INTRODUCTION

The nutritional and potentially therapeutic value of food is a key characteristic in the development of new value-added products manufactured for health conscious consumers (Garcia et al., 1998; Corte, 2008). Frozen yoghurt has been the fastest growing product in the frozen dessert market in recent years (Opdahl and Baer, 1991; Guinard et al., 1994). Frozen yoghurt dessert is a complex fermented frozen dairy dessert that combines the physical characteristics of ice cream with the sensory and nutritional properties of fermented milk products. Yogurt, which is consumed by a wide cross section of people throughout the world, has an established market as a functional therapeutic food (Adhikari et al., 2000). Frozen yoghurt is a low acid product (Schmidt et al., 1997) and can be regarded as a healthy alternative to ice cream for people suffering from obesity, cardiovascular diseases and lactose intolerance due to its low fat content (the fat percentage of regular frozen yoghurt ranges from 3.5% to 6%) (Milani and Koocheki, 2011).

In the present scenario, the herbal products are gaining more popularity over synthetic products in the world market. This is occurring due to some side effect of synthetic products on the body (Amirdivani, 2008). In spite of well-practiced knowledge of herbal medicines and occurrence of a large number of medicinal plants, the share of India in the global market is not up to the mark (Rajkumar, 2009).

Yoghurt contains substantial amounts of live lactic acid bacteria (Ghosh and Raymond, 2003). However, numbers of viable yoghurt cultures differ widely among brands of frozen yogurt, for the retention of the usefulness of lactic acid bacteria, it is necessary not only to promote its growth but also to inhibit death of its cells and further, it is required to maintain a high viable cell count in the final product during storage, for maintenance of these physiological effects at high levels, it is important to retain useful bacteria, such as lactic acid bacteria, in a viable state (Schmidt et al., 1997).

One reason for low counts in frozen yogurt is death of the culture by freezing (Donkor et al., 2006). Freezing with agitation kills a large portion of added lactobacilli. Death of yogurt bacteria in frozen desserts reduces the potential to produce health benefits. The freezing step is especially critical as it negatively affects both viability and physiological state of the bacteria. The formation of ice crystals induces mechanical damage that leads to cellular death (Dave and Shah, 1996).

The objective of the present study was to increase the viable count of yoghurt culture and to maintain the viable count in the final product by protecting the yoghurt culture from adverse effect of freezing and subsequent storage at low temperature.

MATERIALS AND METHODS

This study was carried out at Warner School of Food and Dairy Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, U.P., India.
Yoghurt Culture

(Streptococcus salivarius ssp. Thermophilus NCDC 074 and Lactobacillus delbrueckii ssp. Bulgaricus NCDC 009) were obtained from National Collection of Dairy Culture (NCDC), Dairy Microbiology Division at NDRI Karnal, Haryana, India.

Herbs

(Ajowan, Black Pepper, Cardamom, Garlic, Ginger and Mint) were obtained from the local market in Allahabad, U.P., India.

Selection of Herbal Extract

Six herbs viz., Ajowan, Black Pepper, Cardamom, Garlic, Ginger and Mint extract of 10º brix were selected to enhance the viability of probiotic culture by the application of Disc Diffusion method (Baur and Kirby, 1966).

Procedure Adopted for Manufacturing Frozen Yoghurt

Both control (T₀) and experimental (T₁, T₂, T₃, and T₄) frozen yoghurt mix were standardized to 5.0 % fat, 8.7% serum solids, 12 % sugar, stabilizer and emulsifier 0.5% and total solids adjusted to 26 %. Herbal extract (10ºbrix) was added at 0.0 %, 0.5%, 1.0%, 1.5% and 2.0% for treatments T₀, T₁, T₂, T₃ and T₄ respectively.

The mix was homogenized and then pasteurized and cooled to 42°C and yoghurt starter culture was added at 2.5% (1:1 ratio). The mix was incubated at 42°C till we achieve an acidity of 0.45%. Before the mix is aged at 5°C and frozen in a batch freezer to an overrun of 70%. The frozen yoghurt was filled in cups and kept in deep freezer for hardening at a temperature of -18°C.

Analysis of Yoghurt Culture

Selective enumerations of yoghurt culture (Streptococcus salivarius ssp. thermophilus NCDC 074, Lactobacillus delbrueckii ssp. bulgaricus NCDC 009) were done according to Shah (2000).

RESULTS AND DISCUSSION

The highest mean selective enumeration (10⁷ cfu/g) of Streptococcus thermophilus score before freezing was 76 in group T₃ followed by 40, 30, 11 and 2.8 in groups T₂, T₄, T₁ and T₀ respectively (Fig. 1). The differences in the values were significant (P< 0.05), except groups T₀-T₁ and T₂-T₄.

The highest mean selective enumeration (10⁷ cfu/g) of Lactobacillus bulgaricus score before freezing was 29.8 in group T₃ followed by 21.5, 18.1, 18 and 4.5 in groups T₂, T₄, T₁ and T₀ respectively (Fig. 1). The differences in the values were significant (P< 0.05), except groups T₁-T₂, T₁-T₄ and T₂-T₄.

The highest mean selective enumeration (10⁷ cfu/g) of Streptococcus thermophilus count after freezing (0 days) was 70.5 in group T₃ followed by 33, 28.5, 9.2 and 1.3 in groups T₂, T₄, T₁ and T₀ respectively (Fig. 2). The differences in the values were significant (P< 0.05).

The highest mean selective enumeration (10⁷ cfu/g) of Lactobacillus bulgaricus score after freezing (0 days) was 27 in group T₃ followed by 17, 15.8, 15.1 and 3 in groups T₂, T₄, T₁ and T₀ respectively (Fig. 2). The differences in the values were significant (P< 0.05), except groups T₂-T₄ and T₃-T₄.

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The highest mean selective enumeration (10⁷ cfu/g) of Lactobacillus bulgaricus count after freezing (0 days) was 27 in group T₃ followed by 17, 15.8, 15.1 and 3 in groups T₂, T₄, T₁ and T₀ respectively (Fig. 2). The differences in the values were significant (P< 0.05), except groups T₂-T₄ and T₃-T₄.

The highest mean selective enumeration (10⁷ cfu/g) of sum of yoghurt culture (Lactobacillus bulgaricus + Streptococcus thermophilus) count before freezing was 105.8 in group T₃ followed by 61.5, 48.1, 29 and 7.3 in groups T₂, T₄, T₁ and T₀ respectively.
respectively (Fig. 3). The differences in the values were significant (P < 0.05).

The highest mean selective enumeration (10^7 cfu/g) of sum of yoghurt culture \textit{(Lactobacillus bulgaricus + Streptococcus thermophilus)} count after freezing (0 days) was 97.5 in group T_0 followed by 44.3, 24.3 and 4.3 in groups (Fig. 3). The differences in the values were significant (P < 0.05).

The highest average total viable count (10^7 cfu/g) after 10 days of storage between control and experimental treatment was 60 in group T_0 followed by 19.9, 17.8, 10.8 and 2.5 in groups T, T_p, T_s, and T_v respectively (Fig. 3). The differences in the values were significant (P < 0.05) except groups T_pT_v.

The highest average total viable count (10^7 cfu/g) after 20 days of storage between control and experimental treatment was 39.8 in group T_0 followed by 10.6, 10.3, 6.5 and 1.1 in groups T_p, T_s, T_v and T_v respectively (Fig. 3). The differences in the values were significant (P < 0.05) except groups T_pT_v.

The highest average total viable count (10^7 cfu/g) after 30 days of storage between control and experimental treatment was 23.6 in group T_0 followed by 4.6, 4.5, 2.1 and 0.6 in groups T_p, T_s, T_v and T_v respectively (Fig. 3). The differences in the values were significant (P < 0.05).

Rajkumar (2009) reported that ginger extract and honey with cryoprotective agent (Glycerol) enhanced the viable cell count of frozen yoghurt. Donkor et al. (2006; Singh, 2009). By analysing different levels of herbal extract we have found that viable count of frozen yoghurt was enhanced by the use of herbal extract.

Singh (2009) suggested that herbal extract (Ajowan, Cardamom, Garlic and Mint) enhances the growth of yoghurt culture. During storage the death of yoghurt culture was found non-significant and herbal extract can be recommended as growth promoter (Heenan et al., 2004; Rajkumar, 2009; Radhika, 2010) for the yoghurt culture.

Chowdhury et al. (2008) prepared yoghurt with tulsi leaf (Ocimum sanctum), pudina leaf (Mentha arvensis) and coriander leaf (Coriandrum sativum) based. Tulsi yoghurt had the maximum α-galactosidase activity than other herbal yoghurt.

Some researchers have attempted to improve the viable count, sensory and nutritive characteristics of frozen yoghurt by adding probiotics during storage. Krasaekoot et al. (2003) studied that encapsulation of probiotic culture protects the bacteria in the product's environment and improve their survival during freezing. Donkor et al. (2006) reported that presence of probiotic organisms \textit{(Lactobacillus acidophilus LAFTI L10, Bifidobacterium lactis LAFTI B94, and L. paracasei LAFTI L26)} enhanced proteolysis significantly in comparison with the control batch the increased proteolysis improved survival of \textit{L. delbrueckii} ssp. \textit{bulgaricus} Lb1466 during storage resulting in lowering of pH and production of higher levels of organic acids, which might have caused the low cell counts.

Frozen yoghurt with high viable count can be manufactured by addition of different level of herbal extract. These herbal extracts improve the rate of survival of yoghurt culture on freezing and storage of frozen yoghurt. Therefore, we can recommend that use of herbal extract in frozen yoghurt enhances the viable count of yoghurt culture.

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