Study on Probiotic Ice Cream

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Abstract. Probiotics are microorganisms that have claimed health benefits when consumed. The emergence of antibiotic-resistant bacteria and natural ways of suppressing the growth of pathogens has contributed to the concept of 'probiotics'. Probiotic bacteria not only compete and suppress 'unhealthy fermentation' in human intestine, but also produce a number of beneficial health effects of their own. The aim of our work was to obtain ice cream which contains probiotic viable microorganism cells using molasses as prebiotic for these bacteria. The main objective of this paper was to study the ability of different strains of probiotics to remain viable in the matured ice cream compared to icecream stored at -5°C. Five samples of ice cream were prepared with the same recipe using molasses as a sweetener. These icecream samples were inoculated with different bacteria strains: *Lactobacillus plantarum*, *Bifidobacterium infantis* 4BN, *Lactobacillus casei* 4BN, *Bifidobacterium breve* 4BN and a mix prepared from the strains mentioned above. For each ice cream sample, the dilutions 10^{-5} and 2×10^5 cells/ml were prepared and the number of bacteria were counted by Thoma camera using the microscope. The better rate of proliferation were obtained for *Lactobacillus plantarum* strain and the mix strain proliferated the best, both in matured icecream and in ice cream stored at -5°C. In this study, we also found that molasses are growth promoters for *Lactobacillus casei*, *Lactobacillus plantarum* *Bifidobacterium breve*, *Bifidobacterium infantis*.

Keywords: ice cream, probiotics, molasses, Trypane-Blue, FT-IR spectrophotometry

INTRODUCTION

The aims of this study were to investigate the survival of probiotic bacteria in ice cream during storage at low temperatures and to evaluate the proliferation rate of microorganisms using molasses as prebiotic.

Properties of some lactic acid bacteria, called probiotics are long known and are the subject of many studies. Probiotic bacteria not only provides competition of substrates and suppress "undesirable fermentation" in the gut, but exert their own beneficial effects. This may affect the structure and function of the gastrointestinal tract, and can be used as a manager of bacteria in the intestinal microflora. Other effects include prevention of intestinal infections, expression of antitumor activity and increased use of lactose (Kirjavainen and Gibson, 1999; Glodin, 1998).

Probiotic bacteria are commonly used as the active ingredient in functional foods such as bio-yogurts, nutritional supplements and other products that stimulate health. Dairy products incorporating probiotic bacteria are gaining popularity and an increase in market share.

Ice cream is a valuable food product, with significant caloric energy. In terms of nutrition, ice cream contains three macronutrients that must be present in any diet: carbohydrates, proteins and lipids. Due to its contents, ice cream can be considered a good vehicle for probiotic bacteria. The manufacturing process and the storage of ice cream could lower the survival of microorganisms contained. To develop truly effective probiotic frozen

products we must ensure that the bacteria remain viable at the end of the technological process of production and throughout the storage period.

It is important to show whether, after different periods of storage, probiotic cultures are still able to provide the same health benefits observed in other products already shown in products with shorter shelf life which are stored at higher temperatures, such as yogurt and acidophilic milk.

Increase in bifidobacteria has been influenced by the type of sweetener used. Although bacteria of the genus *Bifidobacterium* are known to be fastidious, having a difficult increase milk, production of the organic acid increased as a result of the addition of molasses which was an unexpected result.

Molasses is considered a bifidogenic factor, as all oligosaccharides. Substrates with low polymerization degree may be preferred for supporting the *Bifidobacteria*. In contrast, carbohydrates with a high polymerization degree were considered poor substrate for the bacteria. Very little is yet known about the mechanism of carbohydrate ingestion by *Bifidobacteria* (Kleessen *et al.*, 1997). Furthermore, understanding the substrate preference by bacteria of the genus *Bifidobacterium*, will facilitate the development of probiotics, prebiotics and simbiotics.

It has been shown that the molasses has proven effective as a prebiotic due to the increase in the number of bifidobacteria and lactic acid bacteria, stimulating and enhancing the production of acetic acid and lactic acid, adding possible beneficial effects by inhibiting the growth of pathogenic bacteria, by the production of acids short-chain fatty acids, decreasing the pH and producing antimicrobial compounds, as well as by competition on substrates for growth and adhesion (Benno *et al.*, 1984, Gibson and Wang., 1994, Hudault *et al.*, 1997).

MATERIALS AND METHODS

Probiotics are microorganisms that after oral administration are able to colonize the digestive tract and keep or cause an increase in the natural flora of the digestive system, preventing pathogen adherence to the intestinal wall and ensuring optimum utilization of food security.

The research was conducted in the Laboratory of Biochemistry, Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Also, we collaborated with Alpin 57 Lux in Sebes, Alba County.

In this study we considered the following definitions: viable *Lactobacillus* bacteria are those bacteria of the genus *Lactobacillus* which are capable of replication in specific growth conditions of *Lactobacillus* cultures. Non-viable *Lactobacillus* bacteria are the bacteria of the genus *Lactobacillus* which are mostly unable to grow under specific conditions of *Lactobacillus* cultures.

The specific conditions for the growth of *Lactobacillus* bacteria refers to a combination of pH, medium and temperature, where viable cultures (10^6 /ml) in diluted solution would grow to a density of at least 10^7 bacteria /ml within a specific period of time.

The samples of ice cream were divided into two categories: ice cream samples inoculated with lactic acid bacteria and then frozen and ice cream samples inoculated with lactic acid bacteria, allowed to mature for 24 h hours at 4°C and then frozen.

For the preparation of each sample it was used 1 egg, 60 ml of 1.5% milk and 25 g of molasses. Molasses was provided by the factory S.C. Zahăruț S.A. Ludaș.

Ice cream mix was prepared according to the following scheme, shown in Fig. 1.

Fig.1. Diagram of the ice cream mix preparation

The milk was inoculated with five types of lactic acid bacteria culture. Sample 1, contained milk inoculated with *Lactobacillus plantarum*, sample 2 contained milk inoculated with *Bifidobacterium infantis* 4BN, sample 3 contained milk inoculated with *Lactobacillus casei* 4BN, sample 4 contained milk inoculated with *Bifidobacterium breve* 4BN and sample 5 contained milk inoculated with a mix of cultures mentioned above.

Milk used in the manufacture of ice cream samples was inoculated with 1 g of lactic acid bacteria culture to 200 ml of milk.

Molasses in ice cream has a fine structure; homogeneous consistency except the presence of small ice crystals and clumps of fat, the color was uniform brown, sweet taste, pleasant and mild molasses flavor and a fine and pleasant molasses smell, foreign odors missing.

The counting of viable bacteria in inoculated milk and in the two categories of icecream samples were performed by the method of Trypan blue. This was carried out as follows:

- concentration is obtained by diluting to 10^{-5} - 2×10^{-5} cells / ml
- from the dilution obtained above 0.5 ml of cell suspension was placed in a test tube
- addition of 0.1 ml 0.4 % Trypan Blue and then stirred
- allow standing for 5 min at 15-30°C (room temperature)
- is attached to a hemocytometer for cell counting
- using a microscope distinguishes the viable cells that excluded the dye from the unviable, that have changed color.

For the calculation of viable cells, it was used the following formula:

$$N_{cv} = A \times 4.000.000 \times \frac{C}{B} \quad (1)$$

Where:

A = plate count;

B = the number of squares (80);

C = dilution.

For IR spectroscopy analysis it was used a Shimadzu IRPrestige21 device and the domain recorded was 600-4000 cm⁻¹.

It was performed FT-IR comparative analysis of fructose, glucose, sucrose, molasses and ice cream samples. FT -IR spectra patterns of bacteria are highly reproducible fingerprint type and species specific. Using this method, unknown microorganisms can be identified easily and quickly once there is a library with reference spectrum.

For identification, the infrared spectrum of each sample was compared with the standard spectra of glucose, fructose, sucrose and molasses in order to establish their presence in icecream.

RESULTS AND DISCUSSIONS

Tab. 1 summarizes the results of microscopic counting of viable cells from the samples prepared above.

Tab. 1

The number of viable bacteria determined microscopically by Trypan Blue method

Samples	Inoculated Milk	Cured ice cream	Frozen ice cream
Sample 1 – <i>Lactobacillus plantarum</i>	8,5 x 10 ⁶	10,5x10 ⁶	8,5 x 10 ⁶
Sample 2 – <i>Bifidobacterium infantis</i> 4BN	3,5 x 10 ⁶	3 x10 ⁶	4,5 x 10 ⁶
Sample 3 – <i>Lactobacillus casei</i> 4BN	6,5 x 10 ⁶	6 x 10 ⁶	5 x 10 ⁶
Sample 4 – <i>Bifidobacterium breve</i> 4BN	5,5 x 10 ⁶	5,5 x10 ⁶	4 x 10 ⁶
Sample 5 – Mix cultures	9 x 10 ⁶	11,5x10 ⁶	7,5 x 10 ⁶

Although the amount of the bacterial culture used to inoculate the milk in ice cream composition was the same for each sample, after Trypan blue analysis significant differences in the viable bacterial cell counts can be observed. These differences arise both within the same samples - milk, cured and frozen icecream – but also between different categories of samples.

It can be seen that *Lactobacillus plantarum* and the culture mix had the best rate proliferation. Only in the frozen ice cream sample, the mix of cultures had a lower growth (7.5x10⁶) than *Lactobacillus plantarum* (8.5 x10⁶). Both in samples of milk and cured ice cream, the mix of cultures had the higher proliferation, followed by *Lactobacillus plantarum*, *Lactobacillus casei* 4BN and *Bifidobacterium breve* 4BN. In the first two categories of samples *Bifidobacterium infantis* 4BN had the lower rate proliferation. When considering only the samples of frozen ice cream, the lower proliferation was observed with bacteria of the genus *Bifidobacterium breve* 4BN followed by *Bifidobacterium infantis* 4BN.

It can be observed that in the case of bacteria *Lactobacillus plantarum* the best cell proliferation occurs in the sample of cured ice cream as compared to milk and frozen ice cream sample. *Bifidobacterium infantis* has the best proliferation in the frozen ice cream, suggesting a better resistance to freeze. In the samples inoculated with bacteria mix the most significant proliferation occurs in the case of cured icecream, followed by the inoculated milk. The lower rate proliferation of the mix culture occurred in the frozen icecream, but the result is significantly higher than that obtained for the proliferation of *Bifidobacterium infantis* 4BN cultures, *Lactobacillus casei* 4BN and *Bifidobacterium breve* 4BN in frozen icecream.

In order to show the presence of sucrose as the major compound, FT-IR spectrum of sucrose standard was compared to that of icecream sample. In Fig. 3 are shown by comparison the FT-IR spectra of sucrose standard, molasses sample and icecream sample.

Fig. 3. FT-IR spectra of sucrose standard compared to that of molasses and ice cream sample

In the standard spectra of glucose, fructose and sucrose the characteristic vibration of carbonyl at 1600 cm^{-1} and the associated hydroxyl groups at 3450 cm^{-1} can be noticed. The FT- IR spectra of icecream and molasses samples demonstrated the presence of sucrose as the major element, which proved that this compound was found in all five samples.

The analysis of FT-IR spectra of probiotic bacteria used to inoculate milk show two distinct bands at 2845 cm^{-1} and 2929 cm^{-1} . These bands are due to asymmetric stretching vibration of methylene and methyl groups (Kummer *et al.*, 1998), groups which are characteristic for the fatty acids in the cell wall of the bacteria.

In addition, the absorption peak at 1730 cm^{-1} , which can be observed in the spectra of all the analyzed bacteria, is characteristic of stretching vibration of $\text{C}=\text{O}$ bond of the ester groups of lipids and fatty acids (Kansiz *et al.* 1999). The vibration band between 1790 cm^{-1} and 1310 cm^{-1} comprises stretching vibration of $\text{C}=\text{O}$ bond in protein bonded amide (1620 cm^{-1}), bending vibrations of N-H bond (1530 cm^{-1}), symmetric and asymmetric vibration of the $-\text{CH}_2$, $-\text{CH}_3$ groups (1430 cm^{-1} , 1372 cm^{-1}) (Philip, Hermann 2001). Vibration range 1300 - 900 cm^{-1} is characteristic of proteins, nucleic acids, cell membranes.

Specific bacteria fingerprint include stretching vibration of $\text{P}=\text{O}$ group from phosphodiester bonds in nucleic acids at 1190 cm^{-1} and 1030 cm^{-1} , while the C-O-C bond vibrations from polysaccharides which are associated with glycopeptides and lipopolysaccharide from the cell wall can be identified in the 1200 cm^{-1} to 900 cm^{-1} range (Kansiz, 1999).

Fig. 4 present the IR spectra of bacteria with probiotic activity analyzed in this study.

Fig. 4. Comparison of IR spectra of analyzed probiotics

The following figure represents the FT-IR spectra of inoculated milk samples and one of the ice cream samples.

Fig. 5. FT- IR spectra of *Lactobacillus plantarum* inoculated milk compared to that of ice cream sample obtained from the same inoculated milk

The IR spectra of the samples of milk inoculated with different types of bacteria highlight their presence between 900-1200 cm^{-1} region. In the ice cream sample the presence of bacteria is better highlighted, which shows that their number increased. Also, in all the samples the presence of sucrose was observed between 1500-1650 cm^{-1} .

CONCLUSION

The addition of probiotics in ice cream samples prepared in the laboratory had different consequences depending on the type of microorganisms and the technology of

preparation of ice cream. Thus, in the cured ice cream, the proliferation of microorganisms was in most cases better than in the frozen icecream. This can be explained by the fact that the cultures were maintained at positive temperatures (4 °C) for 24 hours, for cured ice cream. In contrast, for the frozen ice cream, the mix was stored at negative temperatures immediately after homogenization.

Regardless of the type of microorganism or the process used for obtaining the ice cream, the number of probiotic cells was significant in all samples according to Trypan blue determination. Thus, we can say that the ice cream with molasses is a good vehicle for probiotics and these microorganisms can survive to negative temperatures.

However, the study on the survival of probiotics in ice cream and their influence should be continued and adapted to industrial production processes involving pasteurization of the mix and adding different flavors, fruits, colouring agents, cocoa, different types of nuts or toppings.

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