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Full Length Article

Viability of Probiotics in Frozen Yogurt with different levels of Overrun and Glycerol

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Abstract

The effects of overrun and glycerol as a cryoprotectant on viability of probiotics in frozen yogurt were investigated. Frozen yogurt was prepared with different types of probiotics (*Lactobacillus acidophilus* and *Bifidobacterium lactis*) along with normal yogurt starter culture (*Streptococcus salivarius ssp. thermophiles* and *L. delbrueckii ssp. bulgaricus*). Frozen yogurt mixture was supplemented with 1.5, 3 and 4.5% glycerol and was frozen with 60, 80 and 100% overrun. Viability of probiotic bacteria after freezing and during storage period of 12 weeks was investigated. Supplementation of glycerol in the mixture has improved the survival of *L. acidophilus* and *B. lactis* during the frozen storage. At the end of storage period the overall loss in viability of the *L. acidophilus* with 60, 80 and 100% overrun was 1.81, 1.96 and 2.24 log CFU/g, respectively. The *B. lactis* viability loss with 60, 80 and 100% overrun was 2.11, 2.26 and 2.26 log CFU/g, respectively. All the supplemented probiotics maintained the minimum concentration of 10^7CFU/g in frozen yogurt during frozen storage, which is required for beneficial health effects in the host.

Keywords: Probiotics; Glycerol; Overrun; Frozen yogurt

Introduction

The changing life style has also changed the eating behavior of the people. Now people prefer to consume the food that not only meet their nutritional requirement but also improve their health. This preference leads to the development of functional foods. Probiotics are one of the major constitutes of the functional foods. Probiotics may be defined as the live microorganism which when administered in a sufficient quantity help in improving the health of the host (FAO/WHO, 2001). The minimum concentration of probiotics recommended to attain the desirable health benefits is 10^6-10^7 CFU/g or mL of food at the time of consumption (Hekmat and Mcmahon, 1992; Gomes and Malcata, 1999; Boylston *et al*., 2004). Bacteria form *Lactobacillus* and *Bifidobacterium* genera are most commonly used as probiotics in dairy products (Mohammadi *et al*., 2011).

Ice cream is a very popular dairy product that can be used as a vehicle to deliver the healthy bacterial cultures to the consumers. It can be manufactured with various concentrations of different ingredients and then mixed with various ratios of probiotic cultures (Hekmat and Mcmahon, 1992; Davidson *et al*., 2000). There are a lot of factors that determine the growth and viability of the probiotic microbes in frozen dairy products. The factors are total solids, pH, acidity, time and temperature during freezing, physical damage during mixing, storage time and temperature and overrun (Akin *et al*., 2007; Homayouni *et al*., 2008).

Overrun is normally referred as the incorporation of air in the ice cream, to give it the desirable characteristics. It provides the lightness in the body, smoothness in the texture and affects the melting properties of ice cream (Sofjan and Hartel, 2004).

Freezing is very important step in frozen yogurt production. In the freezing process water is converted into ice crystals. During this process many species of bacteria lose viability due to damage in their cell wall and membrane caused by growing ice crystals in and outside the cell. Further this damage also change cell membrane permeability and intracellular dehydration that results in the inactivation of microbes (Gill, 2006; Akin *et al*., 2007). This effect can be minimized by using different types of cryoprotectants. The cryoprotectants accumulate within the cell and decrease the osmotic difference with their external environment (Kets *et al*., 1996). However, there is little information available on the use of cryoprotectant like glycerol in the frozen yogurt. So a study plan was designed to prepare the frozen yogurt having live probiotic culture and glycerol was supplemented in various concentrations in the mixture before freezing. Different levels of overrun were

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used to see the effect of air incorporation on viability of probiotics during freezing and storage in frozen yogurt. Viability of each bacterium in frozen yogurt was evaluated at regular interval up to three months of frozen storage.

Materials and Methods

Strains of Probiotics

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

Frozen Yogurt Preparation

The experiment was 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 mixture 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 and Emulsifier system, Danisco Inc. USA) in 42.90 lb. milk, with continuing agitation at 45°C. The mixture was pasteurized at 80°C for ten min, homogenized at 60°C in APV Homogenizer (Gaulin Inc. Model 400/200, MG-3 TPS, WI, USA) at 2000 psi single stage. After homogenization one fourth of this mixture was used to make the yogurt by adding yogurt starter culture and other one fourth was used to propagate the probiotics by adding the LA-5 and BB-12 at the rate of 0.02% and incubating at 43°C for 4 h. The yogurt and probiotic fermented milk was remixed and divided into four parts. First part was taken as control part while in second, third and fourth part food grade glycerol (Sigma Chemical, Inc. USA) was supplemented in 1.5, 3.0 and 4.5% concentrations, respectively. Each of the mixture was aged at 4°C for 24 h and was frozen in a continuous ice cream maker (Technogel, Freezer 100, Italta) by injecting sterilized air in 60, 80 and 100% under pressure. Yogurt ice cream was packed in 500 mL cups and hardened at -35°C for 24 h. All the samples were stored at -20°C in a room refrigerator for further study.

Enumeration of Bacteria

The viability of bacteria in yogurt ice cream was determined before freezing, post-freezing and after 3, 6, 9 and 12 weeks of storage period by serial dilutions in sterilized peptone water and by pour plate method. Live cells of *L. acidophilus* were enumerated by MRS agar (Oxoid Co. USA) with 1% sorbitol supplementation and *Bifidobacterium lactis* with MRS-NNLP agar (NNPL solution: Neomycin sulphate 100 mg, Paromomycin 200 mg; Nalidixic acid 15 mg, LiCl 3 g per liter of media). 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 (Tharmaraj and Shah, 2003; Tabasco *et al*., 2007). The colony were counted from 25-250 and expressed as colony forming units per gram (CFU/g).

Statistical Analysis

The data obtained in the experiments were statistically analyzed using the SPSS statistical programme, version 19.0 (SPSS Inc. Chicago, IL 60606). The effect of different formulations on viability of bacteria was measured by general linear model. Effect of storage time on viability of bacteria was analyzed by one way ANOVA. The comparison among treatment means were done through Duncan's Multiple Range Test and statistical significance was determined at $p < 0.05$. The whole experiment was conducted twice and each test was run in triplicate.

Results

The viability of the *L. acidophilus* during freezing and storage is given in Table 1. The initial bacterial count of *L. acidophilus* in the mixture was 10.62 log CFU/g. The probiotic viability loss during frozen storage was significantly observed for 12 weeks ($P<0.05$). The viability reduction in control (without glycerol) sample with 60% overrun was 17% and with increasing the overrun amount this loss increased up to 18% (with 80% overrun) and 21% (with 100% overrun). Glycerol supplementation helped in better survival of the probiotics and the decreased the probiotic loss. The highest effect was observed with 4.5% glycerol supplementation that decreased the microbial reduction to almost 23% (with 60% overrun), 16% (with 80% overrun) and 23% (with 100% overrun), respectively (Fig. 1). During the storage period the microbial reduction increased after 6 weeks, during the 9th week of storage this loss was from 7 to 16% and in 12th week from 13 to 21% (Table 1).

The initial count of *B. lactis* in frozen yogurt mixture was 10.76 log CFU/g (Table 2) and overrun has shown more effect on viability of *B. lactis* than *L. acidophilus* during freezing process. The viability loss of *B. lactis* was observed significant ($P<0.05$) during the storage period. The overall loss in viability of *B. lactis* after 12 weeks of storage was 19% with 60% overrun while the microbial reduction increased with 80% overrun (to 21%) and 100% overrun (to 24%). Glycerol supplementation decreased the viability loss of frozen yogurt below 0ºC. The glycerol supplementation 4.5% have shown more effect than all the other concentration used and decreased the microbial reduction to almost 15% (with 60% overrun), 19% (with 80% overrun) and 25% (with 100% overrun), respectively (Fig. 2).

Table 1: Viability of *Lactobacillus acidophilus* in yogurt ice cream with different overrun and glycerol levels

Glycerol	Overrun	After Freezing	3 Weeks	6 Weeks	9 Weeks	12 Weeks
	60%	10.21 ± 0.12^{abA}	9.66 ± 0.03^{abcAB}	9.84 ± 0.10^{bcdB}	9.42 ± 0.09^{bcdC}	8.81 ± 0.07^{bcD}
Control	80 %	10.24 ± 0.06^{abA}	9.63 ± 0.08^{abB}	9.74 ± 0.04^{bB}	9.13 ± 0.02^{abC}	8.66 ± 0.12^{bD}
	100%	10.15 ± 0.07^{aA}	9.92 ± 0.05^{aAB}	9.61 ± 0.08^{aB}	8.93 ± 0.02^{AC}	8.38 ± 0.04^{aD}
	60%	10.43 ± 0.11^{abA}	10.30 ± 0.03^{cdeB}	9.93 ± 0.09^{deC}	9.58 ± 0.12^{deC}	8.89 ± 0.09^{bcD}
G 1.5%	80 %	10.41 ± 0.04^{abA}	10.25 ± 0.08^{bcdeA}	9.87 ± 0.02^{bcdB}	9.45 ± 0.07^{cdC}	8.78 ± 0.04^{bcD}
	100%	10.34 ± 0.06^{abA}	10.10 ± 0.17^{abcdB}	9.78 ± 0.09^{bcC}	9.02 ± 0.02^{aD}	8.63 ± 0.11^{bE}
	60%	10.47 ± 0.04^{abA}	10.42 ± 0.09^{deA}	9.95 ± 0.03^{deB}	9.64 ± 0.10^{deC}	8.94 ± 0.03^{cD}
G 3%	80 %	10.45 ± 0.16^{abA}	10.37 ± 0.04^{deA}	9.86 ± 0.07^{bcdB}	9.47 ± 0.03^{cdC}	8.86 ± 0.06^{bcD}
	100%	10.40 ± 0.18^{abA}	10.17 ± 0.09^{abcdA}	9.75 ± 0.08^{bB}	9.23 ± 0.06^{abcC}	8.73 ± 0.09^{bcD}
	60%	10.58 ± 0.09^{bA}	10.44 ± 0.12^{eA}	10.02 ± 0.11^{eB}	9.81 ± 0.07^{Eb}	9.24 ± 0.05^{dC}
G 4.5%	80 %	10.53 ± 0.10^{abA}	10.40 ± 0.09^{deA}	9.92 ± 0.07^{cdeB}	9.70 ± 0.9^{deB}	8.97 ± 0.03^{cC}
	100%	10.53 ± 0.08^{abA}	10.11 ± 0.03^{abcdB}	9.88 ± 0.06^{bcdC}	9.57 ± 0.11^{deD}	8.87 ± 0.07^{bcE}

Means ± standard deviation
^{abc}Means within the same column with different letters are significantly different ($P<0.05$)
^{ABC}Means within the same row with different letters are significantly different ($P<0.05$)
G = Glycerol (%)
Initial bacterial count =10.62±0.13

Table 2: Viability of *Bifidobacterium lactis* in yogurt ice cream with different overrun and glycerol levels

Glycerol	Overrun	After Freezing	3 Weeks	6 Weeks	9 Weeks	12 Weeks
	60%	10.48 ± 0.14^{aA}	10.08 ± 0.05^{aB}	9.81 ± 0.07^{bcdC}	8.99 ± 0.09^{abD}	8.65 ± 0.10^{bcdE}
Control	80 %	10.46 ± 0.09^{aA}	9.97 ± 0.02^{aB}	9.75 ± 0.09^{bcB}	8.92 ± 0.05^{abC}	8.50 ± 0.06^{bD}
	100%	10.43 ± 0.06^{aA}	9.92 ± 0.05^{aB}	9.51 ± 0.12^{aC}	8.80 ± 0.03^{aD}	8.11 ± 0.11^{aE}
	60%	10.54 ± 0.11^{aA}	10.46 ± 0.03^{cdA}	9.89 ± 0.07^{cdeB}	9.55 ± 0.05^{cdC}	8.81 ± 0.06^{defD}
G 1.5%	80 %	10.54 ± 0.04^{aA}	10.14 ± 0.07^{abB}	9.80 ± 0.09^{bcdC}	9.41 ± 0.10^{cdD}	8.74 ± 0.08^{cdefE}
	100%	10.44 ± 0.02^{aA}	9.95 ± 0.040^{aB}	9.70 ± 0.11^{bB}	8.70 ± 0.05^{aC}	8.62 ± 0.07^{bcC}
	60%	10.62 ± 0.06^{aA}	10.56 ± 0.02^{cdA}	9.95 ± 0.03^{deB}	9.65 ± 0.12^{cdC}	8.91 ± 0.11^{fgD}
G 3 %	80 %	10.57 ± 0.07^{aA}	10.36 ± 0.09^{bcA}	9.84 ± 0.05^{bcdeB}	9.49 ± 0.11^{cdC}	8.84 ± 0.10^{efgD}
	100%	10.49 ± 0.02^{aA}	9.99 ± 0.04^{aB}	$9.80\pm0.08^{bcd\,B}$	9.25 ± 0.2^{bcC}	8.71 ± 0.09^{cdeD}
	60%	10.74 ± 0.08^{aA}	10.69 ± 0.02^{dA}	$10.14\pm0.11^{f\,B}$	9.81 ± 0.03^{dC}	8.99 ± 0.06^{gD}
G 4.5%	80 %	10.72 ± 0.11^{aA}	10.63 ± 0.14^{dA}	9.99 ± 0.05^{eB}	9.71 ± 0.07^{dC}	8.91 ± 0.09^{fgD}
	100%	10.67 ± 0.10^{aA}	10.57 ± 0.11^{cdA}	9.90 ± 0.09^{cdeB}	9.54 ± 0.13^{cdC}	8.78 ± 0.06^{cdefD}

Means ± standard deviation
^{abc}Means within the same column with different letters are significantly different ($P<0.05$)
^{ABC}Means within the same row with different letters are significantly different ($P<0.05$)
G = Glycerol (%)
Initial bacterial count =10.76±0.10

During the storage the highest viability loss was observed in 9th week from 8 to 19% and 12th week from 16 to 24% (Table 2).

Discussion

These results are similar to Magarinos *et al*. (2007), who reported the decrease in viability of *L. acidophilus* during freezing and hardening was 0.63 log CFU/g (with 108% overrun) and 0.75 log CFU/g (with 106% overrun) in *B. lactis* concentration. The loss in mix culture of *L. acidophilus* and *B. lactis* was 0.82 log CFU/g with 108% overrun while in another study the viability loss of *L. acidophilus* were 14% (with 60% overrun) and 24% (with 90% overrun) after 60 days of storage period (Ferraz *et al*., 2012).

These results indicate the different types of stress play a great influence on the viability of probiotics in the frozen yogurt. The frozen yogurt mixture is subjected to mechanical sharing, cold stress and forcefully incorporation of air that affect the viability negatively. Ice crystal formations in and outside the cell and accumulation of the solutes in extracellular medium cause lethal injuries by damaging the cell membrane and cell wall that result in the cell death (Gill, 2006). The supplementation of glycerol in this study may bind much of the free water present in the mixture and prevent the formation of large ice crystals during freezing process. This would help in better survival of the probiotics in frozen yogurt.

On the other hand frozen yogurt contain high amount of oxygen that may be incorporated during various steps like, continuous agitation during mixing of ingredients, in homogenization process and during process of freezing air is injected under pressure in ice the cream maker. During storage oxygen may also penetrate from the packaging material. This oxygen rich environment can affect the viability of probiotic bacteria (Brunner *et al*., 1993; Ishibashi and Shimamura, 1993; Miller *et al*., 2002). Oxygen tolerance is different in different strains of probiotics (Kawasaki *et al*., 2006). Probiotics are gut origin where the environment is anaerobic, The oxygen as such does not harm the bacteria but when it is converted into different reactive compounds like superoxide (O_2^-) and hydroxyl anion (OH^-) in the

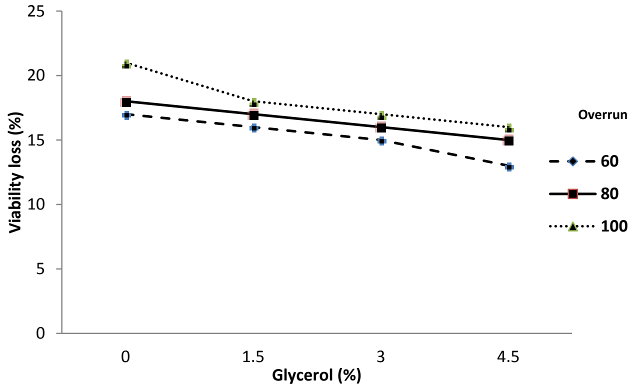

Fig. 1: Viability loss (%) of *L. acidophilus* after 12 weeks of storage period with different overrun and glycerol levels

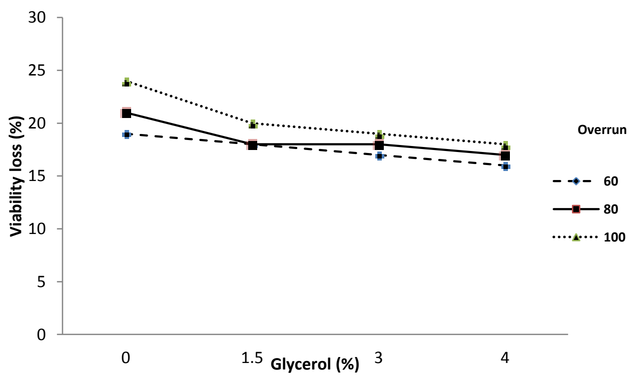

Fig. 2: Viability loss (%) of *B. lactis* after 12 weeks of storage period with different overrun and glycerol levels

cell and leads to bacterial death (Talwalkar and Kailasapathy, 2003; Corcoran *et al*., 2008). These toxic compounds upon reacting with the major components of the cell like nucleic acid, lipids and proteins cause death of the cell (Ferraz *et al*., 2012). The solution to these problems along with using cryoprotectant, using the less oxygen sensitive strains of probiotics and is microencapsulation of the probiotics in frozen dairy products (Godward and Kailasapathy, 2003; Homayouni *et al*., 2008; Burgain *et al*., 2011).

Conclusion

The results have shown that the oxygen stress is one of the important factors that negatively influence the viability of probiotics in frozen yogurt. The viability has decreased with increasing the overrun amount and vice versa. Sixty present overrun has shown the minimum loss in the *L. acidophilus* and *B. lactis* viability. The damage caused by ice crystal formation can be minimized by supplementation of cryoprotectant. The glycerol supplementation as a cryoprotectant helped in the better survival during frozen storage. The glycerol addition has shown its highest effect in frozen yogurt with 100% overrun.

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