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Probitication of Over Ripe Mango Beverages by Fermentation with *Bifidobacterium Longum* BB536

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ABSTRACT

This study was carried out for probitication (addition of probiotic) to over ripe mango beverage with probiotic *Bifidobacterium longum BB536* and evaluates related physicochemical changes during fermentation. Two mango varieties (Baladi and Alphonso) were used in formulation of beverage. On the other hand, re-constituted skim milk was used as a control. The over ripe mango and skim milk beverages were sterilized at 121 °C for 15 minutes, 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 strain BB536 was attained 18 h fermentation; amounting 8.91, 8.74,7.92,7.87, and 7.66 log CFU/ml in re-constituted skim milk and Alphonso mix, skim milk, Alphonso, re-constituted skim milk and baladi mix, and Baladi, , respectively. These high viable count values of strain BB536 exceed the minimum number of probiotic required to presence in foods; 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 acidity production during fermentation process. While the moisture content was significantly ($P<0.05$) increased due to breakdown of macro-component and release of water. Therefore, probotication of over ripe mango beverages (Baladi and Alphonso) were successful. They are suitable medium to deliver strain B536 to consumers at high levels, comparable to that of the re-constituted skim milk (The control) the main carrier of probiotics.

Keywords: **Probitication,** *Bifidobacterium*, Fermentation, Physiochemical, Mango.

INTRODUCTION

The Mango (Mangiferaindica L.) is the most economically important fruit in the *Anacardiaceae* (family). In Sudan, mango is the most popular fruit; it is the cheapest, plentiful and most nourishing among fruits. Mango is good for consumers who are interested in diet, health, fitness and natural food because it is nutritional value considered high. Mango contains energy-giving fructose which is low on the glycaemic index, and high on dietary fiber, minerals and vitamins including vitamin A and C and traces of B-complex.

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 (Chavan *et.al.*, 1988; Dirar, 1993). Normally, conventional starter cultures are used for food fermentation. Nevertheless, utilization of

friendly beneficial bacteria such as probiotics in Sudanese food fermentation is widely encouraged (Kabier et al., 2005). Probiotics are live microorganisms that when ingested in adequate amount confer a health benefit to host (FAO / WHO, 2001).

Among probiotics, *Bifidobacteriumlongum* BB536 is successfully approved strain. It has been found mainly in human faeces and it may be considered as the most common species of *bifidobacteria*, 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 *B. longum BB536*(Kojima et al., 1996; Nambak *et al*., 2003). Thus there is a considerable interest in incorporating this health's promoting bifidobacteria into food. 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).

A wide range of foods have been fermented or enriched with probiotics as possible carriers of these beneficial microorganisms and successfully placed on the market. Several species of *Lactobacillus* and *Bifidobacterium* have become the most commonly used probiotic strains in these food products. Moreover other potential Probiotic such as *Saccharomyces cerevisiae* (boulardii), *Enterococcus*, *Bacillus*, and *Escherichia* are also applied (Perricone *et. al*., 2015; Holzapfel *et. al*., 1998).

Today most probiotics available are dairy based, but other sources were introduced such as cereals to overcome some of the disadvantages associated with fermented dairy products like lactose intolerance, allergy and the impact in cholesterol levels (Prado *et. al*., 2008). Nowadays, consumers have become more aware of health because of the wide availability of scientific evidences on various aspects related to food quality and safety (Gobbetti *et. al*., 2010). Although the proper handling techniques, reduction of post-harvest losses, delaying of fruit ripening and extending the shelf-life are crucial for the development of sound mango industry in Sudan, most of the losses occur at the marketing stage as a result of over ripening. Therefore, the objective of this study was to evaluate successful incorporation of *Bifidobacterium longum BB536* into over ripe mango beverage and assess related physicochemical changes during fermentation.

MATERIAL AND METHODS

Raw Material

Over ripe mango fruits were purchased from Central Fruits and Vegetables markets in Khartoum North (Khartoum State, Sudan). Skim milk was obtained from Department of Animal Production (College of Agricultural Studies, Sudan University of Science and Technology, Sudan.

Preparation of Mango Beverage

Sound over ripe mango fruits were first selected, weighted, sorted, and then thoroughly washed under running water. Cleaned fruits were peeled, sliced by a sharp clean stainless steel knife. The slices were blended with water in ratio of (1:4 w/v) for 5 minutes at medium speed using an electric blender. The slurry was filtered using a double layer cheese cloth to obtain mango beverage, which were sterilized at 121 °C for 15 minutes for fermentation purpose.

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 and

incubation anaerobically at 37 °C for 24h. 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 minutes and incubation at 37 °C for 24 h.

Growth Medium and Fermentation Conditions

The sterilized over ripe mango beverages, Baladi, Alphonso, re-constituted skim milk and baladi mix, re-constituted skim milk and Alphonso mix and 10% re-constituted skim milk (control) were inculcated with 3% active *Bifidobacterium longum*BB536 culture After that the mixure was incubated anaerobically at 37 ºC for 24 h to obtain the fermented beverage.

Enumeration of *B.longum* BB536 **Viable Count**

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 fermented beverage was 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 viable count was calculated as Colony Forming Unit per ml (CFU/ml).

Determination of pH Value

The pH value of the different fermented beverages was determined using a pH-meter (model HI 8521 microprocessorbench 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 was determined at room temperature using digital Refractometer with degree Brixº scale 0-100 according to AOAC (1990) method.

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 was150 ml. The sample was then vigorously agitated and filtered. Twenty-five milliliters of the filtrate were pipette into aporcelain 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. Then acidity of different beverages samples were calculated.

Determination of Moisture Content

Moisture content was determined according to the method of AOAC (1990). Five grams of each 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 being re-weighed to calculate moisture.

Statistical Analysis

One way ANOVA was 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

Probotications of different over ripe mango beverages with *Bifidobacterium longum* BB536

The results in Table1 showed that there were significant increases ($p<0.05$) in *Bifidobacterium longum* BB536 viable counts during fermentation of different mango beverages. The maximum growth of *B. longum* BB536 was attained at 18 h in all types of fermented beverages. The maximum growth obtained in re-constituted skim milk and Alphonso mix, re-constituted skim milk, Alphonso, re-constituted skim milk and baladi mix and Baladi beverages were 8.91, 8.74,7.92, 7.87, and 7.66 log CFU/ml, respectively. The rates of *B. longum*BB536 increased in different fermented beverages were 6.05, 5.95, 5.25, 5.14, and 5.12% in fermented re-constituted skim milk and Alphonso mix, re-constituted skim milk, Alphonso, re-constituted skim milk and baladi mix and Baladi, respectively. The highest of growth *B. longum* BB536 was recorded for Alphonso, and re-constituted skim milk and Alphonso mix due to their high total sulible solid (TSS) revealed in Table 3. In this respect, mango was reported to contain sugars specifically monosaccharide's and disaccharides (Charalampopoulos *et. al.*, 2002 and Rathore *et al.*, 2012). On the other hand, after 18 h fermentation (maximum growth), the strain declined in all types of fermented mango 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). Higher maximum growths for other *Bifidobacterium* strains was in peanut milk and re-constituted skim milk supplemented with fructooligosaccharides amounted 8.39 logCFU/ml after 24h incubation reported as stated by Kebier *et. al.* (2014). However, Wang *et. al.* (2002) and Laine *et al.* (2003) reported lower growths of *Bifidobacterium* strains in soymilk and oat based medium amounted about 7 log CFU/ml after24 h incubation. Other study by Arora *et al.* (2003) revealed viable number count of 7.58×10^8 log cfu/ml *Lacidophilus* in fermented food mixture formulated from mango. The international standard FIL/IDF describe that the probiotic products should contain 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 total strain BB536 viable counts in the final fermented over ripe mango beverages was in range of 10^8 to10^9cfu/ml.; which were above the minimum level of 10^6cfu/ml required to maintain the intestinal balance population and to ensure that the consumer will derive health benefits (Ukwuru and Ohaegbu, 2018). Therefore, probitications of mango beverages via fermentation was successful.

Table1: **Probitication of different over ripe mango beverages with *Bifidobacterium longum BB536* (log CFU/ml) via fermentation**

Period (h)	Fermented beverages				
	Baladi 100%	**Alphonso 100%**	**Re-constituted skim milk and Baladi mix**	**Re-constituted skim milk and Alphonso mix**	**re-constituted Skim milk 100% (control)**
initial	2.54±0.04 ^{e}	2.67±0.03 ^{e}	2.73±0.01 ^{e}	2.86±0.01 ^{e}	2.79±0.02 ^{e}
6	4.82±0.01 ^{d}	5.77±0.05 ^{d}	4.89±0.05 ^{d}	5.75±0.02 ^{d}	5.85±0.01 ^{d}
12	6.85±0.06 ^{b}	7.61±0.02 ^{b}	5.91±0.04 ^{c}	6.85±0.03 ^{c}	7.92±0.00 ^{b}
18	7.66±0.03 ^{a}	7.92±0.00 ^{a}	7.87±0.02 ^{a}	8.91±0.04 ^{a}	8.74±0.03 ^{a}
24	6.61±0.02 ^{c}	6.86±0.01 ^{c}	6.82±0.03 ^{b}	7.88±0.01 ^{b}	7.65±0.04 ^{c}

* 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.
Re-constituted skim milk and Baladi mix = 50% Baladi beverage + 50% re-constituted skim milk.
Re-constituted skim milk and Alphonso mix = 50% Alphonso beverage +50% re-constituted skim milk.

Changes in pH levels during fermentation of different over ripe mango beverages

The initial pH of beverages was lower due to acidity of fruits; however, mixing with re-constituted skim milk slightly increased the pH level (Table 2). During fermentation process with strain *Bifidobacterium longumBB536* there was significant (P<0.05) decrease in pH levels of all types of beverages by extended fermentation period to 24 h. The decreases in pH are due to increased acids production during fermentation process as a result of fermentation of sugar by *Bifidobacterium longum* BB536, and production of acetic and lactic acids. Study by Rozada-Sánchez *et al.* (2008) on *Bifidobacterium spp*. reported that in production of a potentially probiotic malt-based beverage for 14 hours' fermentation the pH ranged between 4.30 and 4.10. Buruleanu (2009) also found that pH value of the carrot juices was decreased from an initial value of 6.45 below 4.3 after 48 hours' fermentation with *Bifidobacterium sp.* Angelov *et al. (*2005) also revalued that the pH of a fermented beverage ranged between 4 and 4.5.

Table2: Changes in pH levels during fermentation of different over ripe mango beverages

Period (h)	Fermented beverages				
	Baladi 100%	Alphonso 100%	Re-constituted skim milk and Baladi mix	Re-constituted skim milk and Alphonso mix	re-constitutedSkim milk 100% (control)
initial	4.08±0.01 [a]	4.04±0.04 [a]	4.61±0.06 [a]	4.80±0.05 [a]	5.29±0.05 [a]
6	4.02±0.03 [a]	3.93±0.07 [b]	4.50±0.05 [b]	4.47±0.03 [b]	5.03±0.03 [b]
12	4.04±0.04 [a]	3.91±0.05 [b]	4.30±0.04 [c]	4.31±0.02 [c]	4.80±0.01 [c]
18	3.82±0.06 [b]	3.90±0.01 [b]	4.26±0.03 [c d]	4.22±0.06 [d]	4.70±0.02 [d]
24	3.55±0.05 [c]	3.83±0.02 [c]	4.21±0.01 [d]	4.12±0.03 [e]	4.63±0.07 [e]

* 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.
Re-constituted skim milk and Baladi mix = 50% Baladi beverage + 50% re-constituted skim milk.
Re-constituted skim milk and Alphonso mix = 50% Alphonso beverage +50% re-constituted skim milk.

Total soluble solid (TSS) levels during fermentation of different over ripe mango beverages

Table 3 showed the changes in TSS during fermentation of different formulated beverages with *B. longum* BB536. There were significant (P<0.05) decreases in TSS levels in all types of fermented beverages by extended fermentation period. The rates of TSS decreases at maximum growth were 8.00, 5.85, 4.50, 3.04 and 3.02 in fermented Alphonso, Baladi, re-constituted skim milk, baladi mix and Alphonso mix, 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 stated by Muyanj *et al.* (2010).

Table 3: Total soluble solid (TSS) (%) levels during fermentation of different over ripe mango beverages

Period (h)	Fermented beverages				
	Baladi 100%	Alphonso 100%	Re-constituted skim milk and Baladi mix	Re-constituted skim milk and Alphonso mix	re-constituted skim milk 100%(control)
initial	7.00±0.04 [a]	9.00±0.10 [a]	6.00±0.03 [a]	5.05±0.05 [a]	5.50±0.10 [a]
6	6.50±0.10 [b]	8.36±0.05 [b]	5.05±0.05 [b]	4.48±0.08 [b]	5.33±0.31 [a]
12	6.00±0.05 [c]	8.30±0.03 [c]	4.02±0.08 [c]	4.02±0.08 [c]	4.80±0.04 [b]
18	5.85±0.02 [c]	8.00±0.06 [d]	3.04±0.04 [d]	3.02±0.03 [d]	4.50±0.05 [b c]
24	5.15±0.13 [d]	7.50±0.04 [e]	2.80±0.02 [e]	2.82±0.02 [e]	3.22±0.08 [c]

* 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.
Re-constituted skim milk and Baladi mix = 50% Baladi beverage + 50% re-constituted skim milk.
Re-constituted skim milk and Alphonso mix = 50% Alphonso beverage +50% re-constituted skim milk.

Titerable acidity of different fermented over ripe mango beverages

As presented in Table 4, there were significant (p<0.05) increases in titratable acidity by extended fermentation period of mango beverages to 24 h. At maximum growth of strain BB536 (18 h), the rates of titratable acidity increase were 0.05, 0.04, 0.03, and 0.02 in fermented re-constituted skim milk, Baladi, Alphonso, Alphonso mixure, and baladi mixure respectively. The increases in acidity are due to fermentation of sugar by *Bifidobacterium longum* BB536, and production of acetic and lactic acids (SefaDedeh *et al.*, 2003). The increase in acidity causes decrease in the number of strain BB 536 growth because all *Bifidobacterium spp.* are less acid tolerant (Shah, 2007).

Table 4: **Titerable acidity (%) of different fermented over ripe mango beverages**
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Period (h)	Fermented beverages				
	Baladi 100%	Alphonso 100%	Re-constituted skim milk and Baladi mix	Re-constituted skim milk and Alphonso mix	re-constituted Skim milk 100% (control)
initial	0.29±0.01 [a]	0.35±0.12 [a]	0.31±0.01 [a]	0.28±0.02 [a]	0.35±0.09 [a]
6	0.30±0.02 [a]	0.36±0.02 [b]	0.32±0.02 [a b]	0.30±0.03 [b c]	0.36±0.12 [b]
12	0.31±0.06 [a]	0.37±0.04 [b]	0.32±0.07 [b]	0.32±0.11 [b c]	0.38±0.10 [c]
18	0.33±0.03 [a]	0.38±0.10 [b]	0.33±0.13 [b]	0.33±0.04 [b]	0.39±0.05 [d]
24	0.32±0.05 [a]	0.36±0.11 [b]	0.33±0.06 [b]	0.31±0.05 [c]	0.38±0.02 [d]

* 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.
Re-constituted skim milk and Baladi mix = 50% Baladi beverage + 50% re-constituted skim milk.
Re-constituted skim milk and Alphonso mix = 50% Alphonso beverage +50% re-constituted skim milk.

Moisture content of different fermented over ripe mango beverages

Table 5 showed the moisture content of over ripe mango fermented beverages. The result revealed significant (p<0.05) increases in moisture content of beverages by extend fermentation period to 24h. The rate of moisture increases were 1.00, 1.40, 2.40, 3.00 and 3.53% in fermented Baladi, Alphonso, baladi mixure, Alphonso mixure and re-constituted skim milk, 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) or as a result of reduction of other components of beverages.

Table 5: Moisture (%) content of different fermented over ripe mango beverages

Period (h)	Fermented beverages				
	Baladi 100%	Alphonso 100%	Re-constituted skim milk and Baladi mix	Re-constituted skim milk and Alphonso mix	re-constitutedSkim milk 100% (control)
Initial	79.60±0.10 ^{b}	81.00±1.00 ^{b}	86.00±0.01 ^{b}	85.00±0.29 $^{b c}$	89.00±0.13 ^{b}
6	79.70±0.04 ^{b}	82.50±0.09 $^{a b}$	88.00±0.03 ^{b}	84.00±1.14 ^{b}	90.60±0.04 ^{b}
12	80.10±0.06 ^{b}	82.70±0.07 $^{a b}$	89.00±0.05 ^{a}	86.00±0.06 $^{a b c}$	90.00±0.12 ^{b}
18	81.00±1.00 $^{a b}$	84.00±0.51 ^{a}	89.53±0.07 ^{a}	87.40±0.10 $^{a b}$	90.00±0.05 ^{b}
24	82.00±1.02 ^{a}	83.00±0.06 $^{a b}$	89.60±0.10 ^{a}	88.00±1.00 ^{a}	90.70±0.20 ^{a}

* Values are mean ± SD for triplicates independent runs.
** Values that bear different superscript letter in the same column are significantly different at $p<0.05$.
Re-constituted skim milk and Baladi mix = 50% Baladi beverage + 50% re-constituted skim milk.
Re-constituted skim milk and Alphonso mix = 50% Alphonso beverage +50% re-constituted skim milk.

CONCLUSION

The results obtained in this study indicated significant ($p<0.05$) increases in viable numbers of *Bifidobacterium longum* BB 536 during fermentation of over ripe mango beverages. The attained maximum viable numbers of strain BB536 fulfilled the requirement of probiotic food. Fermentation processes of over ripe mango beverages were also revealed significant ($p<0.05$) decreases in pH and TSS; whereas moisture and acidity were increased. Therefore, probotication of over ripe mango beverages (Baladi and Alphonso) were successful. They are suitable medium to deliver strain B536 to consumers, besides conferring additional nutritional value to the diet.

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