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Probiotics viability in frozen yogurt supplemented with oligofructose and glycerol.

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Abstract— *Functional foods are very common nowadays throughout the world due to their nutritional and health benefits. The present study was designed to see the viability of yogurt culture and probiotics in frozen yogurt when supplemented with oligofructose and glycerol. Different types of yogurt culture (Streptococcus thermophilus and Lactobacillus bulgaricus) and probiotics (Lactobacillus acidophilus and Bifidobacterium lactis) were used for the preparation of frozen yogurt mixture. Furthermore, oligofructose (2%, 4%, and 6%) along with glycerol (1%, 2%, 3%, and 4%) were also used to improve the cultures viability. During analysis the viable count before freezing, after freezing and after every three weeks regular interval was done up to 12 weeks. The results have shown that the viability loss of S. thermophilus and L. bulgaricuswere recorded 0.3 and 0.36 log cyclesduring freezing which were further decreased as1.14 and 1.68log cyclesrespectively, at the end of storage. While, in L. acidophilus and B. lactiscultures this reduction wasobserved0.5 and 0.14log cyclesin freezing process which further reduced as 2.29 and 2.81log cycles respectively after 12 weeks.*

Keywords— Lactobacillus acidophilus, Bifidobacterium lactis, oligofructose, frozen yogurt.

I. INTRODUCTION

Probiotics are well known solution to different gastrointestinal infections caused by antibiotic resistant microorganisms. Furthermore, they also help to cure different other types of other health ailments i.e.lactose intolerance, colon cancer, high serum cholesterol level and also boost the immune system (Cheikhyoussefet al. 2008; Cleusixet al. 2008; Delzenneet al.2011; Kumaret al. 2011;Takeda et al.2006). Probiotics also enhance the acceptability of different food items as they produce different types of flavors during fermentation process. The most common type of probiotic bacteria usually used in different dairy products belong to the genera *Lactobacillus* and *Bifidobacterium* (McFarland & Elmer, 2006). Probiotics can only be beneficial to the consumers when taken in amount of 10^6 -10^7 CFU/g or mL of food.

Frozen yogurt belongs to the category of ice cream which has physical attributed similar to ice cream while sensory properties related to cultured dairy products (Soukoulis et al. 2009). This product can be used as a medium to deliver the live culture to the consumers body. But during its processing like ice cream various steps like mixing, homogenization, freezing and air incorporation are involved which create stressful condition for the live cultures and reduced their viability.

Oligofructose is a common type of prebiotics which is non-digestible in our body. It is in-between polysaccharides and simple sugars and has 2-9 repeating unit of fructose. It is hygroscopic in nature, bind much of the free water and improve the physical characteristics of the food. It also impart good effects on health by reducing body weight gain (Parnell& Reimer, 2009), help in the growth of different selective microbes in the gut which produce different types of short chain fatty acids that promote the health of the consumers (Eswaran et al. 2013).

The current research was aimed to prepare frozen yogurt with probiotics and to improve their viability during processing the effect of different concentration of oligofructose and glycerol were investigated

II. MATERIALS AND METHODS

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 research was conducted at Creamery (Milk plant), School of Food Science, Washington State University, USA. The mix was prepared after weighing the skim milk powder, sugar, cream, stabilizer and emulsifier in a required amount, then pasteurized(80°C,10 minutes)and homogenized in APV Homogenizer (Gaulin Inc. Model 400/200, MG-3

TPS, WI, USA). In one quarter of this mixture yogurt starter culture and in another quarter probiotics cultures were propagated. Oligofructose (Orafti P95, Beneo-Orafti, Morris Plains, NJ, USA) was supplemented 2%, 4% and 6% respectively, after that each of the three parts were further divided into five parts and food grade glycerol (Sigma-Aldrich Co. St. Louis, MO, USA) was added at 0%, 1%, 2%, 3% and 4%. Each of the mixture was aged at 4°C for 24 hours and then was frozen in a batch ice cream maker (TAYLOR, model 103, Rockton, IL, USA) separately and packed in 4 oz cups. The frozen yogurt ice cream was hardened at -35 °C for 24 hours and then stored at -20 °C for further study (Muzammil et al. 2017).

The bacterial population in frozen yogurt were measured before freezing, post-freezing and after three weeks intervals up to 12 weeks of storage study by pour plate method after making serial dilutions in sterilized peptone water. *Streptococcus thermophiles* concentrations were counted on M 17 agar (Oxoid Co. USA) after twenty four hours of incubation at 37°C while concentrations of *Lactobacillus bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium lactis* were counted on MRS agar (Oxoid Co. USA) with pH 4.5(adjusted by 0.1% HCl), MRS agar (with 1% sorbitol) and on MRS NNLP agar for seventy two hours at 43°C respectively(Tabasco et al. 2007;Tharmaraj&Shah 2003;Muzammilet al. 2015).The plates with colonies (25-250) were selected for counting and bacterial concentration were expressed as colony forming units per gram (CFU/g).

After the experiment the collected data were analyzed with SPSS statistical programme, version 19.0 (SPSS Inc. Chicago, IL 60606). General linear model was used to see the effects of oligofructose and glycerol supplementation on cultures viability and one way ANOVA was used to record their effects during storage period at significance level $p < 0.05$. Each trial was performed twice and each experiment was conducted in triplicate.

III. RESULTS AND DISCUSSION

3.1. Yogurt culture viability

The initial count of *S. thermophiles* was 10.84 log cycles (fig-1). The freezing process has shown the loss in viability 5.44 % in control while the same decrease in oligofructose (2, 4 and 6%) supplemented samples were 2.78%, 1.75% and 1.30%; respectively. In storage study during first 6 weeks there was non-significant decrease (*P*>0.05), while in 9 and 12 weeks the loss 0.34 and 0.48 log cycle were observed (*P*<0.05). After the storage period of 12 weeks the overall loss was observed 10.84%, 9.5% and 7.66% with oligofructose (2, 4 and 6%) supplementation; respectively. The glycerol supplementation (fig-2) has shown most of its effect during freezing process although 1% and 2% glycerol improve the viability but it was non-significant as compared to 3% and 4% (*P*<0.05).

The *L. bulgaricus* concentration before freezing was 10.69 log cycle. The stress in freezing process caused the death of the bacterial cells. The concentration of *L. bulgaricus* decreased to 0.36, 0.28 and 0.19log cycles in oligofructose (2%, 4% and 6%) added frozen yogurt as compared to control 0.63 log cycles (fig-3). During storage period after 9th weeks the maximum reduction 4.78%, 2.70% and 2.36% in microbial viability was observed. The overall loss after 12 weeks of storage in viability was 16.26%, 12.68% and 11.71%; respectively. During freezing process and storage period the glycerol effect on microbial viability was non-significant (*P*>0.05) and at the end of study all the samples have shown almost similar loss in viability(fig-4).

3.2 Probiotics culture viability.

The initial count of *L. acidophilus* in yogurt ice cream mixture, was 10.56 log cycle. In freezing process the live cells decreased to 0.67 log cycles without oligofructose (fig-5). The bacterial population with 2, 4 and 6 % oligofructose decreased to 0.5, 0.36 and 0.24log cycle; respectively. In the storage period of 12 weeks there was significant decrease in *L. acidophilus* viability with the passage of time (P<0.05). The overall loss in frozen yogurt with oligofructose(2%, 4% and 6%) were observed 22.7%, 20.09% and 15.% ; respectively. The added cryoprotectant (fig-6) increased the viability with increasing its concentration and 4% showed the best survival (P<0.05).

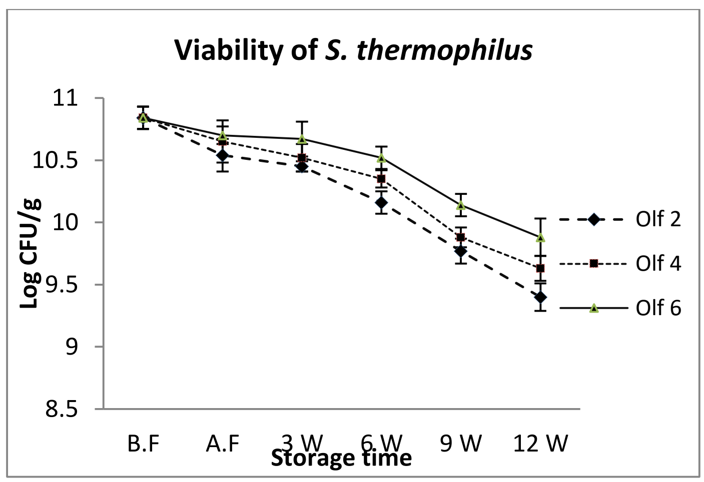

Fig. 1:Survival of S. thermophilus in frozen yoghurt before freezing (B.F.), after freezing (AF) and after 3, 6, 9, 12 weeks of storage, with 2, 4 and 6% oligofructose.

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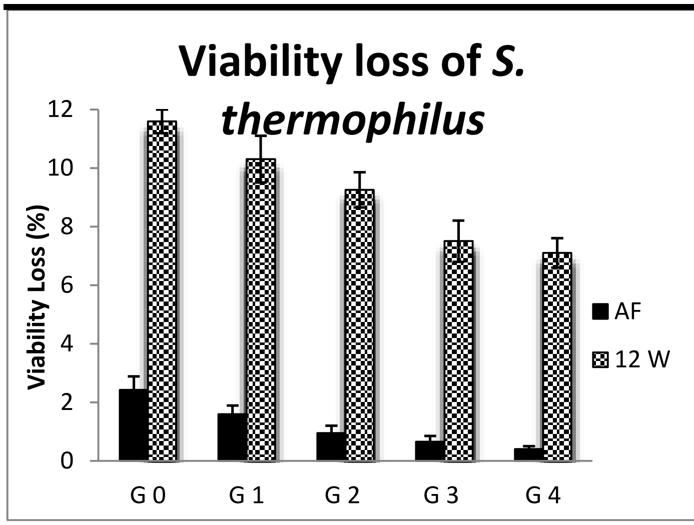

Fig. 2: Viability loss of S. thermophilus in frozen yoghurt with 0, 1, 2, 3 and 4% glycerol, after freezing (AF) and after 12 weeks of storage.

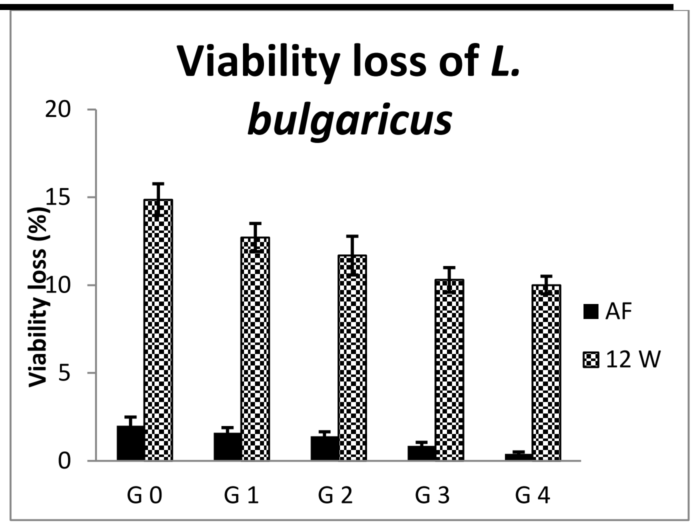

Fig. 4: Viability loss of L. bulgaricus in frozen yoghurt with 0, 1, 2, 3 and 4% glycerol, after freezing (AF) and after 12 weeks of storage.

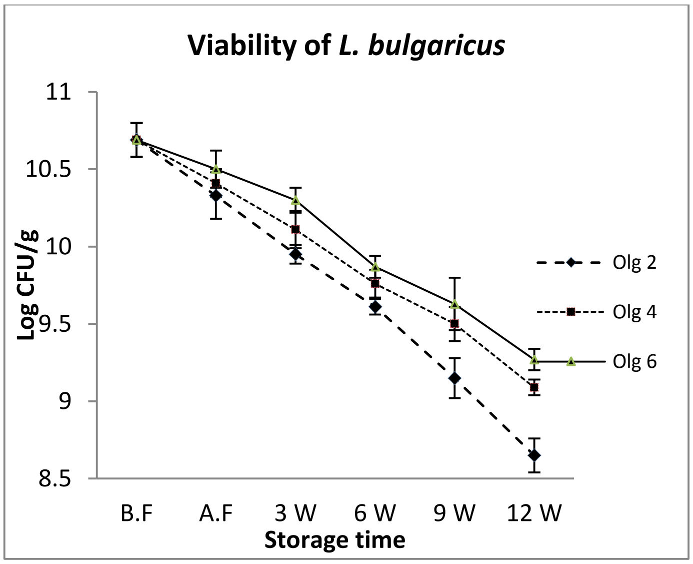

Fig. 3: Survival of L. bulgaricus in frozen yoghurt before freezing (B.F.), after freezing (AF) and after 3, 6, 9, 12 weeks of storage, with 2, 4 and 6% oligofructose.

The *B. lactis*pre-freezing concentration was 10.93 log cycle. In the freezing process the bacterial death was 0.14, 0.09 and 0.06 log cycle with 2, 4 and 6% oligofructose addition as compared to control, 0.6 log cycle (fig-7). Glycerol also helped in decreasing the damage to bacteria caused by the different factors in the freezing ($P<0.05$) (fig-8). During the storage, in first 6^{th}weeks although the loss in B. lactispopulation was very low but it was statistically significant ($P<0.05$). During the storage from 9 to 12^{th}weeks there was almost 0.8 log cycle decreased in *B. lactis* viability with oligofructose and glycerol whereas in control this decreased was 1.15 log cycle.

At the end of 12 week storage the final concentration in control as well as 2, 4 and 6% oligofructose supplemented yogurt was 7.99, 8.61, 8.93 and 9.55; respectively.The similar results were reported by Akalin and Eriser(2008)the viability of probiotics *L.acidophilus* La-5 and *B. animalis*Bb-12 were improved by 9.05 % and 12.12 % with the addition of 4% oligofructose in low fat ice cream. In another study Oliveira et al. (2009) reported the oligofructose perform the best with 7.97 log cycles as compared to control 7.31 log CFU/ml after 24 hours of storage. In the current study the loss in the viability of bacteria during freezing process might be due to stress caused by mechanical mixing and freezing injuries. In the ice cream machine the movement of the blades not only blend the air in the mixture but also brakes large air bubbles. This mechanical sock may interfere with integrity of the live culture resulting their death (Magarinoset al.2007; Ranadheeraet al. 2013). Furthermore the air incorporation also interfere with the anaerobic probiotics which already lack the oxygen scavenging system. The accumulation of metabolites in the cell may cause the death of these microbes.

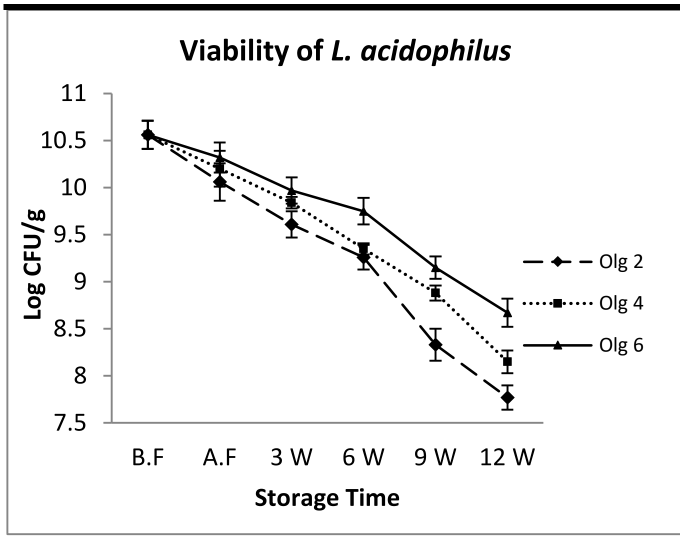

Fig. 5: Survival of L. acidophilus in frozen yoghurt before freezing (B.F.), after freezing (AF) and after 3, 6, 9, 12 weeks of storage, with 2, 4 and 6% oligofructose.

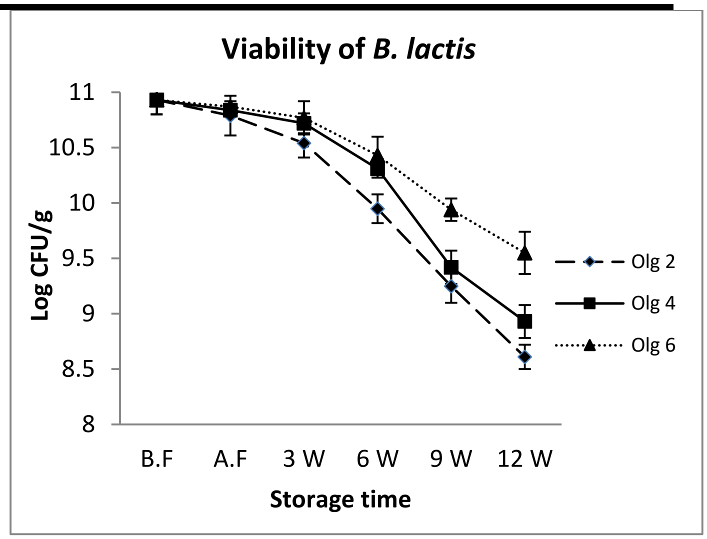

Fig. 7: Survival of B. lactis in frozen yoghurt before freezing (B.F.), after freezing (AF) and after 3, 6, 9, 12 weeks of storage, with 2, 4 and 6% oligofructose.

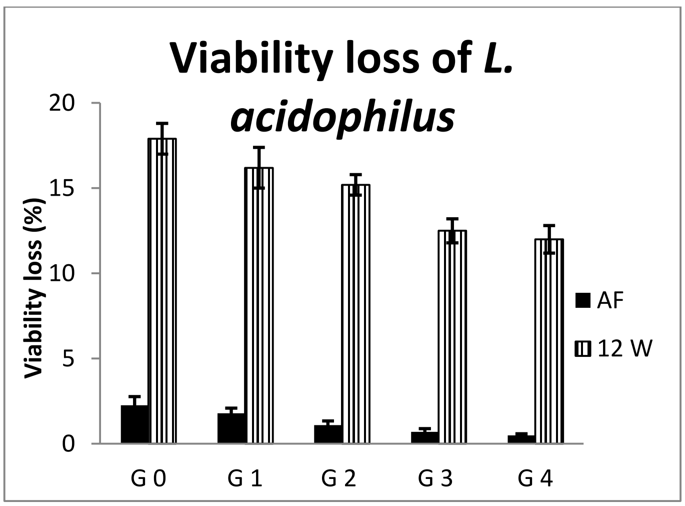

Fig. 6: Viability loss of L. acidophilus in frozen yoghurt with 0, 1, 2, 3 and 4% glycerol, after freezing (AF) and after 12 weeks of storage.

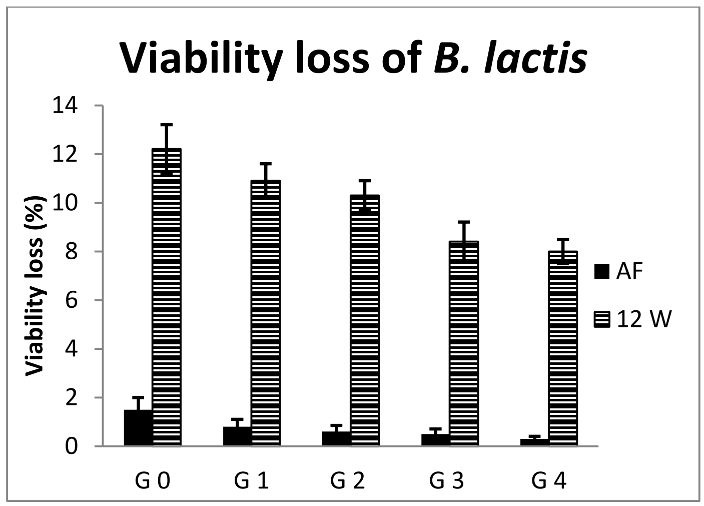

Fig. 8: Viability loss of B. lactis in frozen yoghurt with 0, 1, 2, 3 and 4% glycerol, after freezing (AF) and after 12 weeks of storage.

While the improvement in viability of probiotics may be due to water binding effect of oligofructose and glycerol. They might have decreased the availability of free water and also retard the growth of large ice crystals. The small ice crystal caused the less freeze injury to the bacteria and resulted in the lower viability loss (Muzammil et al. 2015).

IV. CONCLUSION

The results in the current investigation have clearly shown that oligofructose and glycerol supplementation have positive effect on maintaining the viability of probiotics above the recommended level. Oligofructose with 6% supplementation has more protective effect on the tested probiotics (*P*<0.05). oligofructose has shown most of its effect in the freezing process. Out of all the used concentrations (1%, 2%, 3% and 4%), 4% glycerol has shown significant effect to maintain the viability of probiotics(*P*<0.05).

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