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PHYSICOCHEMICAL PROPERTIES OF PROBIOTIC FROZEN YOGURT WITH DIFFERENT LEVELS OF GLYCEROL AND OVERRUN

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ABSTRACT

The study was design to investigate the effects of overrun and glycerol supplementation on physicochemical properties of probiotic frozen yogurt. Frozen yogurt was prepared containing different types of probiotics (*Lactobacillus acidophilus* and *Bifidobacterium lactis*) along with yogurt starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). In frozen yogurt mixture glycerol in 1.5, 3 and 4.5% concentrations were supplemented and frozen with 60, 80 and 100 % overrun. Frozen yogurt was stored at -20ºC after production and hardening. Physicochemical analysis was carried out in the mixture before freezing while melting rate, microstructure and textural analysis were carried out after one week of frozen storage. Melting rate and stickiness significantly decreased with increased overrun levels ($P<0.05$) while glycerol supplementation has shown non-significant effect on melting rate and stickiness ($P<0.05$). Hardness of the frozen yogurt was highest (43.57 Newton) with 60% overrun and 0% glycerol ($P<0.05$). Air cell size and ice crystal subsequently decreased with increased overrun and glycerol levels ($P<0.05$). The 100% overrun and 4.5% glycerol supplementation have shown the better effects on the improvement of physicochemical properties of the probiotic frozen yogurt.

INTRODUCATION

Frozen yogurt is a unique dairy product with physical properties related to ice cream while nutritional and sensory characteristics are similar to fermented dairy products (Soukoulis and Tzia, 2008). Frozen yogurt has evolved when the consumer started preferring low acidic foods over high acidic foods in late 1970s (Chandan and Shahani, 1992). Frozen yogurt may be a good choice for the athletes and people with high risk of obesity. Being low in cholesterol it may be good for consumption in other chronic diseases like cardiovascular problems and lactose intolerance (Marshall et al., 2003; Tamime and Robinson, 2007). Furthermore, the supplementation of probiotic culture into frozen yogurt may increase its beneficial effects on the host. Frozen yogurt may be manufactured with ice cream ingredients like stabilizers, emulsifiers, and sweeteners, solid not fat and fat. Each ingredient has its own effect on physical properties of frozen dairy products. Stabilizers control the recrystallization and heat shock during hardening and frozen storage from temperature fluctuation. Solid not fat and hydrocolloids help in better whipping and holding the air during overrun, shape retention, building body and texture, water binding, improving viscosity and melting properties (Martinou-Voulasiki and Zerfiridis, 1990; Marshall *et al*., 2003). Emulsifiers improve the whipping quality and texture, smoothness in the body and produce drier ice cream with good stand-up quality and melting resistant (Goof, 1988). The overrun normally refer to 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 influences the melting properties office cream. The amount of air along with distribution and size of the air cells lead to the desirable physical properties in ice cream (Sofjan and Hartel, 2004). If small amount of air is incorporated the resulting product will be hard with heavy body and difficult to spoon, if overrun percentage is too high, the resulting product will be fluffy (Alamprese and Foschino, 2011). The quantity of overrun also affects the ice crystal size. If overrun percentage is low than the resulting ice cream has large ice crystal size and vice versa

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(Arbuckle, 1977). Furthermore, the consumers only accept the food products with flavor and texture properties related to the traditional ones, instead of their nutritional and health effects (El-Nagar *et al.,* 2002). So it is very necessary to consider the physicochemical properties along with the nutritional profile and therapeutic value during the development of new dairy product for its good acceptability. However, much of the previous studies were carried out on ice cream. There is little information available on the effects of glycerol supplementation as a cryoprotectant on the physicochemical properties of probiotic frozen yogurt. So the present study was design to investigate the effect of both overrun and glycerol on the physicochemical properties of frozen yogurt.

MATERIALS AND METHODS

Strains of probiotics

The yogurt starter culture YC-X 11 containing *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. Milwaukee, WI, USA).

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. 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 & Emulsifier system, Danisco Inc. USA) in 42.90 lb. milk, with continuing agitation at 45°C and then pasteurized at 80°C for ten minutes, 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 hours. The yogurt and probiotic fermented milk was remixed and divided into four parts. First part (F 1) was taken as control batch while in second (F2), third (F3) and fourth (F4) batch food grade glycerol (Sigma Chemical, Inc. USA) were supplemented in 1.5 %, 3.0 % and 4.5 % concentrations; respectively. Each of the mixture was aged at 4°C for 24 hours. Each batch at the time of freezing was further divided into three parts and was frozen in a continuous ice cream maker (Technogel, freezer 100, Italta) by injecting sterilized air in 60%, 80 % and 100% under pressure; respectively. Frozen yogurt was packed in 500 mL cups and hardened at -35°C for 24 hours. All the samples were stored at -20°C in a room refrigerator for further study.

Physicochemical Analysis of frozen yogurt

The total solids in the frozen yogurt mixture were determined by AOAC method (AOAC 2005, Official method 990.19). Fat contents were determined by Babcock method (AOAC 1995, Official Method 989.05). pH was measured by digital pH meter (Accumet AB-15 pH meter; Fisher Scientific). Titratable acidity was measured by AOAC Method (AOAC 1995, Official Method 942.15). Protein contents in the yogurt ice cream were measured by nitrogen analyzer (LECO. CHN 628, St. Joseph, MI, USA). Samples were run in the triplicate to calculate the nitrogen percentage. Nitrogen % was multiplied with 6.38 (for dairy products) to get the protein percentage.

Melting Rate

The melting rate was calculated by taking 60 g frozen yogurt sample and placing it on a wire mesh over a beaker at 23°C. The melted frozen yogurt was collected in the beaker. After 90 minutes the melted sample and residual sample both were weighed. The melting rate was calculated by dividing the melted sample over time (g/min). Melting rate was calculated after one week of frozen storage.

Texture Analysis

The texture of the yogurt ice cream was measured after one week of frozen storage by texture analyzer (Stable Microsystems Ltd, TA.XT-2, UK). The stainless steel cylindrical probe of 5 mm diameter attached to 25 kg cell with the speed of 1mm/sec was allowed to penetrate up to the depth of 10 mm at room temperature. The hardness was measured as the force required for penetration while the stickiness was measured as the force required for withdrawal of the probe.

Micro structure

The micro structure of the frozen yogurt was analyzed by Scanning electron microscope after freeze drying the samples. The frozen yogurt samples were cut at -20°C into 6 cm cubes and freeze dried in a freeze dryer (Virtis freeze mobile 24 with unitop600L, Vir-Tis SP Industries Co. NY). The shelf temperature was -20°C with 20 Pa vacuum and condenser temperature -60°C. The samples were dried for 24 hours. The freeze dried samples were then analyzed by Scanning electron microscope (SEM, FEI 200F Quanta, FEI Co. Hillsboro, OR, USA) at 20.00 kV and 250x magnification. The analysis was carried out after one week of sample storage. The diameter of 250 air cells and ice crystals were measured with SEM software.

Statistical Analysis

All the data in the experiment was statistically analyzed by the SPSS statistical software programme, (version 19.0, SPSS Inc. Chicago, IL 60606) using one way ANOVA. The comparison among treatment means was done through Duncan's Multiple Range Test at $p < 0.05$. The whole experiment was conducted twice and each test was run in triplicate.

RESULTS AND DISCUSSION

The effect of glycerol supplementation on total solids is given in Table 1. Glycerol supplementation has significantly increased the total solids ($P<0.05$). The percent increase in total solids was found in linear pattern. Fat contents with the supplementation of glycerol also increased significantly ($P<0.05$).

Table 1. Physicochemical analysis of frozen yogurt with different levels of glycerol

Formulation	Total Solids (%)	Protein (%)	Fat (%)	pH	Acidity (%)
F1	30.50 ± 0.4^{a}	3.69 ± 0.8^{a}	8.04 ± 0.5^{a}	5.51 ± 0.02^{a}	0.51 ± 0.05^{a}
F2	31.59 ± 0.7^{b}	3.59 ± 0.6^{a}	8.10 ± 0.9^{a}	5.49 ± 0.03^{a}	0.52 ± 0.04^{a}
F3	32.27 ± 0.9^{bc}	3.48 ± 0.5^{b}	8.19 ± 0.4^{b}	5.51 ± 0.01^{a}	0.51 ± 0.01^{a}
F4	32.70 ± 0.5^{c}	3.41 ± 0.9^{b}	8.26 ± 0.7^{b}	5.51 ± 0.02^{a}	0.51 ± 0.02^{a}

Means ±standard deviation

[a,b,c] Means within the same column with different letters are significantly different (P<0.05).

Conversely, increased concentration of glycerol significantly decreased the protein concentration (*P*<0.05). The decrease in total protein may be due to the supplementation of glycerol that increased the total solids with respect to nitrogen contents in the mixture. The pH and titratable acidity in all the frozen yogurt mixtures before freezing were not different (*P*<0.05).

Melting rate

Melting rate was observed significantly, it decreased with increased overrun levels (*P*<0.05). In F1 the melting rate with 60% overrun was 0.47 g/min that decrease up to 0.07 and 0.29 g/min with 80% and 100% overrun, respectively. The change in melting rates of all other samples (F2, F3, and F4) with different overrun levels was similar to F1 (fig. 1). The glycerol supplementation has shown non-significant effect on melting rate (*P*<0.05). Melting rates in our study were similar to Marshall *et al.* (2003), who reported the melting rate decreased with increased overrun in ice cream. Sofjan and Hartel (2004) also observed the ice cream with 80% overrun melted more rapidly as compared to 100% and 120% overrun. The melting rates of ice cream with 100 and 120% overrun were similar. Furthermore, in the above study 100% overrun was optimum for ice cream but however we use frozen yogurt for which 100% overrun may not be optimal and therefore, further study should be carried out for optimization of overrun in frozen yogurt. The decrease in melting rate in our study may be due to air property, fat globule destabilization and change in air cell size. Since air is a good insulator, it decreased the heat transfer from external environment to inside of the frozen yogurt and prevented the melting. Higher overrun value also destabilized the fat molecules and resulted in small air cells that also decreased the melting rate (Sofjan and Hartel, 2004).

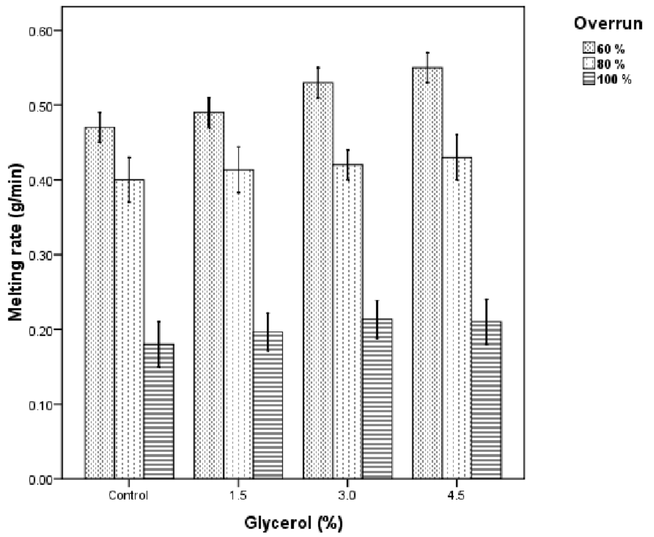

Fig. 1. Melting rate of frozen yogurt with different overrun and glycerol levels

Hardness and stickiness

Hardness of the frozen yogurt significantly decreased with increased overrun levels (*P*<0.05). Hardness of the frozen yogurt in F1 with 60% overrun was 43.57 Newton that decreased up to 10 and 18 N with incorporation of 80 and 100% air; respectively. Glycerol supplementation also significantly decreased the hardness in frozen yogurt (*P*<0.05). The decline in hardness with 60% overrun was 11, 19 and 25 Newton with supplementation of 1.5, 3.0 and 4.5% glycerol (fig. 2). While the decrease in hardness in 80 and 100% overrun was less with glycerol supplementation. The hardness decrease was in the order of overrun 100<80<60 with supplementation of glycerol. Our results were similar to Sofjan and Hartel (2004), who observed the hardness in ice cream with 80% and 100% overrun, was similar and it decreased with 120% overrun. Wilbey *et al.* (1997) also observed the inverse relation between overrun and hardness. While in some studies a direct relationship were also observed (Abd El-Rahman *et al.,* 1997; Prindiville *et al.,* 1999). Furthermore the decrease in hardness with the glycerol supplementation may be due to its water binding property and of fluid modification characteristic which surrounds the air cells and ice crystals. Cryoprotectant by the steric hindrance and water binding quality may prevent the water diffusion from and to the ice crystals (Karaca *et al.,* 2009). This phenomenon hinders the large ice crystals growth and water molecules movement which leads to development of softer matrix.

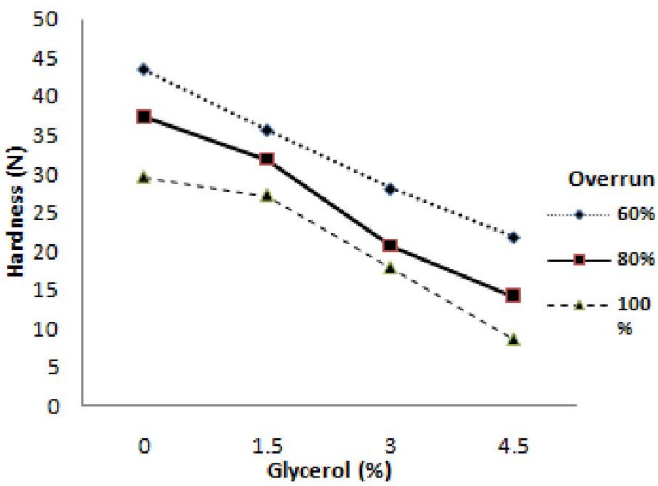

Fig. 2. Hardness of frozen yogurt with different overrun and glycerol levels

The stickiness of the frozen yogurt also changed with increased overrun percentage (*P*<0.05). In frozen yogurt (F1) the decrease in stickiness was 0.27 and .97 N with 80 and 100% overrun as compared to 60% overrun. While in F2 the stickiness decline was observed up to 0.94 and 1.5 N with

80 and 100% overrun with reference to 60% overrun (Fig. 3). Glycerol supplementation has shown non-significant effect on stickiness in frozen yogurt ($P<0.05$). However, in frozen yogurt with 60% overrun the glycerol supplementation has significantly increased the stickiness ($P<0.05$). The combined effect of overrun and glycerol has shown more decreased in stickiness with respect to overrun alone. The stickiness has shown the same pattern like hardness. The decrease in stickiness with increased overrun may be due to formation of more air cells which made the frozen yogurt fluffier. Furthermore, the glycerol supplementation may increase the adhesiveness in the frozen yogurt that was mostly seen with 60% overrun. However, in other samples this effect may be minimized by the increased overrun levels ($P<0.05$).

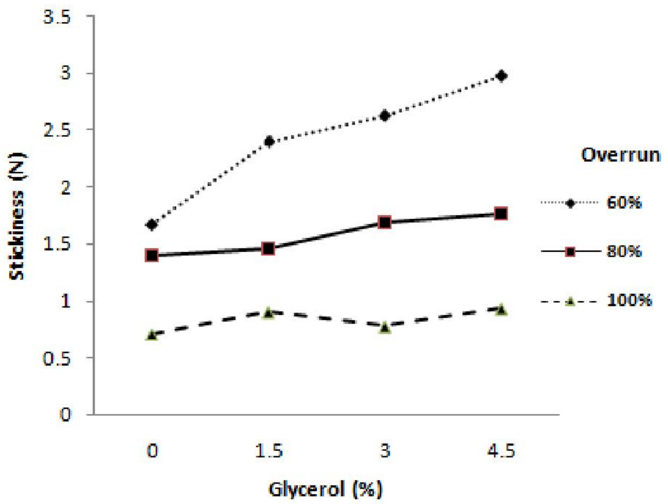

Fig. 3. Stickiness of frozen yogurt with different overrun and glycerol levels

Air cell and ice crystal size

The effect of overrun levels on air cell size is given in fig. 4. Air cell size decreased significantly with increased overrun levels. Air cell size in F1 with 60% overrun was 27.63µm which decreased to 9% (with 80%) and 14% (with 100%) overrun. In all other frozen yogurt samples (F2, F3 and F4) overrun has shown the similar effect. Glycerol supplementation also has shown the similar effect on air cell size. In frozen yogurt with 60% overrun, the glycerol has decreased the air cell size 3.21% (with 1.5% glycerol), 8.31 % (with 3% glycerol) and 14.41% (with 4.5% glycerol).

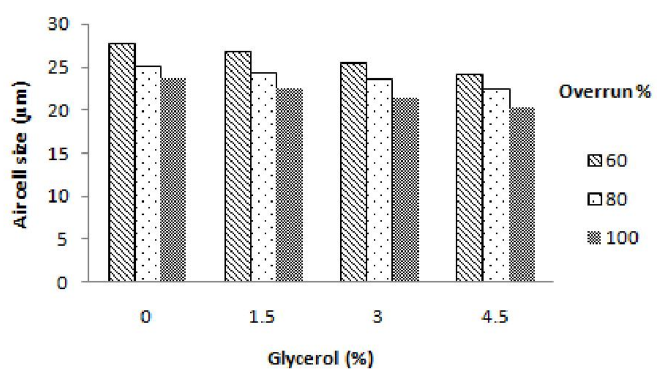

Fig. 4. Air cell size of frozen yogurt with different overrun and glycerol levels

The other overrun levels (80 and 100%) have shown the similar decline in air cell size. Ice crystal size is the most important in the frozen products. It is directly responsible for the other physical characteristics like hardness and acceptability. In our study the ice crystal size has shown inverse relation to the overrun ($P<0.05$). Ice crystal size in F1 frozen yogurt was 25.03 µm which decreased to 5% (with 80%) and 17.4% (with 100) overrun. The similar results were observed in all other frozen yogurt samples (F2, F3 and F4). While the glycerol supplementation also has shown the same effect on ice crystal size (fig. 5). It was observed that ice crystal size in frozen yogurt with 60% overrun decreased to 2, 3.87 and 6.12% with the supplementation of 1.5, 3 and 4.5% glycerol, respectively. The other two overrun levels (80 and 100%) also have shown the similar decreased in ice crystal size.

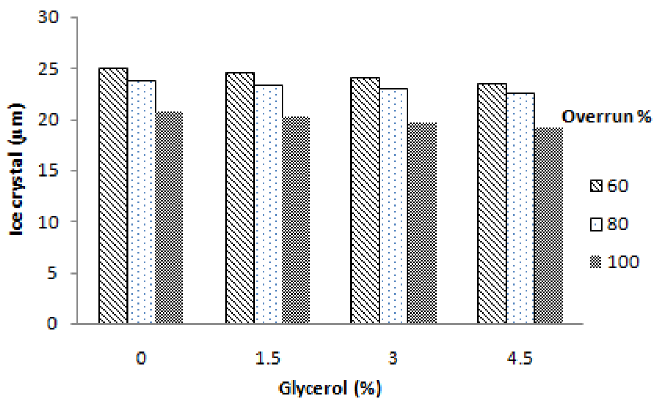

Fig. 5. Ice crystal size of frozen yogurt with different overrun and glycerol levels

The same correlation between air cell size and ice crystal size with overrun levels has been observed in other studies in ice cream (Flores and Goff, 1999; Sofjan and Hartel, 2004). The decrease in size of air cells and ice crystals with increased overrun may be due to the movement of scraped surface impellers of the ice cream maker. During freezing process air is continuously inject under pressure and temperature is lower down which lead to the formation of large air cells and ice crystals. These air cells and ice crystals are broken by impeller movement in to smaller ones and stabilized by fat globules. On the other hand, the glycerol supplementation may increase the viscosity of the mixture. The higher viscosity of the mixture helped in efficient break down of large air cells into smaller ones (Chang and Hartel, 2002). Furthermore, the mixture is not completely frozen in the ice cream maker. The temperature of the ice cream maker may be from -4ºC to -6ºC which led to the production of partially freeze frozen yogurt. The freezing process is completed during hardening at -35ºC. In our study the glycerol supplementation may act as a cryoprotectant and binds much of the free water which prevents the growth of large ice crystals.

Conclusion

The results have shown that the oxygen is one of the important factors which have a great influence on the physicochemical properties of probiotic frozen yogurt. The 100% overrun have shown the slow melting rate with softer product having low stickiness. Furthermore, it also resulted in small air cells with fine ice crystal formation. These properties are directly related to the acceptability of the frozen product. The glycerol supplementation helped in improving the mixture properties. Glycerol has shown a little effect on melting rate and stickiness. Frozen yogurt with 4.5% glycerol was a softer product with reduced size of air cell and ice crystal.

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