemented with 0.05 % L-cystiene. The plates were incubated anaerobically at 37 ^{0}C for 48 h. The growth was calculated as Colony Forming Unit per ml (CFU /MI)

***Determination of pH*:** The pH value of the different fermented beverage was determined using a pH-meter (model HI 8521 microprocessor bench PH/MV/Cº 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 samples were determined at room temperature using digital refractmeter with degree Brix^0 scale 0-100 according to AOAC (1990) method.

***Determination of moisture content*:** Moisture content was determined according to the method of AOAC (1990). Five grams of the sample was weight in sensitive balance, transferred to an oven (Kat-NR-2851, Electrohelios, Sweden) at 105 ^{0} C for 6 h. afterwards, the dishs with dried samples were transferred to desiccators and allowed to cool at room temperature before re-weighing to calculate moisture.

***Safety of fermented beverages*:** All fermented beverages were tested for different microorganisms including: yeast and Mould, *Fecal Streptococcus*, *Staphylococcus aureus*, *E. coli*, and

Salmonella following methods by (Harrigan, 1998).

***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 17.

Results and discussion

The growth of *Bifidobacterium longum* BB536 during fermentation of different banana beverages: The result in table 1 showed that there is significant ($P < 0.05$) increases in *Bifidobacterium longum* BB536 during fermentation of different banana beverages. The maximum growth of strain BB 536 was at 36 h of fermentation in all fermented beverages as compare to strain essential levels at the beginning of fermentation. The viable numbers of the strain at maximum growth in all fermented beverages was more than 6 log CFU/ml, the number required to presence in probiotic food (Vinderola *et al*., 2000); except ripe banana beverage where the maximum growth was 5.78±0.07 log CFU/ml. This low growth is due to high starch content of ripe banana as compared with medium over ripe and over ripe banana. The rates of increases were 3.39, 3.04, 6.28 and 3.03 log CFU/ml in re-constitutes skim milk, ripe banana beverage, medium over ripe banana beverage, over ripe banana beverage, respectively. The growth is due to availability of essential nutrients required for growth. However, the reduction in growth after 36h 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).

Table 1: The growth of *Bifidobacterium longum* BB536 during fermentation of different banana beverages

Time	Fermented beverages			
	Skim milk	Ripe banana	Medium over ripe	Over ripe banana
0	3.50± 0.02^{A}	2.748±0.02^{A}	2.65 ±0.04^{A}	2.75±0.01 ^{A}
6	3.64±0.02^{AB}	2.80±0.02^{A}	3.74±0.02^{A}	2.80±0.02 ^{B}
12	3.80±0.03^{B}	2.88±0.01^{A}	4.78±0.045^{B}	2.88±0.01 ^{C}
18	4.72±0.016^{C}	3.72±0.02 ^{B}	6.65 ±0.20^{C}	3.72±0.02 ^{C}
24	5.64±0.041^{D}	4.85±0.02 ^{B}	6.95±0.02^{C}	4.85±0.02 ^{D}
30	5.46 ±0.59^{E}	4.94±0.04 ^{C}	7.88±0.04^{D}	5.94±0.04 ^{E}
36	6.89± 0.017^{F}	5.78±0.07^{D}	8.93±0.02^{E}	6.78±0.07 ^{F}
42	6.73± 0.032^{F}	5.63±0.02^{D}	8.54±0.06^{E}	6.62± 0.02^{FG}
48	6.52±0.04 ^{G}	4.52±0.05^{D}	7.87±0.02^{D}	4.52± 0.05^{G}

* Values are mean ± SD for triplicates independent runs.

** 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 banana beverages: The result in table 2 Showed that there is a significant ($p < 0.05$)

decrease in TSS of different *Bifidobacterium longum* fermented beverages by continuation of fermentation process. The reduction in TSS could be due to enzymatic activity of the strain during fermentation process (Kabeir *et al*., 2005). The rate of decreases were 2.54, 3.25, 5.26 and 2.40 TSS in re-constituted skim milk, ripe banana beverage, medium over ripe banana beverage, over ripe banana beverage, respectively.

Table 2: Total Soluble Solid (TSS) levels during fermentation of different banana beverages

Time	Fermented beverages			
	Skim milk	Ripe banana	Medium over Ripe	Over Ripe banana
0	6.78± **0.08**^{A}	7.50 ±0.10^{A}	18.23±0.25^{A}	8.10±0.10^{A}
6	6.64 ± 0.04^{AB}	7.17 ±0.15^{B}	17.27±0.23^{A}	7.80 ± 0.10^{B}
12	6.42 ± 0.14^{B}	6.80±0.10^{C}	16.33±0.15^{B}	7.40± 0.10^{C}
18	6.40 ± 0.10^{C}	5.90±0.10^{D}	15.80±0.20^{B}	6.90±0.10^{D}
24	5.00 ± 0.10^{D}	5.60±0.10^{E}	15.00±0.20^{B}	6.50±0.10^{E}
30	4.70 ±0.10 ^{E}	4.78 ±0.08^{F}	13.97±0.25^{C}	6.10±0.10 ^{F}
36	4.24 ± 0.10^{F}	4.25±0.05^{FG}	12.97±0.15^{C}	5.70±0.10^{FG}
42	4.12 ±0.06^{F}	4.45±0.05^{GH}	13.30±0.10^{C}	5.95±0.05^{GH}
48	3.72 ± 0.08^{G}	4.60± 0.05^{H}	12.13±0.15^{C}	6.11±0.10^{H}

* Values are mean ± SD for triplicates independent runs.

** Values that bear different superscript letter in the same column are significantly different at p<0.05.

Moisture content of different banana fermented beverages

The result in table 3 Showed that there is a significant ($p < 0.05$) decrease in moisture of different banana fermented beverage at maximum *Bifidobacterium lonum* BB536 as compared to strain initial level at the beginning of fermentation. The reduction in moisture at maximum growth in all fermented beverages may be due to the increases in biomass of the strain BB 536. The rate of moisture decreases were1.53, 2.46, 2.17 and 2.1% in re-constituted skim milk, ripe banana beverage, medium over ripe banana beverage, over ripe banana beverage, respectively.

Table 3: Moisture content of different banana fermented beverages

Time	Fermented beverages			
	Skim milk	**Ripe banana**	**Medium over Ripe**	**Over Ripe banana**
Initial (0h)	75.19±0.05^{A}	81.07 ± 0.11^{A}	81.43±0.02^{A}	82.48 ± 0.02^{A}
Maximum growth (36)	73.66±0.29^{B}	79.45 ± 0.05^{B}	79.24±0.02^{B}	80.38 ± 0.03^{B}

* Values are mean ± SD for triplicates independent runs.

** Values that bear different superscript letter in the same column are significantly different at p<0.05.

Changes in pH levels during fermentation of different banana beverages: The result presented in table 4 Showed that there is significant ($p < 0.05$) decreases in pH of different *Bifidobacterium longum* BB 536 fermented beverages. The decreases in pH are due to increased acids production during fermentation process as a result of fermenting sugar by *Bifidobacterium BB536* as reported by De Vries *et al.* (1967). Bifidobacterium produces acetic and lactic acids during fermentation. The heights reductions of pH were 1.98, 1.67, 2.58 and 1.72 pH in re-constituted skim milk, ripe banana beverages, and medium over ripe banana beverage, over ripe banana beverage, respectively.

Table 4: Changes in pH levels during fermentation of different banana beverages

Time	Fermented beverages			
	Skim milk	Ripe	Medium over Ripe	Over Ripe
0	5.80 ± 0.10^{A}	6.45 ± 0.05^{A}	5.70 ± 0.10^{A}	6.80 ± 0.10^{A}
6	5.50 ± 0.10^{B}	6.24 ± 0.04^{B}	4.95 ± 0.05^{B}	6.50 ± 0.05^{B}
12	4.90 ± 0.10^{C}	6.08 ± 0.08^{B}	4.45 ± 0.05^{B}	6.22 ± 0.02^{C}
18	4.50 ± 0.10^{D}	5.78 ± 0.08^{C}	4.20 ± 0.10^{B}	5.92 ± 0.02^{D}
24	4.20 ± 0.10^{E}	5.49 ± 0.05^{D}	3.85±0.05C	5.71 ± 0.03^{E}
30	3.95 ± 0.06^{EF}	5.24 ± 0.04^{E}	3.25 ± 0.05^{D}	5.56 ± 0.03^{F}
36	3.82 ± 0.03^{EFG}	4.78 ± 0.07^{F}	3.12 ± 0.02^{D}	5.08 ± 0.07^{F}
42	3.93 ± 0.02^{FG}	4.91 ± 0.02^{FG}	3.15 ± 0.01^{D}	5.24 ± 0.04^{F}
48	4.08 ± 0.08^{G}	5.01 ± 0.08^{G}	3.32 ± 0.25^{D}	5.08 ± 0.07^{F}

* Values are mean ± SD for triplicates independent runs.

** Values that bear different superscript letter in the same column are significantly different at $p<0.05$.

Safety of different fermented banana beverages at maximum *Bifidobacterium longum* BB536 growth: The result presented in table 5 Showed that there is significant ($p < 0.05$) differences in total viable count of tested bacteria regarding safety of the different fermented products including re-constituted skim milk, ripe banana beverage, medium over ripe banana beverage, over ripe banana beverage. Yeast and Mould, *Fecal Streptococcus*, *Staphylococcus Aurous*, *E. coli*, and Salmonella were not found or detected in any of the developed fermented products. The absence of these pathogenic bacteria is due to the bifidogenic effect of strain BB 536 even may be after post fermentation contamination. The strain is reported to produce antibiotic like components against the pathogenic bacterial strains. Therefore, the total viable count of different products represents the *Bifidobacterium* of the products.

Table 5: Safety of different fermented banana beverages at maximum *Bifidobacterium longum* BB536 growth

Types of fermente	Types of tested microorganisms						
	Total viable count of	Yeast and Mould	*Fecal Streptococcus*	*Staphylococcus Aurous* CFU/ml	Coliforms (MPN)	*E. Coli* CFU/ml	Salmonella

d beverages	Bacteria CFU/ml	CFU/ml					
Skim milk	6.33 ±0.14[A]	NF	NF	NF	NF	NF	ND
Ripe banana	6.11±0.05[B]	NF	NF	NF	NF	NF	ND
Medium over ripe	7.99±0.37[C]	NF	NF	NF	NF	NF	ND
Over Ripe banana	6.39 ±0.90[D]	NF	NF	NF	NF	NF	ND

* Values are mean ± SD for triplicates independent runs.

** Values that bear different superscript letter in the same column are significantly different at $p<0.05$.

NF = Not Found

ND = Not Detected

Conclusion

The results obtained in this study indicated significant ($p < 0.05$) increases in viable numbers of the strain BB 536 during fermentation of all banana beverages. The maximum numbers fulfill the requirement of probiotic food. However, there were significant ($p < 0.05$) decreases in TSS, pH, and moisture during fermentation of different beverages due to strain BB 536 metabolic activities. On the other hand, all fermented beverage were safe and free from yeast and Mould, *Fecal Streptococcus*, *Staphylococcus Aurous*, *E. Coli*, and Salmonella due to the bifidogenic affect of strain BB 536 even after post fermentation contamination. Therefore, production of a safe banana fermented beverage with strain BB 536 is successful.

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