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Effect of Cold Storage and Industrial Aromas on the Viability of Bifidobacteria in Fermented Dairy Products

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Abstract: Cultivation and viability of bifidobacteria in milk face several difficulties because milk is not a good medium for growth and surviving of these fastidious microorganisms. Therefore, suitable strains of *Bifidobacterium* should be selected basing on their technological properties. Two of the most important technological factors were examined in this study: the viability of six *Bifidobacterium* strains was evaluated for 21 days of cold storage in milk, as well as the effect of industrial aromas was also assessed for the same period. Very high fatalities were noted for all strains stored at 4°C, where the best viability rate was about 28%, reveling the harmful effect of this factor on the bifidobacteria's surviving, in the other hand, difference in behavior towards artificial aromas was recorded with a best viability rate of about 70% registered for banana and 64.5% for the vanilla.

Key words: Bifidobacteria · Milk · Technological properties · Viability · Cold storage · Artificial aromas

INTRODUCTION

Fermented dairy products are the usual carriers for the delivery of probiotics to humans, *Bifidobacterium* and *Lactobacillus* being the most frequently used bacteria [1, 2]. Several health benefits have been attributed to the consumption of fermented dairy products, such as ensuring the correct balance or good working order of the intestinal flora, regulation of the intestinal immune system or reinforcement of the intestinal barrier [3].

Bifidobacteria are the most important group of probiotic cultures usually incorporated in fermented dairy products, those bacteria contribute a major part of the human intestinal microbiota in healthy humans. They are considered to provide many beneficial effects including improvement of lactose digestibility, anticarcinogenic activity, reduction of serum cholesterol level, synthesis of B vitamins and facilitation in calcium absorption [4].

Lot of technological aspects has to be considered when selecting probiotic strains, such as viability during processing, stability in the product and during storage, good sensory properties and phage resistance [5].

The most important objective of probiotic delivery is that the probiotic strains should be viable at the time of consumption. It has been suggested that the minimum concentration of live probiotic bacteria at the expiry date of the product should be around 10^7cfu mL^{-1} [6].

Many studies have already indicated the impact of several factors on the viability of bifidobacteria in fermented dairy products, including the pH and acid content of the product, the levels of hydrogen peroxide produced by the traditional starter lactic acid cultures and the oxygen levels within the product. However, other crucial factors which could have a significant occurrence on the viability of bifidobacteria in fermented milk are poorly studied. This study aimed to assess the effect of two very important of those factors which are the cold storage at 4°C and the contact with synthetized aromas used in dairy industry.

MATERIALS AND METHODS

Strains Origin: *Bifidobacterium* strains used in this study were derived from two sources:

- Four (4) strains (L_1, L_2, R_1 and R_2) were isolated from healthy breast-fed infant stools, aged less than five months.

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- Two strains (2) (B_N and BB_{12}) were isolated from two probiotic fermented milks produced and sold in Algeria.

Experimental Design

Strains Isolation and Identification: The isolation was performed on MRS medium supplemented with 0.05% of hydrochloric cysteine and 2mg / l of nalidixic acid.

After vigorous agitation using a vortex during 30 s, the samples were diluted in physiological water containing 0.05% cysteine-HCl. 0.1 ml of adequate dilutions were spread on MRS solid medium. Plates were incubated at 37°C for 72 h in jars under anaerobic conditions [7].

The identification of bifidobacteria strains was based on determination of morphologic, biochemical and physical characteristics. The isolates were identified on specie level using sugar fermentation and the profiles were compared with those in standard texts for identification [8].

Viability During Cold Storage: Tubes of 10 ml of skim milk were inoculated with 5% v/v of the young culture of each strain, in accord with the protocol used in dairy industry. After 8 hours of anaerobic incubation at 37°C, the tubes were transferred to cold storage at 4°C and maintained at this temperature for 21 days. The viability assessment is carried out each 7 days (0, 7, 14 and 21 days) [9].

The enumeration of viable cells (CFU/ ml) was effected on MRS agar supplemented with 0.05% HCl-cysteine and 2mg/l of nalidixic acid.

The viability rate was calculated using the equation reported by Ustunol and Gandhi [10].

Viability (%) = (CFU after N days of cold storage / initial CFU) × 100

The rates more than 70% or less than 30% are considered as satisfying or inacceptable respectively, the amounts between those two limits are considered as weak.

Monitoring of Post Acidification During the Cold Storage: The post-acidification of fermented milk by *Bifidobacterium* strains is surveyed by pH measurement throughout the 21 days of cold storage.

Influence of Synthetized Aromas on the Viability of Bifidobacteria: The assessed aromas were banana (isoamyl-acetate) 0.2% and vanilla (ethylvanilline) 0.2% used in dairy industry.

Tubes of 5 ml of MRS broth (supplemented with 0.05% HCL- cysteine) containing tested aromas with the concentrations mentioned above were inoculated with 5% (v/v) of the young culture of each strain. The protocol applied for the counting of viable cells is the same used in the previous test.

The viability rate is calculated using the same equation of Ustunol and Gandhi [10].

RESULTS AND DISCUSSIONS

Strains Identification: The bifidobacteria strains identification was based on macroscopic and microscopic shape.

Bifidobacteria growing on MRS-systeine agar formed small Wight colonies without catalase activity.

Under microscope, bifidobacteria cells are Gram+, with variable shapes but often bifid, this is according to medium composition [11, 12]. Following to the obtained results, we could identify three bifidobacteria species.

Results of bifidobacteria strains identification tests are cited in Table 1.

Viability During Cold Storage: One of the main technological proprieties for strains with probiotic potential is to maintain a good portion of viable cells throughout the cold storage and arrive with a sufficient amount at the time of consumption.

In this study, the viability of bifidobacteria was evaluated for 21 days of cold storage at 4°C, which is in general, the life period for an optimal consumption of the fermented milk.

According to the obtained results, the strains viability decreased gradually all over the cold storage period. After 07 days, an important fall of viability was noted for all the strains, the highest viability was 58.1% registered by BB_{12}. The other strains showed also weak viability (between 38.5 and 53%), except for R_1 the viability was inacceptable (16.22%).

At the 14^{th}day, the strains, BB_{12}, R_2 and L_1maintained the most important portion of viable cells with 50.9, 48 and 39.8% respectively, despite the other strains had a high mortality degree, with a portion of viable cells less than 14%.

Reaching the end of the storage period (21^{st} day), the best viable cells percentage stopped at more than 27%, registered by BB_{12}, L_1 and R_2. On the other hand, the feeblest percentage dropped to less than 10%, noted for B_N, L_2 and R_1.

Table 1: Bifidobacteria strains identification

Species	*B. animalis*		*B. longum*		*B. breve*	
Strains	B_N	BB_{12}	L_1	L_2	R_1	R_2

Table 2: Recapitulation of morphological, physiological and sugar fermentation tests results of the six bifidobacteria strains 2.

Item	L_1	L_2	R_1	R_2	B_N	BB_{12}
Macroscopic shape	Whitish, viscous colonies with regular outline	Punctate, shining, whitish cream colonies	Small, opaque, whitish colonies smooth surface	Small, Whitish, viscous colonies	Whitish, viscous colonies with regular outline	Punctate, shining, whitish cream colonies
Microscopic shape	Spatulated form, Rounded ends, bifid form (V, Y)	Spatulated form, Rounded ends, bifid form	Short rods, Rounded ends, bifid form	Short rods, Rounded ends, bifid form	Spatulated form, Rounded ends, bifid form(V)	Spatulated form, Rounded ends, bifid form
Gram	+	+	+	+	+	+
Catalase	-	-	-	-	-	-
Citrate permease	+	+	+	+	+	+
Urease	-	-	-	-	-	-
Indole	-	-	-	-	-	-
Gelatinase	-	-	-	-	-	-
L-Arabinose	+	+	-	-	-	-
Amidon	+/-	+/-	+/-	+/-	+/-	-
Cellobiose	-	-	+	+	+	+
Fructose	+	+	+	+	+	+
Galactose	+	+	+	+	+/-	+ /-
Lactose	+	+	+	+	+	+
Lévulose	+	+	-	-	+	+
Maltose	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+
Raffinose	+	+	-	-	-	-
Rhamnose	+	+/-	-	-	+	+
Ribose	-	+ /-	+	+	+	+
Sorbitol	+	+	+	+	+	+
Tréhalose	+	+	-	-	+	+
Xylose	+	+	-	-	-	-

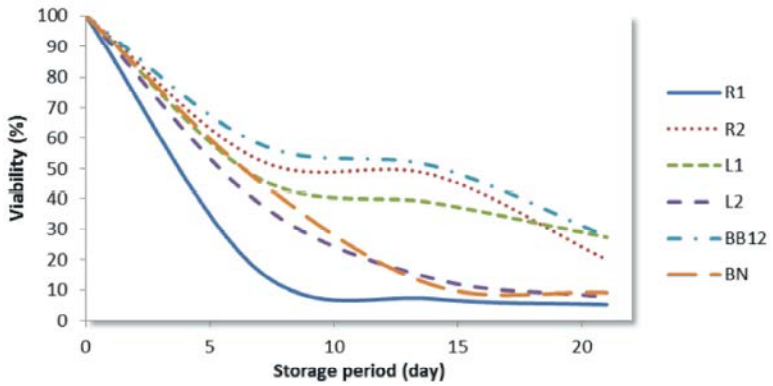

Fig. 1: Viability of bifidobacteria strains during the cold storage at 4°C

This significant loss of viability of bifidobacteria during the cold storage was reported by several authors [13-15].

Many factors can influence the viability of bifidobacteria in fermented milk during the cold storage, including, oxygen, nutriment exhaustion, accumulation of organic acids [16].

Following of post acidification of fermented milk by bifidobacteria stored at 4°C:

The bifidobacteria strains produced a very weak post acidification during the cold storage. The initial milk pH was around of 6. The most post acidifying strain is B_N with a pH arriving to 4.98 after 21 days of cold storage, the other strains have pH between 5.08 and 6.1(Fig. 2).

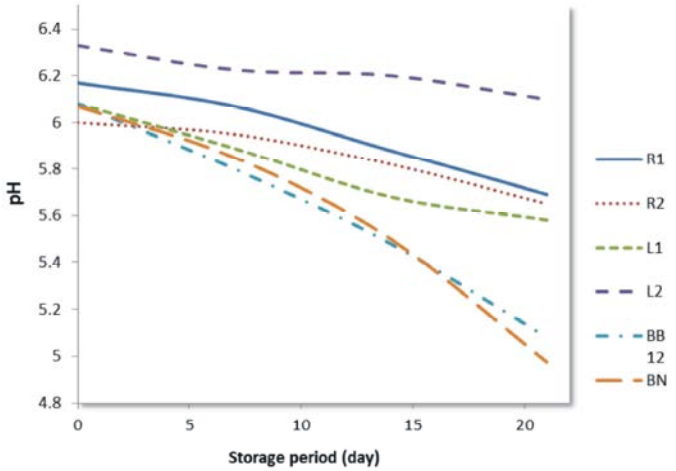

Fig. 2: pH variation of the milk fermented by the strains during the cold storage

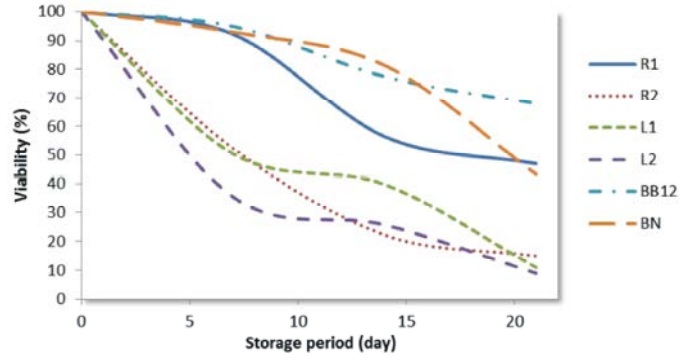

Fig. 3: Viability of bifidobacteria strains through 21 days of cold storage in contact with banana aroma

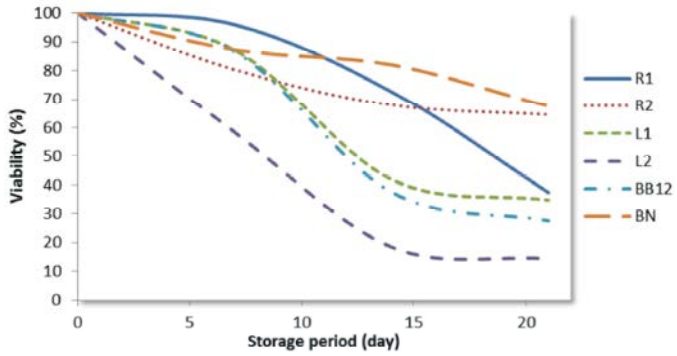

Fig. 4: Viability of bifidobacteria strains through 21 days of cold storage in contact with vanilla aroma

This weak post acidification of bifidobacteria strains has been noted by several authors [13, 17]. This is a much claimed character to do not affect the organoleptic proprieties of final product during the cold storage.

Influence of Synthetized Aromas on the Viability of Bifidobacteria

Banana: The results showed clearly the negative effect of this industrial aroma on the viability of the bifidobacteria strains (Fig. 3).

After 7 days of cold storage, three strains (R_1, B_N and BB_{12}) preserved good fraction of viable cells (over than 90%). Nevertheless, the other strains (L_1 L_2 and R_2) kept middle-to weak viability (less than 52%).

Achieving the 14th day, the viability rate carried on regressing, with best viability of-81.4% registered by B_N and a very weak viability stilled about 22% noted for R_2.

At the end of the storage time, the top viability rate stopped at about 68% reserved for BB_{12}, an acceptable fraction of viable cells has been kept by the two strains B_N and R_1 (close to 50%). However the other three strains showed a very feeble viability rate (around 10%).

Vanilla: At the 7th day of cold storage, the studied strains, except L_2 kept a strong viability (over than 80%).

After two weeks, the best viability rate was 82%, noted for B_N, followed by the two strains R_1 and R_2 with 72.65% and 67.9% of viable cells respectively, on the other hand, a weak viability was showed by the other strains (less than 42%).

Reaching the end of the storage period, B_N and R_2were the most viable strains with 67.45% and 64.45% respectively. Nevertheless, the feeblest viability rate was registered by L_2 (14.4%); the other strains-kept about 30% of the cells alive.

In fact, several studies reported the harmful effect of the synthetized aromas, used in dairy industry on the viability of lactic acid bacteria, in fermented dairy products, even with recommended concentrations [13, 18 and19].

CONCLUSIONS

Bifidobacteria are valuable dietary adjuncts that can be incorporated into dairy products adding additional functional and health attributes to the milk products. Screening of suitable strains of bifidobacteria for application in milk products is a key area in the changing functional dairy product market. Our study aimed to evaluate two of the main technological abilities of some bifidobacteria strains, which are the viability during the cold storage and the behavior towards industrial aromas, the results showed a very harmful effect of cold storage on the surviving of studied strains, conversely for the industrial aromas where certain of *Bifidobacterium* strains preserved a very good viability reaching the 70%, which qualifies them for potential industrial exploitation after other profounder studies.

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