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Viability of Probiotics in Frozen Yogurt Supplemented with Inulin and Glycerol

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Abstract: To evaluate the effect of inulin and glycerol supplementation on the viability of probiotic and yogurt bacterial cultures in frozen yogurt, this study was conducted. The frozen yogurt mixture was prepared with different types of probiotics (*Lactobacillus acidophilus* and *Bifidobacterium lactis*) along with commercial yogurt starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). Different concentrations of inulin (2%, 4%, and 6%) and glycerol (1%, 2%, 3%, and 4%) were also supplemented. The cultural survival rates before and after freezing and with 3 weeks regular intervals up to 12 weeks were examined. The viability loss during freezing process of *S. thermophilus* and *L. bulgaricus* were recorded 0.2-0.3 log cycles. While, in probiotic cultures this was observed 0.2-0.5 log cycles in *L. acidophilus* and 0.1-0.2 log cycles in *B. lactis* concentration. The glycerol 4% supplementation in this study has shown most significant improvement in viability ($P<0.05$).

Keywords: Probiotics, Inulin, Glycerol, Frozen Yogurt

1. Introduction

The antibiotic resistant bacteria are one of the major growing health challenges during the treatment of infectious diseases. The unnecessary use of antibiotics lead to the development of this resistant in different microbes. To overcome this issue, now a day there is increasing trend in microbial solution to the microbial problem. One of the most significant methods of treatment during infectious diseases is the use of beneficial microbes (probiotics).

Probiotic are well known beneficial microbes that help in improving the health of the consumer and prevent from different types of diseases i.e. intestinal infection, diarrhea, lactose intolerance, colon cancer, high serum cholesterol level and also boost the immune system when consumed in a sufficient amount [1-6]. These microbes also develop the different types of flavors and improve the nutritional quality of the product. The minimum value of probiotics required to exert these beneficial effects on the consumer is 10^8-10^9 CFU that can be achieved by consuming 100 grams of the product daily, containing 10^6-10^7 live cells/ml or gram of food [7, 8, 9].

Frozen dairy products can be used as a vehicle to deliver the healthy bacterial cultures to the consumers [10, 11]. While the development of probiotic frozen yogurt is a challenging task because the cells must survive and maintain their viability during freezing process and their storage below 0°C [12]. During the production of frozen yogurt, the live cultures have to face a lot of stress which effects their viability [13]. Frozen yogurt also contains a major portion of water which when freezes, leads to the development of ice crystals which prove lethal for the viability of probiotics.

Inulin is a type of prebiotics, which are not digested in human intestine and can be used as a substrate for the development and growth of beneficial intestinal microorganisms. It also acts as water binding compound that improves the texture and other physicochemical properties of the food products [14]. Inulin like prebiotics may exert a

protective effect on the survival and activity of probiotic bacteria during the storage of probiotic foods, as well as during their passage through the GIT [15-17]. A number of researchers have investigated the effect of inulin as a polysaccharide and also a fat replacer on the viability of different strains of probiotics, but the concentrations used were very low and its effect on yogurt starter culture and with combination of cryoprotectant has not been reported.

Keeping in view the above, a study plan was designed to prepare frozen yogurt containing live probiotic cultures and to enhance their survival during freezing and storage different concentrations of inulin and cryoprotectant like glycerol were supplemented in the mixture.

2. Material and Method

The yogurt starter culture YC-X 11® (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and probiotics *Lactobacillus acidophilus* La-5 and *Bifidobacterium lactis* BB-12 (all in Frozen DVS form) were obtained from Chr. Hansen lab (Chr. Hansen Inc. Horsholm, Denmark).

The experiments were carried out at Creamery (Milk plant), School of Food Science and Human Nutrition, Washington State University, Pullman, WA. USA. A total 60 lb. ice cream mix was prepared by weighing and mixing the skim milk powder 2.54 lb., sugar 6.68 lb. (from local grocery store), cream 8.36 lb.(40% milk fat, Dairy Gold Inc. Seattle, WA 98144, USA) and ice pro 0.3 lb. (Grindsted Ice Pro 20055H, Stabilizer & Emulsifier system, Danisco Inc. USA) in 42.90 lb. milk. Inulin (Orafti, L. G. I., Beneo-Orafti, Morris Plains, N. J., U. S. A.) 2%, 4% and 6% along with food grade glycerol (Sigma-Aldrich Co. St. Louis, MO, USA) 0%, 1%, 2%, 3% and 4% were mixed after dividing into twelve parts [18]. After freezing it was stored at -20 °C for further study.

The viability of bacteria in yogurt ice cream were determined before freezing, post-freezing, 3, 6, 9 and after 12 weeks of storage period by serial dilutions in sterilized peptone water and by pour plate method. *Streptococcus thermophilus* concentration was determined by using M 17 agar (Oxoid Co. USA) and incubated at 37°C for 24 hours. *Lactobacillus bulgaricus* concentration was determined by using MRS agar (Oxoid Co. USA) and pH was adjusted by 0.1% HCl to 4.5, *Lactobacillus acidophilus* was enumerated by MRS agar with 1% added sorbitol and concentration of *Bifidobacterium lactis* was determined by MRS NNLP agar. All these plates were incubated at 43°C anaerobically for 72 hours. Identification of each culture was done by Gram staining and biochemical tests and colony morphology [19, 20, 21]. The colony were counted from 25-250 and expressed as colony forming units per gram (CFU/g).

The data was statistically analyzed using the SPSS statistical programme, version 19.0 (SPSS Inc. Chicago, IL 60606). The effect of different formulations was measured by general linear model and storage time by one way ANOVA. The statistical significance was determined at $p < 0.05$. The whole experiment was conducted twice and each test was run in triplicate.

3. Results and Discussion

3.1. Viability of S. Thermophilus and L. Bulgaricus

The results of viability of *S. thermophilus* are given in figure-1. The initial count of *S. thermophilus* was 10.70 log CFU/g, before freezing. After freezing the loss in viability was observed about 5.33% in control sample. When the microbial reduction was compared to inulin (2%, 4% and 6%) supplemented samples the loss were recorded 3.46%, 2.43%, 2.0%; respectively. During the storage period, in first 3 weeks there was non-significant decrease ($P>0.05$), while in 9 and 12 weeks the loss 0.3 and 0.5 log cycle were observed ($P<0.05$). After the storage period of 12 weeks the overall loss was observed 13%, 12.5% and 11.5% with inulin (2, 4 and 6%) supplementation; respectively. The highest decrease with inulin and glycerol were observed in the 12th week, but this decrease was less as compared to control sample ($P<0.05$). The glycerol supplementation (figure-2) has shown most of its effect during freezing process although 1%, 2% and 3% glycerol increase the viability but it was non-significant as compared to 4% ($P<0.05$). After the storage period the gradual loss were observed in all the samples (G1 to G4). The 4% supplementation has shown only 7.9% loss as compared to 11.5% in control sample.

The initial concentration of *L. bulgaricus* before freezing was 10.63 logs CFU/g which decreased during freezing about 3.38%, 2.44%, and 1.79% in inulin (2, 4 and 6%) supplemented samples as compared to 4.9% in control (figure-3). During storage period the microbial viability significantly reduced but the highest reduction 5.56%, 4.28% and 4.92% was observed after 9th weeks. The overall loss after 12 weeks of storage in viability was 17.12%, 15.8% and 14.3%; respectively. The glycerol supplementation (figure-4) has shown non-significant effect on viability loss during freezing process ($P>0.05$). After 12 weeks of storage the glycerol supplemented samples have shown significant decrease in viability with respect to control, but within the G1 to G4 there was non-significant effect ($P>0.05$).

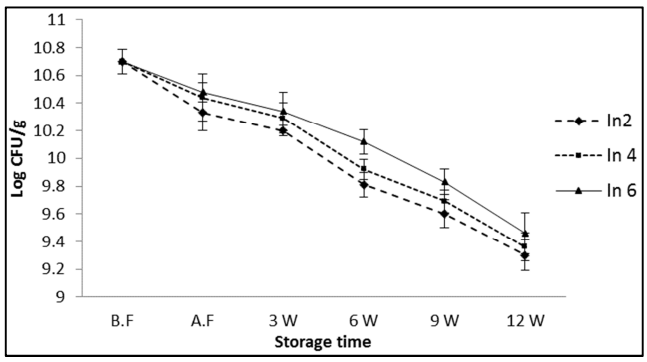

Figure 1. *Survival of S. thermophilus in frozen yoghurt samples (n=6) before freezing (B. F.), after freezing (AF) and after 3, 6, 9, 12 weeks of storage, with 2, 4 and 6% inulin.*

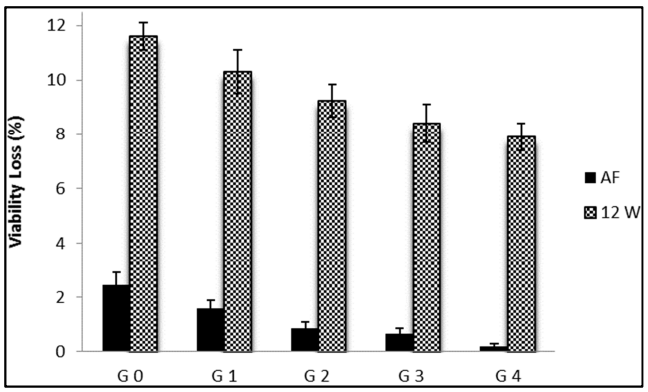

Figure 2. *Viability loss of S. thermophilus in frozen yoghurt samples (n=6), with 0, 1, 2, 3 and 4% glycerol, after freezing (AF) and after 12 weeks of storage.*

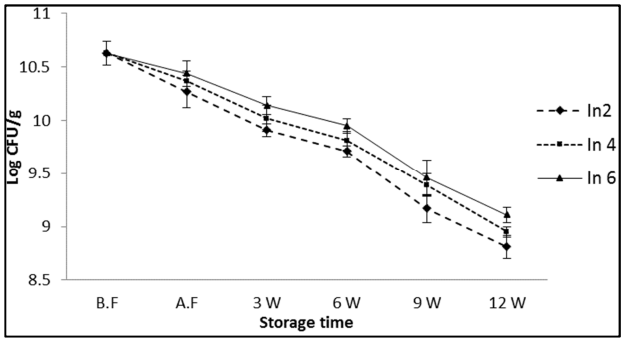

Figure 3. *Survival of L. bulgaricus in frozen yoghurt samples (n=6) before freezing (B. F.), after freezing (AF) and after 3, 6, 9, 12 weeks of storage, with 2, 4 and 6% inulin.*

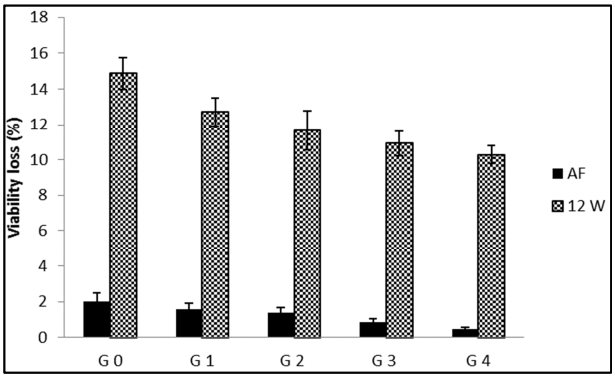

Figure 4. *Viability loss of L. bulgaricus in frozen yoghurt samples (n=6), with 0, 1, 2, 3 and 4% glycerol, after freezing (AF) and after 12 weeks of storage.*

3.2. Viability of L. Acidophilus and B. Lactis

The *L. acidophilus* concentration before freezing was 10.56 log CFU/g and it decreased during freezing process about 4.7%, 3.4% and 2.27% with inulin (2, 4 and 6%) supplementation; respectively, as compared to control 4.17%. At the end of 12 weeks of storage the concentration of *L. acidophilus* decreased up to 26.16%, 21.36% and 17.9%; respectively (figure-5). During freezing process only glycerol 3% and 4% has shown non-significant microbial loss as compared to each other, but significant with respect to control (P<0.05) (figure-6).

The *B. lactis* concentration before freezing was 10.72 logs CFU/g and the microbial loss during freezing was observed 2.0%, 1.6% and 1.2% as compared to control which was 3.64%. During storage period the microbial viability significantly decreased, but the highest reduction (7.35%, 4.55% and 4.1%) was observed after 12th week of analysis (figure-7). The overall microbial reduction during storage after 12 weeks was observed 17.7%, 14.0% and 12.2%; respectively. The glycerol supplementation (figure-8) has shown non-significant effect during freezing process (P>0.05), and after 12 weeks of storage glycerol 3% and 4% has shown most significant effect on the viability (P<0.05). The similar results were reported as loss in *L. acidophilus* La-5 during freezing process 0.5 to 1 log cycles which were further reduced to 1.5 to 2 log cycles during storage period of 60 days [22], while in another study 1.5 to 2.0 log cycles reduction were observed during freezing and 0.3 to 0.9 log cycles after 90 days of storage in both *L. acidophilus* La-5 and *B. lactis* Bb-12 viability [23]. The inulin supplementation also has shown positive effect on maintaining the viability of probiotic in other investigations [24; 25]. However, Pinto *et al.* [26] have observed the results differently, 3.88 log cycles viability loss in first 30 days of storage and 0.25 log cycles in remaining 60 days of storage. Rezaei *et al*. [27] also observed that the samples with 2% inulin significantly improved the viability of *L. acidophilus* and *B. lactis*. The same results were obtained in this study. However, the concentrations of inulin were deafferents.

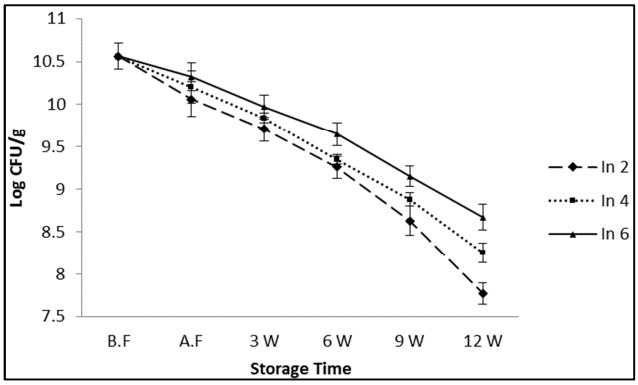

Figure 5. *Survival of L. acidophilus in frozen yoghurt samples (n=6) before freezing (B. F.), after freezing (AF) and after 3, 6, 9, 12 weeks of storage, with 2, 4 and 6% inulin.*

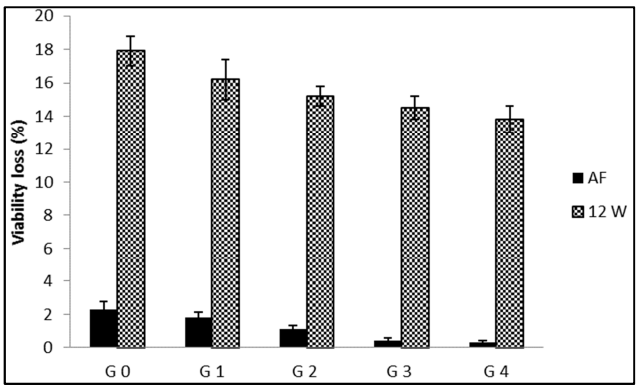

Figure 6. *Viability loss of L. acidophilus in frozen yoghurt samples (n=6), with 0, 1, 2, 3 and 4% glycerol, after freezing (AF) and after 12 weeks of storage.*

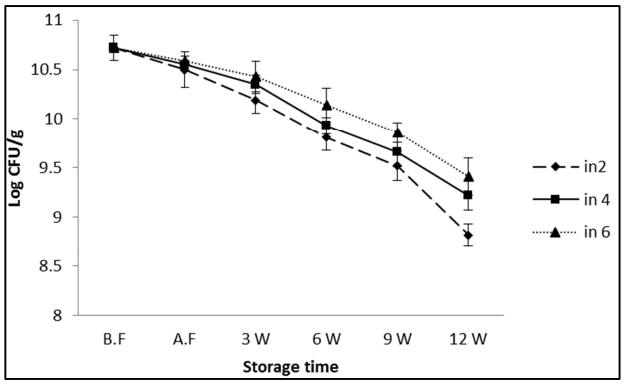

Figure 7. *Survival of B. lactis in frozen yoghurt samples (n=6) before freezing (B. F.), after freezing (AF) and after 3, 6, 9, 12 weeks of storage, with 2, 4 and 6% inulin.*

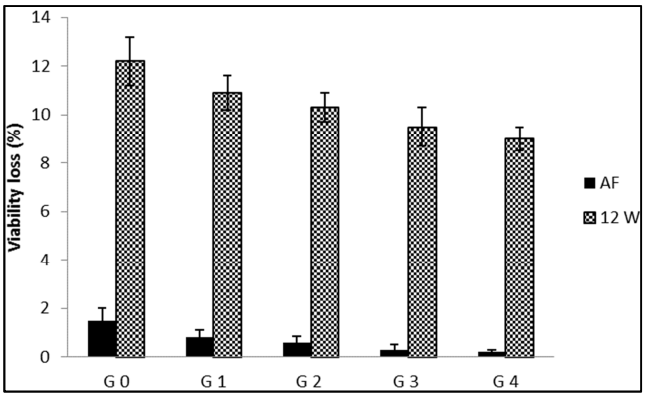

Figure 8. *Viability loss of B. lactis in frozen yoghurt samples (n=6), with 0, 1, 2, 3 and 4% glycerol, after freezing (AF) and after 12 weeks of storage.*

The loss in the viability of bacteria during freezing process might be due to stress caused by mixing of dry and liquid ingredients, production of different acids during incubation. In ice cream machine the frozen yogurt mixture is continuously scraped against the cylindrical surface by the freezer blades. This process not only mixes the air properly but also brakes large air bubbles into smaller size. This mechanical sock may interfere with integrity of the live culture resulting in the death of the cells [13; 28]. On the other hand frozen yogurt is a fluffy product containing 80% - 120% air. As these microbes are mostly anaerobic and lack the oxygen scavenging system. The accumulation of metabolites in the cell may cause the death of the cells. Osmotic difference and thermal shock in the ice cream maker are also lethal to the bacteria [29, 30].

In our study the improvement in viability of probiotics during freezing with inulin and glycerol supplementation may due to hygroscopic nature of these compounds. They might have bound much of the free water and decreased the development of large ice crystals. The small ice crystal causes the less freeze injury to the bacteria and results in the non-significant viability loss [21]. However during storage period, the viability loss may be due to temperature fluctuation. For a long period of storage the minor temperature fluctuation may prove more lethal that damage the microbes [31, 32].

4. Conclusion

The results of our study have shown that inulin and glycerol can be used to improve the viability of the probiotics in frozen yogurt. During freezing process the 6% inulin supplementation has shown more protective effect (P<0.05). The glycerol supplementation also has shown most of its effect during the freezing process. Out of all the glycerol concentration (1%, 2%, 3% and 4%) used in this study, only 4% has shown significant effect in improving the viability as compared to control (P<0.05). So both of them, in combination might have improved the cryoprotectant activity and helped in the better survival of the probiotics in frozen yogurt.

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