

Original Research Article

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Effect of *Bifidobacterium lactis* on Quality of Rice Pudding as a Probiotic Food Carrier

Eman F. Abdel-Latif and M.F. Saad*

Cairo University, Faculty of Veterinary Medicine, Department of Food Hygiene & Control, Giza square, Egypt

*Corresponding author

ABSTRACT

Keywords

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One hundred and ten rice pudding samples were collected from different shops and supermarkets in Cairo and Giza Governorate, Egypt. Collected samples were subjected to microbiological examination. The obtained results were in mean values of 5.6×10^6 cfu/gm, 7.1×10^5 cfu/gm, 9.3×10^4 cfu/gm, and 3.1×10^4 cfu/gm for Mesophilic bacteria, Coagulase Positive Staphylococci (CPS), Total Yeasts & Molds and Aerobic Spore Former respectively. Eighty percent and 6.36% of the examined samples were positive for CPS and *E. coli* respectively. The survival of probiotic bacteria was studied in rice pudding for 14 days. The product was monitored for sensory, physicochemical and *S.aureus* count during its storage. It was found a significant difference between control and probiotic rice pudding samples at day 14 due to occurrence of heterogeneous texture and syneresis. The count of *S.aureus* decreased significantly ($p < 0.05$) at 3rd and 7th day of storage in samples with added *Bifidobacterium lactis*.

Introduction

Milk – based desserts which are the most palatable, nutritious and relatively inexpensive dairy food. These milk based desserts are popular dairy food prepared from ingredients that the milk is the basic constituent (Marwa, 2004). Rice with milk and mehallabia and are almost consumed in Egypt by a wide range of people (Manal, 1993).

Foods that are containing probiotic bacteria come to the focus as having beneficial effect on health. These benefits include improving the gut microbial balance, stimulation of the

immune system, reduction of blood cholesterol level, and reduction in the incidence of cardiovascular diseases, diarrhea, osteoporosis and cancer (Sanders, 2003; Heenan *et al.*, 2004; Madureira *et al.*, 2005; Samarzija *et al.*, 2009).

The viability of probiotic micro-organisms during processing and storage besides the acceptance by the consumers is the major criterion to determine the potency and the market prosperity of the probiotic product. The health benefits are not only dependent on the organism with specific therapeutic properties, but it is also critical that these

live organisms are consumed in adequate amounts to achieve the desired effects (Vinderola *et al.*, 2000).

In recent years researches focus is on alternative products such as milk-based desserts (Favaro-Trindade *et al.*, 2006; Ares *et al.*, 2008; Magarinos *et al.*, 2008). The viability of probiotic bacteria in dairy desserts and their effect on chemical and sensorial characteristics have been studied (Helland *et al.*, 2004; Aragon-Alegro *et al.*, 2007; Cardarelli *et al.*, 2008). However, the information on the effect of probiotic bacteria on growth of pathogen during storage is very limited (Shimamura *et al.*, 2006; Magarinos *et al.*, 2007).

The aim of this study is to evaluate i) microbiological quality of rice pudding sold in some Egyptian markets ii) the economical and public health importance of the isolated microorganisms as well as control measures for improving the quality of such dairy dessert. iii) the survival of probiotic *Bifidobacterium lactis* (*B.lactis*) BB-12 and iv) the impact of this probiotic on sensory attributes and growth of *S.aureus* during storage.

Materials and Methods

Microbiological examination

Collection of samples

One hundred and ten rice pudding samples were collected from different shops and supermarkets in Cairo and Giza Governorate, Egypt. Samples were kept at 4°C in ice box until analyzed.

Preparation of rice pudding homogenate according to ISO (2001)

Ten gram of sample was aseptically transferred with a sterile pipette to 90 ml of diluent 0.1% peptone water (Lab M, 104).

The prepared dilutions were subjected to the following microbiological examinations.

Total Mesophilic Count (TMC) according to ISO (2003a)

One ml of the previously prepared decimal dilutions were inoculated into duplicate plates of Standard Plate Count Agar (Oxoid, CM0463) and incubated at 37°C for 48 hour.

Coagulase Positive Staphylococci Count (CPSC) according to (ISO, 2003b)

From the previously prepared decimal dilutions, 0.1 ml was transferred onto the dry surface of duplicate plates of Baird-Parker medium (Lab M, LAB085). The plates were incubated at 37°C for 48 hours.

Coliforms Count according to (APHA, 2004)

One ml from the previously prepared decimal dilutions was inoculated into a series of three fermentation tubes containing Lauryl Sulphate Tryptose broth (Oxoid, CM0451) were incubated at 35°C for 48 hours. Identification of the isolated Coliforms was done according to (Collins *et al.*, 2004).

Total Yeast and Mold Count according to ISO (2004)

Duplicate plates of Yeast Extract Dextrose Chloramphenical agar (Lab M, LAB119) were inoculated with one ml from the previously prepared serial dilutions. Inoculated plates were incubated at 25°C for 3 up to 5 days.

Identification of the isolated yeasts and molds according to Barnett *et al.* (2000) and Pitt & Hocking (2009)

The yeast isolates were identified using

their morphological and physiological characteristics. Macroscopic morphology and microscopically characters of molds were examined.

Enumeration of Aerobic Spore Forming organisms according to (Vos *et al.*, 2009)

The sample homogenate was placed in water bath at 80°C for 20 minutes, then sudden cooling. 0.1 ml of homogenate was spreaded on dextrose tryptone agar media and incubated at 30°C for 72 hours.

Survival of *S.aureus* and viability of *Bifidobacterium lactis* BB-12 in rice pudding

Preparation of rice pudding

Four rice pudding formulations (Control, *S.aureus*, *S.aureus* + *Bifidobacterium lactis* BB-12 and *Bifidobacterium lactis* BB-12) were produced in triplicate. Each formula of rice pudding was produced in amount to obtain two kg of the final product. After weighing all ingredients (Buffalo's milk, rice, sucrose sugar, starch) individually, they were all mixed together, heated 85°C until the ingredients dissolved completely, and cooled to 40°C in a water bath with continuous stirring. Instantly the mixture reached the desired temperature, calculated amount of probiotic suspension was added, in order to obtain concentration of approximately 7 log cfu/gm in rice pudding at the beginning of the storage (0) day. Also the suspension of *S.aureus* approximately 6 log cfu/ gm was added. The inoculum of probiotic and *S.aureus* strains were evenly distributed in the rice pudding by mixing with a sterile spatula for 50 second. The final product obtained was packaged in individual plastic cups, each one containing approximately 100 g of rice pudding, cooled and then stored at 4±1°C for up to 14 days.

Triplicate rice pudding samples from each trial were taken for sensory evaluation, physicochemical and microbiological analysis immediately after inoculating the probiotic culture in the rice pudding (0 day) and during the storage at 3, 7 and 14 days. The analysis of variance (ANOVA) test, using 99 % confidence intervals, was conducted to test the possible significance ($P \leq 0.05$) among parameters of samples using Fishers Least Significance Difference.

Sensory evaluation

Well-trained five panelists, selected from staff of Department of Food Hygiene and control, Cairo University, carried out the sensory characteristics of the rice pudding trials (Control and *Bifidobacterium lactis* BB-12). Samples were presented in white plastic cups, containing approximately 100 gm rice pudding per cup, already removed from the refrigerator. The judges were evaluated samples using a nine point balanced hedonic scale (1: dislike extremely- 9: like extremely) based on texture (uniform, syneresis), odor, color and taste of each sample, described by Lawless & Heymann (1999) & Cardarelli *et al.* (2008).

Physicochemical analysis

The pH value of rice pudding was applied according to the measuring method no.981.12 of AOAC (2003) using a digital pH meter.

Microbiological analysis

For evaluate the viability of culture in the rice pudding; *Bifidobacterium lactis* BB-12 (CHR-Hansen-Denmark) was enumerated on MRS-LP Agar (Lithium chloride, Sodium propionate) after 3 days of anaerobic incubation at 37±1°C as reported

by Lapierre *et al.*, (1992). *S.aureus* count was determined for all trials throughout storage (ISO, 2003b).

Results and Discussion

From summarized results given in Table (1), it is evident that 100.00% of the examined rice pudding samples were positive for mesophilic bacteria with a mean value of $5.6 \times 10^6 \pm 5.3 \times 10^4$ cfu/gm. Also From the obtained results recorded in Table (1), it was clear that 80.00%, 71.81%, 60.00% and 53.63% of the examined samples were positive for CPS, Coliforms, Total Yeasts & Molds and Aerobic Spore Former with a mean values of $7.1 \times 10^5 \pm 6.7 \times 10^3$ cfu/gm, $6.9 \times 10^5 \pm 6.5 \times 10^3$ MPN/gm, $9.3 \times 10^4 \pm 8.8 \times 10^2$ cfu/gm and $3.1 \times 10^4 \pm 2.9 \times 10^2$ cfu/gm respectively.

Inspection of the results presented in Table (2 & 3) shows that highest percentage of Coliforms (24.51%) and yeasts (38.33%) & molds (44.00%) isolated from examined samples were *Enterobacter aerogenes*, *Candida albicans* and *Aspergillus flavus* respectively.

Coagulase and thermonuclease (TNase) are enzymes produced by staphylococci and are the most accepted indicators of the probable evidence of their enterotoxigenic property (Wong and Bergdoll, 2002). The presence of Coliform species in examined samples is unacceptable as it indicates unsanitary conditions of production, handling and distribution. Moreover, presence of *E. coli* in examined samples is considered indicative of faecal contamination (Quinn *et al.*, 2002; Mhone *et al.*, 2011).

The presence of yeasts and molds in examined samples in this study highlights improper sanitary conditions encountered in the production area. The authors have attributed the presence of these microbes to

improper hygienic practice at surrounding environment. Some species of yeast constitute public health hazards. *Candida* species are ubiquitous fungi that represent the most common fungal pathogens that affect humans (Hidalgo, 2010).

Aspergillus species are known to produce mycotoxins which are carcinogenic to man. Also some species of *Penicillium* have been related to production of citrinin and ochratoxin which causing kidney failure (Palumbo *et al.*, 2011). Results revealed the presence of microbial contaminants which indicates bad milk quality and requires urgent attention as it can cause serious public health risk to consumers.

It is necessary to evaluate the degree of overall acceptability of examined samples versus other available standards than Egyptian standards due to the lack of critical limits of microbiological parameters of rice pudding. Based on TMC and CPSC of the examined rice pudding samples compared to maximum limit of Centre for Food Safety (2007), which stated that the unsatisfactory TMC and unacceptable CPSC are $\geq 10^5$ cfu/gm and $\geq 10^4$ cfu/gm respectively, the percentages of unacceptable examined samples were 73.63% and 78.18% for TMC and CPSC respectively. From the above, it seems necessary that concerned authorities should approve strict regulations for microbiological parameters of rice pudding production and handling in Egypt.

Based on sensory evaluation, control samples were for the most acceptable until day 7. It obtained its lowest score at the end of the storage period (14 days). Use of probiotic bacteria showed the highest score through the days (0, 3 and 7) of storage (softer, palatable and uniform). It was found a significant difference between control and probiotic rice puddings samples at day 14 due to occurrence of heterogeneous texture

and syneresis. No abnormal flavor in the examined probiotic rice puddings was detected (Table 4).

There are several studies indicating that probiotic micro-organisms affecting the

texture and flavor of the food product to which they were added (Aragon-Alegro *et al.*, 2007; Cardarelli *et al.*, 2008; Magarinos *et al.*, 2008).

Table.1 Microbiological parameters of the examined samples (N =110).

Parameters	No. of positive samples	%	Min	Max	Mean	±SEM
Total Mesophilic count (cfu/gm)	110	100.00	10 ²	3.1x10 ⁹	5.6x10 ⁶	5.3x10 ⁴
Total Coagulase Positive Staphylococci count (cfu/gm)	88	80.00	10	3.9x10 ⁷	7.1x10 ⁵	6.7x10 ³
Coliforms count (MPN/gm)	79	71.81	10 ²	9.7x10 ⁷	6.9x10 ⁵	6.5x10 ³
Total Yeast & Mold count (cfu/gm)	66	60.00	10 ²	5.6x10 ⁶	9.3x10 ⁴	8.8x10 ²
Aerobic Spore Former count (cfu/gm)	59	53.63	32	2.8x10 ⁶	3.1x10 ⁴	2.9x 10 ²

*N= Number of samples.

Table.2 Incidence of Coliform organisms isolated from examined samples (N =155).

Types of isolates	No. of isolates	%
<i>Enterobacter sakazaki</i>	20	13.00
<i>Enterobacter cloacae</i>	10	6.44
<i>Enterobacter aerogenes</i>	38	24.51
<i>Escherichia coli</i>	15	9.67
<i>E.coli . inactive</i>	10	6.44
<i>Citrobacter braakii</i>	21	13.50
<i>Citrobacter freundii</i>	32	20.64
<i>Klebsiella pneumniae</i>	9	5.80
Total		100.00

*N= No. of isolates.

Table.3 Incidence of isolated yeasts and molds in the examined samples (N = 135).

Isolated yeasts	No. of isolates	%
<i>Candida albicans</i>	23	38.33
<i>Cryptococcus neoformans</i>	9	15.00
<i>Pichia guilliermondii</i>	2	3.33
<i>Rhodotorula diffluens</i>	3	5.00
<i>Saccharomyces cerevisiae</i>	12	20.00
<i>Zygosaccharomyces bailii</i>	6	10.00
<i>Zygosaccharomyces rouxii</i>	5	8.34
Total	60	100.00
Isolated molds		
<i>Aspergillus flavus</i>	33	44.00
<i>Aspergillus parasiticus</i>	9	12.00
<i>Aspergillus niger</i>	21	28.00
<i>Alternaria alternate</i>	8	10.67
<i>Fusarium oxysporum</i>	4	5.33
Total	75	100.00

*N= No. of isolates.

Table.4 Scores for the sensory attributes of the rice pudding trials.

Trials	Color				Odor				Taste				Texture			
	Storage period (days)															
	0	3	7	14	0	3	7	14	0	3	7	14	0	3	7	14
Control	9a	9a	8a	5b	9a	9a	8a	5b	9a	9a	7a	5b	8a	8a	6b	4b
<i>B.lactis</i>	9a	9a	9a	7a	9a	9a	9a	8a	9a	9a	9a	7a	9a	9a	9a	8a
BB-12																

Values (1-9) equal to mean. Value nine in the scale means the highest score.

Value one in the scale means the lowest score.

Significant differences between the values had the different letter in each row & column ($p < 0.05$).

Table.5 Survival of *S. aureus* in probiotic rice pudding during storage

Formulations Storage period	Day zero	Day 3	Day 7	Day 14
<i>S.aureus</i>				
pH	6.68a	6.77a	6.98a	7.24a
<i>S.aureus</i> count (cfu/gm)	10 ⁶ b	10 ⁷ d	3.3x10 ⁷ d	9.1x10 ⁷ d
<i>S.aureus</i> + <i>B.lactis</i> BB-12				
pH	6.52a	6.50a	6.68a	6.84a
<i>S.aureus</i> count (cfu/gm)	10 ⁶ b	2.2x10 ⁴ c	1.5x10 ⁴ c	3.3 x10 ⁶ b

Significant differences between the values had the different letter in each row ($p < 0.05$).

Table.6 Viability of *B. lactis* BB-12 during storage of rice pudding

Formulations Storage period	Day zero	Day 3	Day 7	Day 14
Control				
pH	6.90a	6.91a	6.93a	6.96a
<i>S.aureus</i> count (cfu/gm)	Less than 10 ² a			
<i>B.lactis</i> BB-12				
pH	6.52a	6.51a	6.53a	6.52a
<i>S.aureus</i> count (cfu/gm)	Less than 10 ² a			
<i>B.lactis</i> BB-12 count	1.6x10 ⁷ a	1.3x10 ⁷ a	1.0x10 ⁷ a	1.5x10 ⁶ b

Significant differences between the values had the different letter in each row ($p < 0.05$).

The changes in pH values of rice pudding during storage for 14 days were given in Table 5 & 6. The pH values obtained in *Bifidobacterium lactis* BB-12 trial samples were non significantly ($p > 0.05$) but lower than those of control.

The impact of using probiotic culture on *S.aureus* in samples was evaluated during storage (Table 5). It could be seen that *Staphylococcus aureus* was determined at a level of 2.2x10⁴, 1.5x10⁴, 3.3x 10⁶ cfu/gm at day 3, 7 and 14 respectively. The count of *S.aureus* decreased significantly ($p < 0.05$) at 3rd, 7th day of storage in comparison to day 14, the count increased significantly ($p < 0.05$) probably due to reduction in count of viable probiotic cells in rice puddings or reduction in formation of anti-microbial substances produced by probiotic strain.

Staphylococcus aureus count was not more than 10²cfu/gm for *Bifidobacterium lactis* BB-12 trial (Table 6); this observation revealed that the use of *Bifidobacterium lactis* BB-12 is profitable on hygienic quality of rice pudding. Several authors have previously reported that cumulative effects of antimicrobial agents such as hydrogen peroxide, bacteriocins, organic acids and various antibiotics are accountable for inhibitory activity of probiotic bacteria (Servin, 2004; Makras & Vyust, 2006 and Parada *et al.*, 2007).

The cell count of *Bifidobacterium lactis* BB-12 was 1.6x10⁷ cfu/ gm at 0 day storage. The variation of the count of probiotic bacteria in rice pudding samples during 14-day storage at 4±1°C were summarized in Table 6. The count of starter probiotic

culture in rice pudding samples resulted in a reduction of 1.45×10^7 cfu/gm in comparison to inoculated amount.

Several studies have shown the reduction of one logarithmic cycle for probiotic bacteria in dairy desserts after 14 days of storage at 4°C (Helland *et al.*, 2004; Magarinos *et al.*, 2008). The recommended level of Bifidobacteria is 10^6 cfu/gm at the time of consumption (Roy, 2001). The minimum therapeutic daily dose is considered as 10^8 - 10^9 viable cells, which could be achieved with a daily consumption of at least 100 gm of fermented products containing between 10^6 and 10^7 viable cells/gm (Blanchette *et al.*, 1996). As it could be seen from Table 6, probiotic bacteria maintained above this count during the whole storage period. Results revealed that probiotic rice pudding pioneered in the present study could be used as carrier of *B. lactis* BB-12 in food.

In conclusion, proper sanitation and hygiene during handling of rice pudding are important factors to protect the consumer and prevent spoilage of the product. There is a lack of information in the Egyptian standards concerning rice pudding therefore; concerned authorities should impose regulations and standards for control rice pudding production, handling and storage. Results indicated that high level of viable *B. lactis* BB-12 in rice pudding is a good source for probiotic delivery with appreciated organoleptic quality, leading to good kinds for its future production.

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