



The Survival of *Bifidobacterium infantis* 20088 and Physicochemical Changes During Refrigeration Storage of Selected Fermented Traditional Sudanese Fruit Beverages

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ARTICLE INFO

Article history:

Received 17 May 2015

Accepted 06 September 2015

Available online, ISSN: 2148-127X

Keywords:

Dom

Gudaim

Fermentation

Bifidobacterium

Survival

Refrigeration

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ABSTRACT

This study was carried out to evaluate survival of *Bifidobacterium infantis* 20088 in fermented beverage formulated from selected traditional Sudanese fruits Gudaim and dom. The viable counts of the strain and physicochemical changes during refrigeration were determined. 10% beverages (w/v) were prepared from dom and gudaim powder. 2.5% (w/w) skim milk was supplemented to each formulation to provide the required nutrient for bacteria growth during the fermentation. After sterilization and cooling, the mixture was inoculated with a 10% culture of *B. infantis* 20088 followed by incubation for 36 h at 37°C. Reconstituted skim milk was used as control. Fermented beverages were held at refrigeration (4°C) for a period of 2 weeks. During the refrigeration storage of the fermented beverages there was significant reduction in *Bifidobacterium infantis* of all fermented beverages. Nevertheless, the strain was maintained high; fulfill the number required to presence in probiotic foods, which was at least 6 log CFU/ml fermented product. There was no significantly difference in TSS and pH as compared to their initial at the beginning of fermentation except in pH of fermented gudaim. Therefore gudaim and dom are suitable carrier to deliver *Bifidobacterium infantis* 20088 to consumer.

Introduction

Sudan is one of the important country in Africa which include different ecological zones rich in rational fruits such as Aradaib (*Tamarindus indica*), Dom (*Hyphaene thebaica*), Gunguleiz (*Adansonia digitata*), Lalob (*Balanites aegyptiaca*), Nabak (*Ziziphus spina-Christi*), Gudaim (*Grewia tenax*). Their utilizations did not go beyond small scale fresh beverages. Fermentation of Sudanese traditional fruits with probiotics and maintain their numbers high during storage, will lead to develop fermented beverages with further enhanced value and improved therapeutic properties. In addition there has been a considerable interest to enhance the survivability of probiotic bacteria that are added in food products (Ziemer and Gibson 1998).

Probiotic bacterium is defined as a living microorganism which when consumed in sufficient number will improve health beyond inherent basic nutrition (Schrezenmeir and de Vrese as, 2001). Strain of *Bifidobacterium*, *Lactobacillus* and non pathogenic yeast such as *Saccharomyces boulardii* are principally used individually or in combination as probiotics (Tomasik and Tomasik, 2003). However, most human origin probiotics are fastidious when used alone; they are characterized by low growth capability in food mediums (FAO/WHO, 2001). Because of the difficulty in maintaining a probiotic

in food carriers for long time, significant research has been focused in discovering new suitable carriers besides milk. Therefore, this study was carried out to examine the survivability of *Bifidobacterium infantis* during refrigeration storage of the fermented traditional Sudanese fruit beverages.

Materials and Methods

Preparation of Tradition Fruits

Dom (*Hyphaenethebaica*) and Gudaim (*Grewia tenax*) were purchased from a local market in Khartoum town randomly without grouping according to their ripeness. The fruits were cleaned by removing the dirt and foreign materials. Dom seed was separated directly from its pulp. While Gudaim fruits were used without separation of seed. The collected pulps of fruits were ground into powder to pass through 250 micron sieve.

Preparation of Fermentation Inoculums

B. infantis was obtained from the stock culture collection of microbiology laboratory (Department of Food Science Technology, Collage of Agriculture Studies, Sudan University of Science and Technology, Sudan). The strain stock was maintained at -20°C in glycerol solution. A working culture was prepared by

activation of the strain in MRS broth, incubated under anaerobic condition at 37°C for 48h. The obtained broth activated again under the same condition to prepare enough stock for the experiment. The working culture was prepared by twice successive transformation in 10% sterilized skim milk (121°C for 15 min) and incubation at 37 C for two days.

Growth Medium and Fermentation Conditions

Gudaim and dom were used for fermentation because they contained the highest level of fiber beside considerable level of sugar (glucose + fructose) which are the most preferable for microorganism. 10% beverages were prepared from dom and gudaim powder. 2.5% (w/w) skim milk was supplemented to each formulation to provide the required nutrient for bacteria growth during the fermentation. After sterilization and cooling, pH of the mixture was adjusted to 6.8 by calcium bicarbonate. The sterilized mixture was inoculated with a 10% culture of *B. infantis* followed by incubation at 37°C to a maximum growth (36h).

The Storage

Fermented beverages were held at 4°C for a period of 2 weeks. During the storage period, viable counts of *B. infantis*, pH, and TSS of the fermented beverages were determined. The colonies that appeared on the plates were then counted. Samples were collected at initial (0 day), one week and after two weeks of storage.

pH and Total Soluble Solids (TSS) Measurements

The pH level during fermentation was determined using a pH meter (Jenway model 351). While the total soluble solid (TSS) was determined using Erma model refractometer.

Enumeration of Viable Cell

MRS was used to enumerate *B. Infantis* of fermented beverages using the plate count technique. Samples were drawn at initial and week intervals during fermentation. 1ml of fermentation broth was diluted in peptone water, followed by plating on Rogosa agar (MRS). The plates were incubated anaerobically at 37°C for 48 h. Growth was calculated as Colony Forming Unit (CFU/ml).

Statistical Analysis

One- way analysis of variance (ANOVA) was performed to examine significant differences between normally distributed data. Tukey test was used to perform multiple comparisons between means. Probability level of less than 0.05 was considered significant ($P < 0.05$). All data were analyzed using MINITAB statistical software (2006) for windows.

Results and Discussion

*Survival of *B. infantis* during refrigeration storage of the fermented beverages*

Table 1 shows the viable counts of *B. infantis* during refrigeration storage. *B. infantis* survived for two week above the required population levels of at least 10^6 CFU/ml in fermented gudaim, dom and skim milk.

The viable number of *B. infantis* in fermented skim milk did not decreased significantly at the end of the second week refrigeration storage. However, the number of the *B. infantis* decreased significantly ($P < 0.05$) at the end of the second week refrigeration storage in both fermented dom and gudaim. Even though, the strain still at a level complying with the standard population requirement for probiotic product to benefit the host. In previous investigation Kabeir et al. (2005) reported that survivability of *B. longum* BB536 under refrigeration storage of fermented Sudanese Medida beverage was not affected for a period of 2 week refrigeration storage. While Akalin et al. (2004) noted a significant reduction on *B. longum* BB46 in yogurt after only one week refrigeration. This indicates that the viability of *Bifidobacterium* in fermented products was dependent on the carrier types and pH of the fermented products during the storage. The maximum *B. infantis* reduction during refrigeration storage was in fermented gudaim. That was correlated well with lowest pH in fermented gudaim. The preservation of any fermented product depends on the temperature of storage. Therefore refrigeration temperature is important to maintain the survivability of *Bifidobacterium* (McMaster et al., 2005) in fermented products.

Reduction of pH During The Storage of Fermented Beverages

Table 2 shows the pH measurement of the fermented traditional fruit beverages during the two week refrigeration storage. There were no significant ($P < 0.05$) changes observed on pH measurement in fermented dom and skim milk during the period of refrigeration storage. However, there was significant ($P < 0.05$) reduction in pH of fermented gudaim. The rates of pH reduction in fermented dom, skim milk and gudaim were 0.05, 0.20, and 0.40, respectively. The combination of low pH due to accumulated acids would result in increasing levels of undissociated acid, which is more harmful to microorganisms and that is a clear factor in the rapidly decreasing bifidobacterial population towards the end of shelf life (Adams & Moss 2000). Sakai and coworkers reported that low pH and storage temperature were the most important factor in *Bifidobacterium* mortality (Sakai et al., 1987). It was reported that the viability of *Bifidobacterium* in fermented milk seriously affected by any drop in pH below 4.3 (Lankaputhra et al., 1996).

Changes in TSS During The Storage of Fermented Beverages under Refrigeration Storages

Table 3 shows TSS of the traditional fermented fruit beverages. Initial TSS of fermented dom and skim milk decreased by storages period, while that for gudaim tend to increase by extend refrigeration. The amounts of reduction in TSS were 0.05 and 0.60 in fermented skim milk and dom. Whereas in fermented gudaim the TSS increase by 0.10%. Over all levels of TSS after two weeks refrigeration storage of each fermented traditional Sudanese fruit beverage did no differ significantly ($P < 0.05$) as compared to their initial at the beginning of fermentation.

Table 1 Viable count of *B. infantis* (CFU/ml) in fermented dom and gudaim during refrigeration storage*

Type FB	FR	After 1 week	After 2 week
Dom	8.3×10 ^{8a}	1.25×10 ^{8b}	7.5×10 ^{7b}
Gudaim	2.40×10 ^{9a}	9.0×10 ^{8b}	5.0×10 ^{8c}
Skim milk	7.0×10 ^{8a}	3.8×10 ^{8b}	6.3×10 ^{6a}

*Type FB: Type of fermented beverages; FR: First reading; Values are mean± STD of duplicate independent runs; Different superscript letters in the same row indicate significant (P<0.05) differences between means

Table 2 pH of the fermented dom and gudaim beverages during refrigeration storages

Type FB	FR	2 week	1 week
Dom	4.10±0.14 ^a	4.05±0.07 ^a	4.15±0.07 ^a
Gudaim	3.95±0.07 ^a	3.55±0.07 ^b	3.60±0.14 ^b
FSM	4.20±0.00 ^a	4.00±0.14 ^a	4.20±0.14 ^a

*Type FB: Type of fermented beverages; FR: First reading; FSM: Fermented Skim milk Values are mean± STD of duplicate independent runs; Different superscript letters in the same row indicate significant (P<0.05) differences between means

Table 3 TSS (%) of fermented dom and gudaim beverages during refrigeration storage

Type FFB	FR	2 week	1 week
Dom	4.75±0.35 ^a	4.95±0.21 ^a	5.35±0.35 ^a
Gudaim	5.20±0.42 ^a	5.10±0.14 ^a	5.10±0.28 ^a
Skim milk	6.65±0.21 ^a	6.45±0.50 ^a	6.70±0.28 ^a

*Type FFB: Type of fermented fruits beverages; FR: First reading; Values are mean± STD of duplicate independent runs; Different superscript letters in the same row indicate significant (P<0.05) differences between means

Conclusion

Bifidobacterium infantis highly survived during refrigeration storage of the fermented beverages. Therefore, fermented dom and godim potentially proved that they could be a good alternative carrier for *B. infantis* as compared to fresh dairy milk which is perishable and

prices high in today's food markets of Sudan. In addition, the fruits are available, cheap and easily to be deliver in dry form. However, further investigation is needed to determine the nutritional values of the fermented beverages.

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