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None-Alcoholic Beverages Formulated From Selected Traditional Sudanese Fruits Fermented With Bifdobacterium Infantis 20088

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Abstract: This study was carried out to develop none-alcoholic beverage from traditional Sudanese fruits dom (Hyphaene thebaica) and gudaim (Grewia tenax) using Bifidobacterium infantis 20088. The fruits were cleaned by removing the dirt and foreign materials. The seeds were separated from fruits except for gudaim fruit was directly used. The growth medium was formulated from10% (w/v) reconstituted skim milk (the control); in addition to 7.5% (w/v) dom and gudaim supplemented with 2.5 g skim milk to provide the required nutrient for bacteria growth during the fermentation.. After sterilization of growth medium and cooling, the mixture was inoculated with a 10% culture of B.Infants followed by incubation for 60 h at 37 ◦C. The results obtained revealed highest growth of B. infantis in gudaim beverage, followed by reconstituted skim milk, and then fermented dom. That is because gudaim contained the highest level of glucose as compared to skim milk and dom fruit. In general there was significant ($p < 0.05$) increase in maximum viable count of each formula as compared with its initial level at the beginning of fermentation in al beverages. The increased viable number was accompanied by a significant $p < 0.05$) reduction in pH and total soluble solids (TSS). Hopefully the strain viable counts fulfill the number required to presence in probiotic foods, which at least 1×106 CFU/ml fermented product. Therefore gudaim and dom are suitable carrier for Bifid bacterium infantis.

Keywords: Sudanese, Gudaim, Dom, Fermentation, Probiotics, Bifidobacterium.

INTRODUCTION

The growing interest in health and diet has recently produced the concept of functional foods. By definition, functional foods are normal foods and parts of the daily diet, but they contain a component that benefits some particular physiological function and reduce the risk of diseases [1]. Nowadays, the wide applications of functional food are in form containing probiotics and non-digestible carbohydrate known as prebiotics [2]. Prebiotics are components present in food, or that can be incorporated into food, which yield health benefits related to their interactions with specific group of microflora exist in the gastrointestinal tract (GI). While the benefits of prebiotics have come to light in more recent years, recognition of probiotic effect dates back to the 19th century when the French scientist Louis Pasteur (1822-1895) postulated the importance of microorganisms in human life, this was further reinforced by work done by Nobel Prize-winner Metchnikoff [3], which led to the concept of probiotics. Probiotic bacterium is defined as a living microorganism which when consumed in sufficient number will improve health beyond inherent basic nutrition [4]. Strain of Bifidbacterium, lactobacillus and non pathogenic yeast such as Saccharomyces boulardii are principally used individually or in combination as probiotics [5]. However, most human origin probiotics are fastidious when used alone, they are characterized by low growth capability in food mediums including the dairy, the main a recommended carrier of probiotics to human [6]. 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). However, the economic value of traditional fruits in Sudan is still low; their utilizations did not go beyond small scale fresh beverages. Fermentation of Sudanese traditional fruits with probiotics will lead to develop fermented beverages with further enhanced value and improved therapeutic properties. This is because; the main carriers of probiotics are the dairy based products. However dairy products are expensive and not consumed by a big slice of population. This has paved the way for researchers to introduce new carriers for probiotics, such as soymilk and malted rice [7, 8]. Therefore, this study was carried out to develop Bifidbacterium infantis probiotic fermented beverages from traditional Sudanese fruit dom and gudaim.

MATEIALS AND METHODS

Preparation of fruits bulbs

Selected traditional Sudanese fruits were used including: Aradaib (Tamarindus indica); Dom (Hyphaenethebaica; Gudeim (Grewia tenax); Gunguleiz (Adansonia digitata); Lalob (Balanitesa gyptiaca); and Nabak (Ziziphusspina_christi). The fruits were purchased from a local market in Khartoum town randomly without grouping according to their ripeness. The traditional fruits were cleaned by removing the dirt and foreign materials. The seeds were separated from the fruits by gently hammering the fruits of gunguleiz, Dom and Nabak. Moreover, that of gunguleiz seed was separated directly from its pulp. Aradaib and lalob, fruit were manual separated from their seed after pealing the hard skin which covers the fruit using a knife .While godaim fruits were used without separation of seed. The collected pulps of fruits were ground into powder to pass through 250 micron sieve.

Proximate composition of traditional fruits

Moisture, fat, protein, and ash was determined according to the official method of [9]. For crude fiber, 2 g of a defatted sample was placed into a conical flask containing 200ml of H2SO4 (0.26N). The flask was fitted to a condenser and allowed to boil for minutes. At the end of the digestion period, the flask was removed and the digest was filtered through a proclaim filter crucible (No.3). After that, the precipitate was repeatedly rinsed with distilled boiled water followed by boiling in 200 ml NaOH (0.23 N) solution for 30 min under reflux condenser and the precipitate was filtered, rinsed with hot distilled water, 20 ml ethyl alcohol (96%) and 20 ml diethyl ether. Finally, the crucible was dried at 105 oC until a constant weight was obtained and the difference in weigh was considered as crude fiber. Carbohydrates were calculated by subtraction of [Moisture (%) + Protein (%) +Fat (%) +Ash (%) + and fiber (%)] from 100.

Growth medium
From among traditional Sudanese fruits, gudaim and dom were used for fermentation because they contained the highest level of fiber. The growth medium was formulated from10% (w/v) reconstituted skim milk (the control); in addition to 7.5% (w/v) dom and gudaim supplemented with 2.5 g skim milk to provide the required nutrient for bacteria growth during the fermentation.

Preparation of fermentation inoculums
Bb.Infants was obtained from the stock culture collection of microbiology laboratory (Department of Food Science Technology, Collage of Agriculture Studies, SUST, Sudan). The strain stock was maintained in 20% glycerol solution. A working culture was prepared by activation of the strain in MRS broth supplemented with 0.05% L-cysteine, incubated under anaerobic condition at 37 ˚C for 24 h. The obtained growth was 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 reconstituted skim milk (121˚C for 15 min) and incubation at 37 ˚C for 24 h.

Fermentation conditions
After sterilization of growth medium and cooling, the mixture was inoculated with a 10% culture of B.Infants followed by incubation for 60 h at 37 ◦C.

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

Enumeration of viable cell
De Mann Rogosa (MRS) agar was used to enumerate B.Infants of fermented beverages using the plate count technique .Samples were drawn at initial and every 12 h intervals during fermentation.1ml of fermentation broth was diluted in peptone water, followed y plating on MRS agar supplemented with 0.05% L-cysteine. The plates were incubated an aerobically at 37 ˚C for 48 h. The viable count of the strain 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' s-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 [10].

RESULTS AND DISCUSSION

Chemical composition of the selected traditional Sudanese fruits
Data on chemical composition of traditional Sudanese fruits were given in table 1. Referring to our results in table 1, all the fruits have low moisture content ranged from 3.13% in dom to 12.06% in lalob. Lipids ranged from 0.1% in gunguleiz to its highest level of 2.6% in nabak. Our result in table 1 showed that some traditional fruits have low protein content of 2.1% such as in gunguleiz up to a high level of 8.3% in gudaium. Crude fiber is widely used to determined insoluble material in dilute acid and dilute alkali under specified conditions. They represent good source of energy for probioits. Table 1 showed that dom has the highest fiber content of 20.13% followed by gudaim, gunguleiz , aradaib , nabak and the lowest fiber content was found in lalob..Ash content of aradaib and lalob did not differ significantly ($p>0.05$). In general carbohydrate content of the traditional fruits is high thus are of nutritional interest.

The growth of Bifdobacteriuminfantis during fermentation of the selected traditional Sudanese fruit beverages
Comparative growth of Bifidobacterium infantis cultured in skim milk, gudaim and dom is shown in table 2. The maximum growth of B. infantis was observed at 36 h fermentation in all fomented beverages. The rate of B. infantis increase in all fermented beverages was significantly ($p>0.05$) difference as compared to strain initial level at the beginning of the fermentation process (Table 2). The highest rate of B.infantis was in fermented gudaim followed by fermented skim milk then the fermented dom. That was due to the highest level of fiber in gudaim as compared to dom (Table 1). In addition the difference in growth rate of bifidobacteria infantis in the different fermented beverages could be attributed to the availability of other nutrients required for growth. Previous investigations on growth of bifidobactera [11, 12] revealed different growth of bifidobacteria in different medium including skim milk and infant formulas. That was interpreted by the metabolic activity of Bifidobacterium in fermented products, which was affected by the composition and availability of nitrogen and carbon sources in growth media [13]. Nevertheless after maximum growth of B.infantis in all fermented beverages presented in Table 2, there was slight decrease in viable count due to reduction in pH (Table 3) and decreases in available nutrients (Table 4). The viability of Bifidobacteria strains is seriously affected by any drop in pH below 4.3 [14]. That is reason why the viable number of B. infantis in all fermented beverages maintained high above 106 CFU/ml fermented products.

pH changes during fermentation of beverages with B.infanti
The result in Table 3 shows decrease in pH due to increase acids production during fermentation process. The accumulated acids produced, reported to have antibacterial activity such as prevent the proliferation of pathogens [15].

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At maximum growth (36 h fermentation) of B. infantis in fermented gudaim, dom, and skim milk beverages; the pH reduction rates were 0.85, 0.60, and 0.30, respectively. However, during whole fermentation period (60 h) the pH reduction rates were 0.95, 1.05, and 1.15 in fermented gudaim, dom, and skim milk, respectively. Further, a statistical analysis showed that the changes in pH of fermented beverages was significant ($p<0.05$) in fermented gudaim and skim milk.

Total Soluble Solids (TSS) changes during fermentation of beverages with B.infantis

Table 4 shows the changes in TSS during fermentation of beverages with B.infantis. There was no significant difference ($p<0.05$) in TSS reduction in all fermented beverages as compared to their initial at the beginning of fermentation. .The amount of TSS reduction at maximum growth of B. infantis in fermented skim milk, dom and gudaim were 2.5, 2.9, 3.75, respectively. This reduction of TSS indicates limitation on available soluble nutrient for strain growth in fermented beverages by extended fermentation periods; together with accumulations of acids may contribute to decrease microbial growth during fermentation.

Conclusion

Fermentation of the selected traditional Sudanese fruits beverages with Bifidobacterium infantis 20088 and its high growth in dom and gudaim indicates that they could be a good carrier for B. infants as compared to fresh dairy milk. Further, the fruits are available, cheap and easy to deliver since they are dry. Further researches to be conducted on nutritional values and functional properties of the fermented beverages to explore their nutritional and health benefits.

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Table 1. *Chemical composition (%) of selected traditional Sudanese fruits**

Types of traditional fruit	Components (%)					
	Moisture	Fat	Protein	Fiber	Ash	**Ca**rbohydrates
Aradaib	11.07 ± 0.33^a	1.40 ± 0.42^b	3.2 ± 0.42^b	6.38 ± 0.60^d	3.37 ± 0.00^c	74.60 ± 0.57^b
Dom	3.13 ± 0.24^d	1.45 ± 0.35^b	2.2 ± 0.28^c	20.13 ± 0.32^a	4.12 ± 0.01^b	$70.00\pm0.\ 00^c$
Gudaim	7.25 ± 0.11^b	1.10 ± 0.00^b	8.30 ± 0.57^a	16.83 ± 0.04^b	2.68 ± 0.06^d	63.85 ± 0.70^d
Gunguleiz	5.73 ± 0.03^c	0.10 ± 0.00^c	2.05 ± 0.1^c	9.83 ± 0.74^c	$7.72\pm0.\ 00^a$	74.58 ± 0.70^b
Lalob	12.06 ± 0.05^a	1.20 ± 0.00^b	4.00 ± 0.28^b	$2.15\pm0.\ 07^e$	$3.63\pm0.29b^c$	76.96 ± 0.69^b

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Nabak	4.96 ± 0.03^{c}	2.55 ± 0.07^{a}	4.05 ± 0.21^{b}	4.850 ± 0.8^{d}	2.72 ± 0.03^{d}	80.9 ± 0.62^{a}

*Values are mean± STD of replicate independent analysis
*Different superscript letters in the same column indicate significant (P < 0.05) differences between means

Table 2. *The viable cells of Bifidobacterium infantis 20088 (CFU/ml) during fermentation period of different beverages**

Fermentation period (h)	Fermented beverage		
	Reconstituted skim milk	Dom	Gudaim
Initial (0)	1.3×10^{6a}	1.5×10^{6a}	7.5×10^{6a}
12	1.3×10^{8b}	1.5×10^{8b}	1.9×10^{8a}
24	1.4×10^{8b}	1.3×10^{8b}	4×10^{8a}
36	1.9×10^{8c}	8.6×10^{7ab}	8×10^{9b}
48	1.2×10^{8b}	7.3×10^{7ab}	1.5×10^{9a}
60	7.2×10^{6a}	6×10^{6a}	2.6×10^{8a}

* Values are mean± STD of duplicate independent runs
*Different superscript letters in the same column indicate significant (P < 0.05) differences between means

Table 3*. pH changes during fermentation of different beverages with Bifidobacterium infantis 20088**

Fermented products	Fermentation period (h)					
	Initial (0)	12	24	36	48	60
Reconstituted skim milk	6.30 ± 0.28^{a}	6.00 ± 0.00^{a}	6.00 ± 0.00^{a}	6.00 ± 0.00^{a}	5.9± 0.00a	5.15 ± 1.48^{b}
Dom	5.60 ± 0.57^{a}	5.15 ± 0.50^{a}	5.05 ± 0.21^{a}	5.00 ± 0.14^{a}	4.90 ± 0.14^{a}	4.75 ± 0.35^{a}
Gudaim	5.80 ± 0.00^{a}	5.10 ± 0.00^{b}	5.10 ± 0.00^{b}	4.95 ± 0.07^{bc}	4.95 ± 0.07^{c}	4.95 ± 0.07^{c}

* Values are mean± STD of duplicate independent runs
*Different superscript letters in the same column indicate significant (P < 0.05) differences between means

Table 4. *TSS during fermentation of different beverages with Bifidobacterium infantis 20088**

Fermented products	Fermentation period (h)					
	Initial (0)	12	24	36	48	60
Reconstituted skim milk	6.70 ± 0.28^{a}	4.55 ± 0.21^{b}	3.85 ± 0.21^{b}	3.80 ± 0.28^{b}	3.60 ± 0.00^{b}	2.75 ± 0.21^{b}
Dom	6.60 ± 0.14^{a}	4.15 ± 0.21^{b}	3.90 ± 0.28^{b}	3.70 ± 0.28^{b}	3.45 ± 0.21^{b}	3.70 ± 0.28^{b}
Gudaim	7.45 ± 0.35^{a}	4.05 ± 0.21^{b}	3.85 ± 0.21^{b}	3.70 ± 0.28^{b}	3.70 ± 0.28^{b}	3.75 ± 0.21^{b}

* Values are mean± STD of duplicate independent runs
*Different superscript letters in the same column indicate significant (P < 0.05) differences between means