SURVIVABILITY OF MICROENCAPSULATED *Lactobacillus acidophilus* LA-5 IN SYNBIOTIC ICE CREAM

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Thesis submitted in partial fulfillment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis, entitled "**SURVIVABILITY OF MICROENCAPSULATED *Lactobacillus acidophilus* LA-5 IN SYNBIOTIC ICE CREAM"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled "**SURVIVABILITY OF MICROENCAPSULATED *Lactobacillus acidophilus* LA-5 IN SYNBIOTIC ICE CREAM"** is a record of research work done independently by **Albert Arockia Raj. P**, under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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My Beloved Family

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Introduction

1. INTRODUCTION

Modern consumers are becoming health conscious and expect the food they eat not only to be nutritive but also be capable of preventing illness. Currently, there is an increasing interest in the addition of probiotic microorganisms to various foods in order to enhance their nutritious and therapeutic values. According to Food and Agriculture Organization (FAO) and the World Health Organization (WHO) probiotics are, "Live microorganisms (bacteria or yeasts), which when ingested or locally applied in sufficient numbers confer one or more specified demonstrated health benefits for the host". The species of bacteria most commonly used in food products for probiotic effects are coming under *Lactobacillus* and *Bifidobacterium*. They have favourable effects when an imbalance of the intestinal microflora occurs. Probiotic microorganisms offer new dietary alternative for the management and stabilization of intestinal microflora.

The documented beneficial health effects through probiotics are anti carcinogenic properties, stimulation of immune system, alleviation of lactose intolerance, serum cholesterol reduction, nutritional enhancement (like calcium absorption, and the production of B-complex vitamins) and prevention of diarrhoea caused by *Escherichia coli* (*E. coli*), *Salmonella* and *Shigella.*

The ability of probiotic microorganisms to survive and multiply in the host strongly influences their probiotic benefits. The bacteria should be metabolically stable and active in the product and survive passage through the upper digestive tract in large numbers to bring about beneficial effects in the host. The standard introduced by several food manufacturers worldwide for any food sold with health claims from addition of probiotics is that it must contain at least 10^6 to 10^7 colony forming unit (cfu) of viable probiotic bacteria per gram. Therefore, it is important to ensure a high survival rate of these microorganisms

during the shelf life of the food product to maintain consumer confidence in probiotic products.

The viability of probiotics in food products is greatly affected by their exposure to detrimental environmental factors such as acidity, pH, dissolved oxygen content, hydrogen peroxide, storage temperature, concentration of organic acids and antibacterial components in the gastrointestinal tract. Their viability can be improved by incorporation of prebiotics (synbiotic approach), microencapsulation and addition of cryoprotectants.

Prebiotics are the non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or limited number of beneficial bacteria in the colon. A prebiotic should resist host digestion and absorption in the stomach and small intestine to reach the large intestine in an unmodified form. In the large intestine it selectively stimulates the growth or activity of one or limited number of potentially beneficial bacteria, particularly bifidobacteria and lactobacilli, while decreasing the number of facultative anaerobic strains such as *E. coli* and Clostridia. Some of the well known prebiotics are fructo-oligosaccharide, inulin and galacto-oligosaccharide.

Synbiotics are the mixtures of probiotics and prebiotics. Synbiotic approach is a further alternative, through which the probiotic would be administered in conjunction with a specific prebiotic. This mix would benefit the host by improving survival and implantation of selected microbial supplements.

Microencapsulation of probiotic bacteria can be used to enhance their viability during processing and also for the targeted delivery in gastrointestinal tract. Microencapsulation is a technology of packaging solids, liquids or gaseous materials in miniature sealed capsules that can release their contents at controlled rates under the influences of specific conditions. Microencapsulation improves the probiotic viability and sensory properties in the food products. It improves the probiotic viability due to its protective effects against detrimental environmental

factors such as high acidity, low pH, molecular oxygen, digestive enzymes and heat processing. Food grade polymers such as alginate, chitosan, carboxymethyl cellulose (CMC), carrageenan, gelatin and pectin are mainly applied using various micro encapsulation technologies like spray drying, emulsion, extrusion and phase separation.

Cryoprotectants are added into probiotic products prior to fermentation or during freeze-drying in order to assist in the adaptation of microorganisms or to maintain its viability. Compatible cryo protectant accumulates within the bacterial cells and reduces the osmotic differences with their external environment.

Ice cream is a delicious, wholesome and nutritious frozen dairy product, made from the combination of components of milk, sweeteners, stabilizers, emulsifiers and flavouring agents. The popularity of ice cream and frozen dessert is attributed to its refreshingly cool and delightfully sweet characteristics besides it being nutritious. Among the milk products, ice cream is gaining momentum as a modern dairy product in India. During 2005, the total ice cream production in India reached to 5,500 lakh litres, with a value output of Rs. 2,400 crores per annum and there is enough scope for more growth in the near future (Bharat Bhushan, 2007).

Ice cream has nutritional significance but possesses no therapeutic properties. The growing interest of consumers on the therapeutic products has led to the incorporation of probiotic cultures into ice cream. The environment provided by ice cream components can favour the survivability of probiotics. Ice cream mixture possesses cryo protective properties due to the presence of casein, sucrose and lactose. Probiotic bacteria die at a slower rate when stored at a low temperature such as $-20^{\circ}C$. The opposite occurs when the maintenance temperature approaches the melting point. So, recommended probiotic dose of 10^6 colony forming unit per gram of product can be maintained for a longer

period in ice cream than any other dairy product stored at refrigeration temperature.

Some of the detrimental factors present in ice cream are oxygen toxicity (due to overrun) and freezing injury. Addition of prebiotics and micro encapsulation may be used to overcome these problems. Hence there is good scope to develop ice cream as a potential carrier of probiotics in conjunction with the microencapsulation process.

The present research work was conducted with the following objectives:-

1. To formulate a synbiotic ice cream using the probiotic culture *Lactobacillus acidophilus* LA-5 and the prebiotic oligofructose.

2. To study the efficiency of micro encapsulation to improve the survivability of probiotic organisms in ice cream.

3. To assess the microbial, physical and chemical properties of the formulated ice cream mix and ice cream along with its consumer acceptability.

Review of Literature

2. REVIEW OF LITERATURE

Ice cream may be defined as a frozen dairy product made by suitable blending and processing of cream and other milk products, together with sugar and flavours, with or without stabilizer or colour and with the incorporation of air during the freezing process.

According to the Prevention of Food Adulteration Act (1954), ice cream is the frozen product obtained from cow or buffalo milk or a combination thereof or from cream and /other milk products, with or without the addition of cane sugar, eggs, fruits, nuts, chocolate, edible flavours and permitted stabilizers not exceeding 0.5 per cent by weight. The mixture must be suitably heated before freezing. The product should contain not less than ten per cent milk fat, 3.5 per cent protein and 36 per cent of total solids. However, when any of the aforesaid preparation contains fruits/nuts/both, the content of milk fat may be proportionately reduced but may not be less than eight per cent by weight. Starch may be added to a maximum extent of five per cent, with a declaration to that effect on the label.

2.1 PROBIOTICS

Probiotic has been defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Fuller 1989).

According to FAO/WHO, the following are the characteristics of probiotics:- 1) it must be alive 2) it must deliver a measured physiological benefit, substantiated by studies conducted in the target host 3) it should not limit the mechanism of action but the delivery of metabolites/products by micro organism to the small intestine be considered as a probiotic activity.

Probiotics have received considerable attention over the past few years for their purported health benefits. Probiotics come in two main forms i.e. foods and dietary supplements.

Foods containing probiotic bacteria fall within the functional foods category, since they provide health benefits over and above basic nutrition. Consumption of probiotics as a part of food (such as dairy products) has the advantage of gaining health benefits of foods, increasing compliance and improving the chances that probiotics reach the intestine alive (as buffer for survival). Consuming probiotics as a dietary supplement (usually tablets, capsules or powder) has the advantage of delivering a high level of bacteria easily, assuming the products are responsibly formulated and stored properly. Probiotic preparations are available as powders or tablets but most commonly as milk based products. Most commonly, *Lactobacillus acidophilus* (*L. acidophilus*), *Lactobacillus casei* (*L. casei*), *Lactobacillus johnsonii* (*L. johnsonii*), *Lactobacillus paracasei* ssp. *paracasei* (*L. paracasei*), *Lactobacillus reuteri* (*L. reuteri*), *Lactobacillus gasseri* (*L. gasseri*), *Lactobacillus rhamnosus* (*L. rhamnosus*), *Bifidobacterium bifidum* (*B.bifidum*), *Bifidobacterium longum* (*B. longum*), *Bifidobacterium animalis* (*B. animalis*), *Bifidobacterium lactis* (*B. lactis*), *Bifidobacterium infantis* (*B. infantis*) and yeast Saccharomyces *boulardii* (*S. boulardii*) have been used as probiotics in humans (Playne, 1994).

2.1.1 *Lactobacillus acidophilus*

L. acidophilus, was first isolated by Moro in1900, from faeces of infants fed with milk. The acidophilus bacterium was isolated from the intestinal tract of animals and human beings, especially from the persons consuming high milk, lactose or dextrin diets. *L. acidophilus* is the most commonly used probiotic, or "friendly" bacteria. It inhabits the human gastro intestinal tract, mouth and vagina and protect against the entrance and proliferation of “harmful" organisms that

could cause disease. This is accomplished through a variety of mechanisms. For example, the breakdown of food by *L. acidophilus* leads to production of lactic acid, hydrogen peroxide, and other byproducts that make the environment hostile for undesirable organisms.

L. acidophilus is a Gram positive, rod shaped (dimensions are in the range of 0.5-1 x 2-10 μm), with rounded ends, occurring in pairs or short chains. It is a non-spore forming, catalase negative organisms devoid of cytochromes. They are anaerobic but are aero tolerant, fastidious, acid tolerant and strictly fermentative. Lactic acid is the major end product of sugar fermentation (Axelsson, 1993).

Metchnikoff (1907) implicated a *lactic acid bacillus* in Bulgarian yoghurt as the agent responsible for preventing intestinal putrefaction and ageing. Later, it was discovered that Metchnikoff's Bulgarian strain did not survive passage through the gastrointestinal tract, prompting substitution of *L. acidophilus* as the most likely candidate to fulfill the primary criteria expected of an intestinal probiotic.

Health benefits attributed to *L. acidophilus* include enhancement of immune system, anti-diarrhoeal properties, lowering serum cholesterol level and effective management of lactose malabsorption (Sanders, 2000).

Large segments of the population across the world are lactose intolerant because of a deficiency of the enzyme lactase. Failure to hydrolyse lactose leads to its fermentation in the large intestine and causes intestinal distress in the consumer. *L. acidophilus* produces lactase, the enzyme that breaks down milk sugar (lactose) into simple sugars. There is good evidence that lactose intolerant subjects can consume significant amounts of lactose from milk or milk products if lactic acid bacteria are present. For this reason, *L. acidophilus* supplements may be beneficial for these individuals (Lin *et al.,* 1991).

An anticarcinogenic effect of *L. acidophilus was* reported by Goldin and Gorbach (1984). Catalytic activity of converting procarcinogens to carcinogens by bacterial enzymes (β-glucuronidase, azoreductase and nitroreductase) and the cytotoxic effects of bile acids on the colonic epithelium are the major causes for colon cancer. Oral dietary supplementation of *L. acidophilus* can cause, significant decline in the faecal levels of these procarcinogenic bacterial enzymes, direct removal of procarcinogens, activation of body's immune system and deconjugation of bile acids. Animal studies have shown that dietary supplementation with *L. acidophilus* decreases the number of colon cancer cells in a dose dependent manner (Lidbeck *et al.*, 1992 and Rao *et al*., 1999).

Being acid resistant, *L. acidophilus* persists in the human stomach longer than other bacteria and exhibit a significant inhibitory effect on the attachment of *Helicobacter pylori* (*H. pylori*) to the gastric epithelial cell lines (Kabir *et al.,* 1997). Wang *et al*. (2004) evinced that ingesting *L. acidophilus* LA-5 exerts a suppressive effect on *H. pylori* infection in both animals and humans. *L. acidophilus* LA-5 has prophylactic action against candidal vaginitis (Hilton *et al.*, 1992) and traveler's diarrhoea (Black *et al*., 1989).

L. acidophilus also *plays* a major role in preventing and controlling intestinal infections (Gilliland and Speck, 1997), lowering serum cholesterol levels (Harrison and Peat, 1975), helps in relieving constipation and other digestive disorders (Salminen and Deighton, 1992).

L. acidophilus remains as a resident in the intestinal tract of humans until death. But this residency is affected when the gut flora is disturbed by exogenous factors such as antibiotic treatment, radiation exposure, hormone therapy, intestinal diseases and starvation.

2.1.2 Probiotic Ice Cream

Cultured ice cream (yoghurt ice) was introduced in Denmark in the sixties, but at that time consumers found the taste to be too acidic. Over the past years cultured ice cream has evolved to a milder taste profile and application of flavors has become rather common.

Duthle *et al*. (1982) indicated through their preliminary study, that ice cream is probably a good way to provide *L. acidophilus* bacterium to consumers than milk. Ice cream presented no flavour problems and kept the survival rate of *L. acidophilus* greater than the recommended level of two million colonies per gram for a longer period (more than 28 days) than the acidophilus milk.

Kaul and Mathur (1982) developed and assessed an unfermented ice cream containing *L. acidophilus*. Cell preparation of *L. acidophilus* was added directly into freezer along with the ice cream mix and the survivability of the organism after freezing was found to vary between 93 and 96 per cent. Reduction in the lactobacillus population seemed to be more pronounced during the first week of storage period of 60 days at –20ºC after which count was almost stable. There was no perceptible change in the sensory analysis of the sweet acidophilus ice cream with addition of 0.03 per cent of *Lactobacillus* cell preparation. However, as the amount of added *Lactobacillus* cell preparation was increased from 0.03 per cent to 0.04 per cent the flavour scores declined significantly. No significant differences were observed in the body, texture, melting quality and colour between control and sweet acidophilus ice cream.

Hekmat and McMahon (1992) prepared probiotic ice cream by fermenting a standard ice cream mix with *L. acidophilus* and *B. bifidum* cultures and stored at –29°C for 17 weeks. The total colony counts after fermentation of the ice cream mix to pH 4.9 were 5 x 10^8 cfu/g for both the organism. After one week of frozen storage, bacterial counts obtained were 1.5 x 10^8 cfu/g for *L. acidophilus* and 2.5 x 10^8 cfu/g for *B. bifidum*. Seventeen weeks after freezing, these counts

had decreased to 3 x 10^6 and 1 x 10^7cfu/ml, respectively. Probiotic ice cream was prepared with a pH of 5.0, 5.5, and 6.0 by mixing fermented mix with unfermented mix, to determine consumer preferences and was compared with standard ice cream (pH 6.5). The preferred pH of probiotic ice cream, based on overall acceptance, was 5.5. They demonstrated that probiotic ice cream is a suitable vehicle for delivering beneficial microorganisms such as *L. acidophilus* and *B. bifidum* to consumers.

Ma (1995) produced ice cream containing *B. bifidum* using conventional procedures modified by inoculation of four per cent *B. bifidum* together with one per cent lactic acid bacteria and cultured at 42°C to pH 4.8. The final probiotic ice cream had the texture of ordinary ice cream, but with a sweet-sour flavour. One gram of the final probiotic ice cream contained 10^6 active cells of *B. bifidum* and lactic acid bacteria.

Christiansen *et al*. (1996) succeeded in manufacturing probiotic ice cream by simply mixing commercial *L. acidophilus* and *B. bifidum* cultured milks with unfermented ice mix. Further fermentation was prevented by keeping the mixture below 5°C. After 16 weeks of frozen storage the ice cream contained the same high levels of viable organisms as an ice cream produced from a fermented ice cream mix.

Inoue *et al*. (1998) observed that there was no appreciable change in the structure, acidity and pH values of ice cream type frozen yoghurts (fat content 10.6 per cent with varying pH values) during a storage period of six months at –35ºC. In addition, there was no increase in thiobarbituric acid values of the products during storage. Viable lactic acid bacteria decreased in number with increasing storage period. The product having a pH value of 5.5 was the most preferred ice cream type frozen yoghurt.

Ravula and Shah (1998) assessed the effect of acid casein hydrolysate and cysteine on the viability of yoghurt bacteria *Streptococcus salivarius* ssp

thermophilus (*S. thermophilus*) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (*L. bulgaricus*) and probiotic bacteria *L. acidophilus and B. bifidum* in fermented frozen dairy desserts during a storage period of twelve weeks at –18ºC. The results suggested that the acid casein hydrolysate and cysteine stimulated the growth of *L. acidophilus* and *B.bifidum,* which resulted in improved viability of these organisms.

Davidson *et al*. (2000) prepared frozen yoghurt by fermenting the low-fat ice cream mix with probiotic bacteria (*L. acidophilus* and *B. longum)* and traditional yoghurt culture (*S. thermophilus* and *L. bulgaricus*). Fermentation was stopped when the pH reached 5.6 or when the titratable acidity reached 0.15 per cent greater than the initial value. Mix was frozen and stored for eleven weeks at –20ºC. Frozen storage of the product had little or no effect on culture survival and bacterial cultures remained at levels sufficient to offer the suggested therapeutic effects. Supplementation with probiotic bacteria had little effect on flavour or compositional characteristics of frozen yoghurt. So, it was concluded that frozen yoghurt can serve as an excellent vehicle for dietary incorporation of probiotic bacteria.

Alamprese *et al.* (2002) analyzed the survivability of *L. johnsonii* La1 and influence of its addition in ice cream produced with different sugar and fat concentrations. They found that when probiotic bacteria were added to ice cream mixes in the level of 10^7 cfu per g, it did not modify the overrun and firmness of the product. The survival rate of *L. johnsonii* La1 was also high up to eight months of storage, regardless of formulation.

Godward and Kailasapathy (2003) studied on the preparation of ice cream with incorporation of probiotic bacterial cultures (*L. acidophilus* 2401 and *B. infantis* 1912) in the forms of free, freshly encapsulated, encapsulated and freeze dried and co-encapsulated and freeze dried cultures. The survival of probiotic bacteria was monitored over a period of 24 weeks of storage of the ice

cream at -20ºC. The result showed that free cells survived better than freshly encapsulated cells in ice cream. Co-encapsulation enhanced the survival of both strains as compared with individual encapsulation of the same strain. Freshly encapsulated cells showed greater survival than those that were freeze dried after encapsulation. Addition of encapsulated culture did not show any effect on the amount of air incorporated into the ice cream.

Heenan *et al.* (2004) incorporated probiotic microorganisms (above10^6 cfu/g) into a non-fermented vegetarian frozen soy dessert and assessed for the probiotic survivability and sensory acceptability. Culture like *L. acidophilus* LA1, *L. rhamnosus* 100C, *L. paracasei* 01, *B. lactis* BBDB2 *and B. lactis* BB12 survived the six month storage trial at populations of 10^7 cfu/gram or greater. *S. boulardii* 74012 did not retain sufficient viability and decreased below desirable level of 10^6cfu/gram. Product inoculated with *L. acidophilus* LA1 could not be distinguished from the control sample. It was concluded that frozen soy dessert was a suitable food for the delivery of bacterial probiotic strains with excellent viability and acceptable sensory characteristics.

Rao and Prakash (2004) manufactured a probiotic kulfi of acceptable quality using probiotic cultures *B. bifidum* and *L. acidophilus*. The kulfi contained high levels of viable probiotic organisms, even after four weeks of frozen storage.

Salem *et al*. (2005) was successful in producing probiotic ice cream by mixing fortified milk fermented with probiotics (*L. acidophilus, B. bifidum, L. reuteri*, *L. gasseri* and *L. rhamnosus*) into the ice cream mix, followed by freezing and stored at –26ºC for 12 weeks. The probiotic ice cream showed faster melting, increase in acidity and viscosity and decrease in freezing point and overrun than control ice cream. Although there was a decrease in the number of viable cells, the ice cream proved to be a probiotic food during 12 weeks of storage, since the probiotic count remained above the recommended minimum

limit of 10^6 cfu per gram. Supplementation with probiotic bacteria has been found to exert a little effect on flavour or compositional characteristics of ice cream.

Taha *et al.* (2005) evaluated ice cream as a carrier for mixed culture (1:1:1) of probiotic bacteria; *B. bifidum* Bb-12, *L. acidophilus* LA-5 and *L. casei* 01. The mixed culture was either added to ice cream mix one hour before freezing (T1) or used to ferment the ice cream mix without sugar to reach 0.5 per cent acidity and then mixed with the sugar and left overnight before freezing (T2). Control ice cream was prepared without probiotics. Ice cream samples from different treatments were stored at -18 to -20°C for three months. Initial freezing reduced slightly the viability of probiotic bacteria, being more obvious in T1 samples. During storage, further reduction occurred in the viability of all strains. However, the probiotic count of ice cream was higher than the recommended minimum limit of 10^6 cfu/g. Ice cream from T2 treatment had the highest overrun and melting resistance as compared with T1 and control samples. All ice cream samples had acceptable organoleptic properties. The obtained results suggest that ice cream can be used as a carrier for probiotics without impairing its quality.

Trindade *et al.* (2006) analysed the stability of probiotic microorganisms and vitamin C in fermented acerola ice cream. Six varieties of fermented acerola ice creams were prepared, containing different starter cultures (*B. longum*, *B.lactis, S. thermophilus* and *L. bulgaricus*) and with final pH (5 and 4.5). Mixes were frozen and stored for 15 weeks at –18ºC. The viable counts for probiotic cultures remained above the recommended minimum limit of 10^6 cfu per gram during the storage period of 15 weeks even in products with pH 4.5. Vitamin C concentration remained around 140 mg / 100g of product. The sensory attributes like aroma, taste, texture and overall acceptance obtained score in the range of 5.15 to 7.22.

Magarinos *et al.* (2007) conducted a research work to determine the survivability of *L. acidophilus* La-5 and *B. lactis* Bb-12 in probiotic ice cream. The probiotic ice cream was prepared with four per cent inoculation of each culture and stored at –25^{o}C for 60 days. The survival rate of *L. acidophilus* La-5 was 87 per cent at the end of the study.

Trindade *et al.* (2007) prepared twelve varieties of fermented yellow mombin (*Spondias mombin* L) ice creams with five and ten per cent cream by incorporating *L. acidophilus* 74-2, *L. acidophilus* LAC4 and yoghurt culture. They found that the probiotic count of the ice creams with pH 4.5 and 5 was higher than the recommended level of 10^6 cfu/g even after 105 days of storage at -18 ^{o}C. The probiotic ice cream with pH 4.5 and cream five per cent containing *L. acidophilus* LAC4 received significantly higher sensory score. Through this study, they concluded that the yellow mombin ice cream was a suitable food for the delivery of *L. acidophilus* strains, with excellent viability and acceptable sensory characteristics.

Homayouni *et al.* (2008[a]) investigated the growth and survival of probiotic strains (*L. acidophilus*, *L. casei*, *B. bifidum and B. longum*) in simulated ice cream condition. They found that *Lactobacilli* strains proved to be highly resistant in comparison with *Bifidobacterium* strains in different sucrose concentrations, different redox potentials and refrigeration temperature. Growth and survival rate of *L. casei* was found to be highest among the probiotic strains used in simulated ice cream condition.

2.2 PREBIOTICS

Prebiotics pose an alternative approach to overcome the survivability and colonization difficulties that abound with probiotics. Prebiotics are non digestible carbohydrates that resist hydrolysis and absorption in upper parts of the gastrointestinal tract and exploit selective enzyme production by those gut microorganisms that may impart health benefits to the host. Certain

carbohydrates oligosaccharides and polysaccharides occur naturally and meet the criteria of prebiotics (Gibson and Roberfroid, 1995).

Prebiotics have a number of functional effects on the gastro intestinal tract, such as improved glucose tolerance, improved bioavailability of minerals such as calcium, magnesium and iron, delayed gastric emptying, reduced fat and cholesterol absorption via binding of bile acids and modulation of microbial fermentation with increased short chain fatty acid production, decreased pH and ammonia production (Roberfroid, 1996). The combination of these effects could potentially result in improved host health by reducing intestinal disturbances, cardiovascular disease and intestinal cancer. The prebiotic group that has received the maximum attention in research is the oligosaccharides. Among them, fructooligosaccharide (FOS)/oligofructose have been extensively studied (Sangeetha *et al*., 2005).

A number of foods such as chicory, onion, artichoke, garlic and asparagus contain relatively high concentrations of fructooligosaccharide (Gibson and Roberfroid, 1995). Fructooligosaccharide and other oligosaccharides can also be produced enzymatically, which is advantagious for large-scale commercial production (Crittenden and Playne, 1996). Fructooligosaccharide increases the number of bifidobacteria and lactobacilli, increases short chain fatty acid concentrations and decreases clostridia, fusobacteria and bacteroides and pH (Fuller and Gibson, 1997). Buddington *et al*. (1996) have demonstrated that fructo oligosaccharides are prebiotic at four g/day.

2.2.1 Prebiotics in Ice Cream

The results of the study conducted by Wang and Gibson (1993) suggested that the addition of oligofructose or inulin to the diet may cause an improvement in the composition of the gut microflora. This may arise because of a stimulation of bifidobacterial numbers, in comparison with other bacterial genera. Oligofructose and inulin are not hydrolysed during passage to the colon, thus their

calorific value is likely to be reduced. The increase in the concentration of fructose based oligosaccharides in the diet may alter the gut microflora in such a manner that number of *Bifidobacteria* may be selectively stimulated.

Crittenden and Playne (1996) gave an overview of application of oligosaccharides in food industry. The major use of oligosaccharides is in beverages, probiotic yoghurt and yoghurt drinks to produce synbiotic products, confectionary products, desserts such as jellies and ice creams, bakery products including biscuits, breads and pastries, spreads such as jams and marmalades and infant formulae.

Wouters (1998) reported that inulin could replace 100 per cent of the fat and oligofructose could be used as a partial or complete substitute for sugar in ice cream. Inulin improves stability and texture of ice cream without impairing its flavour during storage. Oligofructose masks the aftertaste of artificial sweetener in ice cream. Both inulin and oligofructose affect the freezing point and delay melting but have no effect on maximum overrun. Inulin and oligofructose, classified as dietary fiber and prebiotic substances contain only 1-1.5 kcal /g and are used as ingredients in low energy and diabetic ice cream.

Povolny and Smith (1999) studied the effects of substitution of inulin for corn syrup (42 Dextrose Equivalent) in reduced fat ice cream using sensory analysis. Three combinations of inulin and corn syrup were evaluated for iciness, chewiness, sweetness and vanilla flavour intensity. Replacing 50 per cent or 100 per cent of corn syrup (42 Dextrose Equivalent) with inulin increased chewiness. However, sweetness and vanilla flavour intensity perception of the ice cream were reduced. Storage stability data showed that partial/full replacement of corn syrup (42 Dextrose Equivalent) with inulin inhibited ice crystal formation over a six week thermal abuse period.

Devereux *et al.* (2003) reported that inulin and oligofructose were used successfully as fat replacers in plenty of food products like ice cream, cakes,

cookies and sausages at levels from four to thirteen grams, to achieve a significant reduction in fat content (20 to 80 per cent relative).

2.3 SYNBIOTIC

The synbiotic concept combines efficacious probiotic strains with specific prebiotic compounds in a single product (Ashwell, 2002). Synbiotic is defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplement in the gastrointestinal tract (Gibson and Roberfroid, 1995). In synbiotics there is synergistic relation between viable beneficial bacteria and their selective substrate.

2.3.1 Synbiotic Ice Cream

Modler *et al*. (1990) used ice cream as a carrier to incorporate *Bifidobacterium* spp and fructo oligosaccharides (FOS) into the human diet. Three *Bifidobacterium spp* (*B*. *longum, B. brevi* and *B. infantis)* were mixed with two types of bifidogenic factors, neosugar (synthetic FOS) and Jerusalem artichoke flour (natural FOS) in the ice cream. Approximately 90 per cent survival of all bacterial species was observed over 70 days period of storage at -17^{o}C. Bifidogenic factors had no detrimental effect on bacterial counts. Ice cream with neosugar was similar to the control ice cream with respect to sensory characters but ice cream with Jerusalem artichoke flour had undesirable flavour and appearance.

Akin *et al.* (2007) studied the effect of inulin and sugar levels on viability of probiotic bacteria and the physical and sensory characters of probiotic ice cream. Fermented milk supplemented with inulin (at one per cent and two per cent levels) was used to prepare the probiotic ice cream with different sugar levels (15 per cent, 18 per cent and 21 per cent (w/w)). Increasing sugar concentration stimulated physical and sensory properties of the probiotic ice

cream. The addition of inulin improved viscosity, first dripping and complete melting times without affecting the sensory properties. Viable bacterial count was highest at 18 per cent sugar concentration. The counts of *L. acidophilus* and *B. lactis* decreased to 10^5 cfu/g in the control sample, whereas the counts were 10^6 cfu/g in samples supplemented with inulin. The result suggested that the addition of inulin stimulated the growth of *L.acidophilus* and *B. lactis*, which resulted in improved viability of these organisms.

Akalin and Erisir (2008) reported the effect of supplementation of oligofructose or inulin on the rheological characteristics and survival of *L. acidophilus* La-5 and *B. animalis* Bb-12 in low fat ice cream stored at –18ºC for 90 days. Inulin increased the firmness, prolonged the first dripping time and brought only lowest change in melting properties, thereby improved the textural properties of ice cream. Oligofructose significantly increased the viability of *L. acidophilus* La-5 and *B. animalis* Bb-12 in ice cream mix. It is due to the structure of oligofructose which is conducive to cell viability during storage.

2.4 MICROENCAPSULATION

Micro encapsulation is defined as a technology of packaging solids, liquids or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under the influences of specific conditions (Kailasapathy and Masaondole, 2005).

From microbiological point of view, micro encapsulation can be defined as the process of entrapment/enclosure of cells of microorganisms by means of coating them with proper hydrocolloid(s) in order to isolate the cells from the surrounding environment, in a way that results in appropriate cell release in the intestinal medium (Sultana *et al*., 2000).

Among the agents that release the bacterial cells from the microencapsulated beads are pH changes, mechanical tensions, heat, enzymatic

activities, osmotic pressure, slow diffusion of the moisture through the capsule layers, presence of some chemical components and storage time (Gouin, 2004).

Micropropagation of probiotic cells has been shown to preserve them from detrimental environmental factors such as high acidity and low pH (Wenrong and Griffiths, 2000), bile salts (Lee and Heo, 2000), cold shock induced by the processing conditions such as deep freezing and freeze drying (Shah and Ravula, 2000), molecular oxygen in case of obligatory anaerobic microorganisms, heat shock caused by spray drying, bacteriophages (Steenson *et al*., 1987) and chemical antimicrobial agents (Sultana *et al*., 2000). However, other advantages such as increase in stability of sensory properties and its improvement (Gomes and Malcatta, 1999) and immobilization of the cells for their homogenous distribution throughout the product (Krasaekoopt *et al*., 2003) can also be achieved.

Main components used for micro encapsulation of probiotics are alginate and its combinations with prebiotics (FOS, Hi-maize etc), starch, xanthan-gelan mixture, carrageenan and its mixtures, gelatin, cellulose acetate phthalate, chitosan and whey proteins (Mortazavian *et al*., 2007).

The survival and multiplication of probiotics in the host strongly affect their probiotic benefits. Many studies have shown low viability of probiotics in dairy products including yoghurt and fermented milk (Iwana et *al*., 1993, Shah and Lankaputhra 1997 and Schillinger, 1999).

Protection of the probiotics has been proposed for various dairy fermentations, with micro encapsulation in hydro colloidal beads for improving probiotic viability in both the food products and the intestinal tract (Prevost and Divies 1988, Lacroix *et al.,* 1990 and Champagne *et al*., 1992).

2.4.1 Micro Encapsulation with Alginate

Alginate is a linear hetero polysaccharide extracted from different types of algae, with two structural units consisting of D-manuronic and L-guluronic acids. Calcium alginate has been widely used for the encapsulation of lactic and probiotic bacteria, mainly in the concentration range of 0.5 – 4 per cent (Sheu and Marshall, 1993).

Alginate capsules easily form gel matrices around bacterial cells, they are not poisonous to the body (safe or biocompatible), need only cheap, mild process conditions for their performance, can be easily prepared and performed (simplicity and ease of handling) and properly resolve in the intestine and release entrapped cells. Alginate gel matrix appropriately surrounds the bacterial cells with a diameter of 1-3µm and the pore sizes formed at the surfaces of alginate beads do not exceed 7nm (Klien *et al*.,1983).

2.4.2 Probiotic Ice Cream with Microencapsulated Bacteria

Sheu *et al.* (1993) proved the improvement in the survivability of culture bacteria in frozen desserts by micro entrapment. *L. bulgaricus* cells were entrapped in beads of calcium alginate and evaluated for their ability to survive freezing process. Cells survived freezing (without agitation) in ice milk mix much better than in distilled water and entrapped cells survived more than did cells that were not entrapped. The percentage of survival for entrapped and non-entrapped cells in continuously frozen ice milk was approximately 90 per cent and 40 per cent respectively. Addition of entrapped *Lactobacilli* had no measurable effect on the sensory characteristics of the ice milk.

Kebary *et al*. (1998) showed that *Bifidobacterium* spp. survived in high numbers in frozen ice milk in beads made from alginate than those made from κ-carrageenan.

Shah and Ravula (2000) reported that the survival of probiotic bacteria in fermented frozen desserts improved with encapsulation. Encapsulation thus enhances the shelf life of probiotic cultures in frozen dairy products.

Talwarkar and Kailasapthy (2003) reported the protective role of micro encapsulation against oxygen toxicity in probiotic yoghurt. The actual process of micro encapsulation seems to play a significant role in deciding the oxygen – alginate – bacteria interaction.

Kailasapathy and Sultana (2003) analysed the survivability of *L. acidophilus* DD910 and *B. lactis* DD920 in non fermented ice creams prepared with free probiotic cultures and with alginate encapsulated probiotic cultures and by incorporating alginate encapsulated probiotic cultures in ice cream mix fermented with *S. thermophilus*. The count of *L. acidophilus* showed an average of 2.25 log decrease for free cells, 2.06 log and 2.27 log decrease for encapsulated state in non fermented and fermented ice creams respectively after 24 weeks of storage at –20°C. Through this study, they revealed that encapsulation of probiotic bacteria does not significantly increase their survival in ice cream.

Kailasapathy and Sureeta (2004) showed that microencapsulation with whey protein can improve the survivability of *L. acidophilus* CSCC 2409, when incorporated in yoghurt stored at 4°C over a period of four week period. The number of free cells of *L. acidophilus* was reduced by 2.7 log numbers, while the whey protein encapsulated *L. acidophilus* were reduced by two log numbers at the end of the storage period.

Chen *et al.* (2005) performed a research work with the object to improve probiotic microencapsulation using prebiotics and used modern optimization technique to determine optimal processing conditions, performance and survival rates of probiotics. Optimisation with response surface methodology indicated that one per cent sodium alginate mixed with one per cent peptide and three per

cent fructo oligosaccharides as coating material would produce the highest survival in terms of probiotic count. The storage results also demonstrated that addition of prebiotics in the walls of probiotic microcapsules provided improved protection for the active organisms.

Homayouni *et al*. (2008[b]) showed that encapsulation in calcium alginate beads improved 30 per cent survivability of probiotic bacteria *L. casei* and B. *lactis* in the non-fermented synbiotic ice cream stored at -20°C for 180 days. One per cent of Hi-maize resistant starch was used as prebiotic.

2.5 LOW FAT PROBIOTIC AND SYNBIOTIC ICE CREAM

Haynes and Playne (2002) observed the survivability of probiotic cultures (*L. acidophilus*, *L. paracasei* and *B. lactis*) when added in the form of frozen concentrates in the full fat ice cream and low fat ice cream (prepared by using resistant starch Hi- maize), over a period of twelve months stored at -25°C. Initial freezing and churning significantly affected ($P<0.05$) the survival rate of *L. acidophilus* than other probiotic organisms. In the stored ice cream *B. lactis* survived better than *L. paracasei* and *L. acidophilus* both of which showed similar rate of decline till the end of storage period. The full fat ice cream offered no extra protection for cultures over the low fat ice cream during storage, with the low fat formulation showing improved survival of all three cultures during the freezing process. The addition of Hi-maize resulted in slightly lower number of viable organisms of all cultures after twelve months of storage.

Basyigit *et al.* (2006) investigated the viability and survival of human derived probiotic *Lactobacilli* (*Lactobacillus agilis*, *L. acidophilus* and *L. rhamnosus*) in two different ice cream formulations having six per cent of fat. Ice cream with sucrose and ice cream with aspartame were prepared and each of these was divided into two sub groups one with direct addition of the probiotic culture and other with milk fermented by the same probiotic culture. The probiotic cultures remained unchanged in ice cream stored for up to six months

regardless of the sweeteners used. Using probiotic cultures in ice cream mixes did not alter the characteristics of the product.

Aryana and Summers (2006) investigated the effects of a mixed culture of *L. acidophilus*, *L. casei* and *Bifidobacterium* spp. on the physicochemical and sensory characteristics of fat and sugar free ice cream. Flavour, body and texture scores were reduced at medium to high levels of probiotic culture incorporation. This would need to be taken into consideration when selecting the level of probiotic bacteria to be incorporated into fat and sugar free ice creams.

2.6 PROPERTIES OF ICE CREAM MIX

2.6.1 Physico - chemical Properties of Ice Cream Mix

2.6.1.1 pH of Ice Cream Mix

According to Alamprese *et al.* (2002) addition of microorganisms into the probiotic ice cream mix has influence on the pH of the ice cream mix. The pH of the ice cream mix was between 6.55 and 6.64.

Salem *et al*. (2005) showed that pH of ice cream mix with ten per cent added fermented milks were around 6.26 to 6.42. Although the pH of the added fermented milk was between 4.36 (*L. reuteri*) and 5.66 (*L. acidophilus)*, the pH of the total mix was high due to the high buffering capacity of the ice cream mix.

Trindade *et al*. (2006) prepared probiotic fermented acerola ice cream and the fermentation was interrupted when the pH reached 5.5 to 5.0. However, with addition of acerola pulp before freezing, pH values were reduced to 5.0 and 4.5 respectively.

Akin *et al*. (2007) reported that the pH of milk which was initially 6.59 - 6.62 decreased to 5.81 - 6.00 during preparation of probiotic ice cream mix

using ten per cent of fermented milk supplemented with or without one to two per cent of inulin.

Akalin and Erisir (2008) reported that the pH of probiotic ice cream mix, synbiotic ice cream mix with inulin, synbiotic ice cream mix with oligofructose and control ice cream mix were 5.52, 5.47, 5.52 and 6.90 respectively.

2.6.1.2 Titratable Acidity of Ice Cream Mix

The apparent or natural acidity of ice cream mix is caused by milk proteins, mineral salts and dissolved CO_2. A high acidity is undesirable as it contributes to excess mix viscosity, decreased whipping rate, inferior flavor and a less stable mix. If fresh milk components of excellent quality are used, the mix can be expected to have a normal acidity. Arbuckle (1966) reported that the normal ice cream mix containing 11 per cent milk solids not fat (MSNF) would have an acidity of 0.198 per cent. According to Bureau of Indian Standard specifications (IS: 2802-1964) maximum titratable acidity permitted in ice cream is 0.25 per cent.

De (1980) suggested that normal acidity of the ice cream mixes should not be more than 0.25 per cent.

Inoue *et al*. (1998) showed that the acidity of ice cream type frozen yoghurt was essentially constant during storage at -35°C.

According to Alamprese *et al*. (2002) the acidity of the probiotic ice cream mix prepared with *L. johnsonii* La1 was influenced neither by aging nor by storage, irrespective of the temperature of storage at -16°C or -28°C for up to three months. Microorganisms, sugar and fat have no effect on acidity of the mix. The acidity of the mix was between 0.010 and 0.015 per cent of lactic acid.

Haynes and Playne (2002) described that the addition of cultures did not adversely affect the product acidity.

Salem *et al*. (2005) showed that acidity of ice cream mix with ten per cent added fermented milks were around 0.24 to 0.27 per cent.

According to Basyigit *et al*. (2006) the acidity of the ice cream mix made by fermentation was higher than those made by direct addition of cultures. This can be explained by conversion of lactose to lactic acid during fermentation.

Akin *et al*. (2007) reported that acidity of probiotic ice cream increased as inulin content was increased. It was concluded that an increase in inulin content of the fermented milk had stimulated the metabolic activities of starter bacteria and improved development of acidity.

2.6.1.3 Specific Gravity of Ice Cream Mix

Naidu *et al.* (1986) studied the effect of utilization of whey in cream and observed that specific gravity of mix ranged from 1.054 to 1.123.

Rao *et al.* (1988) prepared frozen dessert using sweetened fermented milk and compared its physico-chemical properties with standard ice cream. Specific gravity of control and treatment mixes ranged from 1.092 to 1.102. They also found that the specific gravity of the frozen dessert mixes increased with increasing levels of fermented milk.

Arbuckle (1966) reported that specific gravity of ice cream mixes vary from 1.054 to 1.123.

Christiansen *et al.* (1996) found that the ice cream mixes prepared with 25 per cent and 50 per cent cultured milk showed no significant differences in their densities.

The specific gravity or density of ice cream mix varies with composition. The specific gravity of a mix may vary from 1.054 to 1.123 g per ml, with average for a 10 per cent mix of approximately 1.1g per ml.

Salem *et al.* (2005) showed that the specific gravity of the ice cream mix was not altered by the addition of fermented milk with different cultures.

2.6.1.4 Fat Content in Ice Cream Mix

Milk fat is an important component of ice cream. The use of the correct percentage is essential not only to balance the mix properly but also to satisfy the legal standards. The fat component of frozen dairy dessert mixes increases the richness of flavour, produces a characteristic smooth texture by lubricating the palate, helps to give body and aids in producing desirable melting properties. The fat in a mix also aids in lubricating the freezer barrel while the ice cream is being frozen. Limitations on excessive use of fat in a mix include cost, a decreased whipping ability, decreased consumption due to excessive richness and high calorific value (Marshall *et al.,* 2003).

2.6.2 Microbial Properties of Ice Cream Mix

2.6.2.1 Probiotic Count of Ice Cream Mix

Haynes and Playne (2002) performed preliminary trials to ascertain the optimal stage of culture addition and showed that addition and mixing prior to overnight ageing resulted in lower counts than when cultures were added immediately prior to freezing.

Salem *et al.* (2005) showed that when fermented milk was added to an ordinary ice cream mix at the rate of ten per cent (v/v) and the number of viable probiotic bacteria in the complete ice cream mix was decreased by one log unit.

According to Magarinos *et al.* (2007) as a consequence of dilution of culture used to inoculate the various ice cream mixtures, there was a decrease of 1.9 log units in *L. acidophilus,* corresponding to a 20.7 per cent log decrease with respect to initial concentration.

2.6.2.2 Coliform Count in Ice Cream Mix

The ice cream mix must be prepared in hygienic condition in order to avoid contamination and also to satisfy the Bureau of Indian Standard specifications (IS: 2802-1964). The resultant ice cream should contain not more than 90 per gram for coliform count. Since presence coliforms in the food products are used as the indicator of unhygienic manner of preparation.

Patwari and Chavan (1995) detected coliforms, lactic acid bacteria and micrococci in ice cream and attributed this to unclean equipment and utensils used for the production of ice cream.

Bostan (2002) conducted a study on the microbiological quality of industrial ice cream and observed that ice cream prepared using good quality raw materials in hygienic condition had an acceptable microbial quality.

2.7 PROPERTIES OF ICE CREAM

2.7.1 Physico – chemical Properties of Ice Cream

2.7.1.1 pH of Ice Cream

Christiansen *et al.* (1996) reported that the pH of ice cream prepared with 25 per cent of cultured milk (fermented with *L. acidophilus* and *B. bifidum*) was 5.8 and with 50 per cent of same cultured milk was 5.4 while the pH of the control ice cream was 6.6.

Inoue *et al.* (1998) reported that changes in pH of the ice cream type frozen yoghurt during storage were small and it was inversely related to the lactic acid content of the products.

Haynes and Playne (2002) observed that pH of full fat and low fat probiotic ice cream remained virtually unchanged over the storage period of 12 months. The pH of full fat probiotic ice cream was within the range of 6.31 to 6.40 after hardening and 6.31 to 6.38 at the end of 12 months of storage period. The pH of low fat ice cream was between 6.46 and 6.53 after hardening and between 6.44 and 6.48 at the end of storage of twelve months of storage. The higher level of pH range in the low fat probiotic ice cream was due to its higher milk solids not fat content (11.4 per cent) than that of full fat ice cream (9.5 per cent).

According to Kailasapathy and Sultana (2003) the pH of the fermented ice cream (pH- 4.5) remained virtually unchanged over 24 weeks of storage period.

Basyigit *et al*. (2006) showed that the pH of ice cream prepared by the addition of fermented milk was between 5.0 and 5.5 and that of ice cream prepared by addition of pre grown cultures without fermentation was between 5.7 and 6.6.

Trindade *et al*. (2006) showed that the pH of probiotic fermented acerola ice cream did not vary during storage period of 15 weeks at -18°C.

Akalin and Erisir (2008) reported that the pH of probiotic ice cream, synbiotic ice cream with inulin, synbiotic ice cream with oligofructose and control ice cream as 5.45, 5.35, 5.45 and 6.90 respectively.

2.7.1.2 Fat Content in Ice Cream

According to Prevention of Food Adulteration Act (1954) and Bureau of Indian Standard specifications (IS: 2802-1964) ice cream should contain minimum ten per cent fat. Guinard *et al*. (1996) reported that ice cream with higher fat content had better flavour and texture ratings as determined by sensory panel.

Christiansen *et al*.(1996) produced acceptable quality of ice cream by incorporating 12.0 per cent fat in control ice cream, 9.5 per cent in probiotic ice cream with 25 per cent cultured milk and 7.3 per cent fat in probiotic ice cream with 50 per cent cultured milk.

Adapa *et al*. (2000) found that structure development in ice cream is often attributed to the macromolecules present in ice cream mix such as milk fat, protein and complex carbohydrates.

2.7.1.3 Overrun of Ice Cream

Overrun is defined as the volume of ice cream obtained in excess of volume of mix and is expressed as percentage. The increased volume is due to incorporation of air into ice cream during freezing process.

Arbuckle (1966) reported that the overrun for packed ice cream ranged from 70 to 80 and softy ice cream from 30 to 50 per cent.

Christiansen *et al*. (1996) reported that the overrun of probiotic ice creams with 25 per cent and 50 per cent cultured milk were same (74 per cent and 75 per cent) and distinctly lower than that of the control (88 per cent). This indicates that the decrease in pH is responsible for the reduced air incorporation rather than total solids and fat content.

According to Alamprese *et al*. (2002) the overrun of the probiotic ice cream is inversely correlated to fat content and the effect of fat on overrun is higher in formulation with lower sugar concentrations. Overrun is not being affected by addition of micro organisms in the ice cream. The overrun reported was between 22.5 and 27.6.

Haynes and Playne (2002) reported that overrun of low fat probiotic ice cream varied between 48 per cent and 58 per cent.

Marshall *et al*. (2003) described that ice cream containing high amount of air (high overrun) tends to melt slowly. Air cells act as an insulator.

Salem *et al*. (2005) reported that overrun of probiotic ice cream prepared with addition of ten per cent of fermented milk using *L. acidophilus* was 65.52 per cent. The overrun in the resultant ice cream is affected by different factors such as the state and nature of proteins, acidity and freezing point in the mix. Differences in overrun of probiotic ice cream are attributed to the different levels of acidity in the mix with different probiotic cultures which affected the freezing point and/or the nature of proteins.

Yilsay *et al.* (2006) observed a reduction of overrun in the low fat ice cream containing whey protein concentrate. The overrun of ice cream containing 12 per cent of fat was 105 per cent and overrun of low fat ice cream with 0.5 percent milk fat and six per cent of whey protein concentrate was 98 per cent.

Akin *et al*. (2007) reported that addition of inulin (one per cent and two per cent levels) into the probiotic ice cream had an insignificant effect on overrun values of the ice cream samples.

Khillari *et al.* (2007) reported that overrun of low fat ice cream made by replacing 20, 40 and 60 per cent of the fat with whey protein concentrate

decreased the overrun value to 39.6, 38.3 and 36.0 per cent respectively while the overrun of the control ice cream was 40.1 per cent.

Akalin and Erisir (2008) analysed the increase in overrun (31.7 per cent) in the synbiotic ice cream by adding oligofructose at four per cent level than the control (23.6 per cent). The addition of *L. acidophilus* and fermentation of the ice cream mix has not significantly affected the overrun value (27.6 per cent) in the probiotic ice cream.

2.7.1.4 Whipping Ability of Ice Cream

Schmidt *et al*. (1993) observed that the use of maltodextrin based fat replacer in low fat ice cream resulted in mixes, which incorporated less air than the control mix.

Arbuckle (1966) reported that the diameter of air cells in ice cream ranges from 30 to 150 μm.

Moorthy and Balachandran (2000) reported that the whipping properties of the ice cream mixes determined ease with which the air is incorporated into the ice cream and fineness of dispersion of air cells. They also observed that the whipping properties are affected by process variables such as fat, MSNF, stabilizer and emulsifier including homogenization and ageing.

Pinto *et al*. (2004) reported that the acidity of the mix influences the viscosity, which in turn affects the whipping ability of the mix.

2.7.1.5 Meltdown Time of Ice Cream

Rao *et al.* (1988) found that the melting resistance of ice cream increased due to addition of sweetened fermented milk. The quantum of increase was in positive correlation to the magnitude of addition of fermented milk.

Modler *et al.* (1990) observed that the meltdown characteristics were not significantly different among the ice creams prepared with *Bifidobacterium* and different levels of fructo oligosaccharide (zero per cent, half per cent, one per cent and two per cent).

Christiansen *et al.* (1996) showed that control and ice cream with 25 per cent of commercial cultured milk fermented with *L. acidophilus* and *B. bifidum* were comparable in all over melting properties. However, the ice cream with 25 per cent cultured milk possessed the best melting resistance. The appearance of the melted control ice cream was slightly foamy and that of probiotic ice cream (with 25 per cent cultured milk) was more liquid probably due to difference in pH. The appearance of melted probiotic ice cream (with 50 per cent cultured milk), was little fluffy due to protein flocculation and it reported to have distinctly different meltdown properties.

Alamprese *et al.* (2002) revealed that the cultures did not influence the melting rate of probiotic ice creams even though the fat concentration was inversely proportional. Probiotic ice creams produced with high fat concentration were softer and showed lower melting rates.

El- Nagar *et al.* (2002) also reported that addition of inulin reduced melting rate of yog-ice cream.

Salem *et al.* (2005) described that the probiotic ice cream with *L. acidophilus* was close to control ice cream in respect of melting behaviour. The melting resistance was influenced by the freezing points and viscosity.

Akin *et al.* (2007) found that increased addition of inulin to ice cream mix increased melting times. The results for melting point suggested that inulin may act as a stabilizer due to its capacity of binding water. Inulin with its ability to reduce the free movement of water molecules appears to retard the melting of ice cream.

Akalin and Erisir (2008) observed the slower change in the melting properties of probiotic ice cream when compared to the control ice cream. The most remarkable improvement in melting properties was obtained in the probiotic ice cream added with inulin than oligofructose. The change in melting properties decreased in all types of ice cream as storage time increased. Addition of inulin led to the lowest change in melting properties and longest first dripping time as well as increase in firmness of probiotic ice cream than with addition of oligofructose.

2.7.1.6 Weight per Litre of Ice Cream

As per Bureau of Indian Standard specifications (IS: 2802-1964) minimum weight in gram per litre for plain and fruit ice cream are 525 and 540 respectively.

Salem *et al.* (2005) reported that there was an increase in the weight per litre by adding the probiotic cultures to the ice cream mix compared to the control.

2.7.2 Microbial Properties of Ice cream

2.7.2.1 Probiotic Count of Ice Cream After Freezing

Modler *et al*. (1990) described that the reduction in probiotic count during freezing is attributed to the incorporation of oxygen due to agitation of the ice cream mixture and also due to the incorporation of air during overrun. Agitation is necessary in order to achieve an even distribution of microbial agents.

Laroia and Martin (1991) showed that over 90 per cent of *L. acidophilus* and *B. bifidum* survives after freezing, in frozen yoghurt mixtures, with a pH between 5.6 and 5.8.

Hekmat and McMahon (1992) indicated that freezing process caused a reduction of at least one log cycle in the total count of *L. acidophilus* colonies, in a probiotic ice cream mix containing 12 per cent fat with pH 4.9.

Jay (1992) explained that the micro organisms which are better equipped to survive freezing are those that can dehydrate themselves more quickly. Such cells are able to reduce the number of intracellular ice crystals, which can break the cell's cytoplasmic membrane. In addition, milk fat and air bubbles act as insulators, because they reduce the transfer of heat through the frozen foam both the components restrict the growth of ice crystals, minimizing the damage that could be caused to microbial cells.

Mashayekh and Brown (1992) observed that the freezing and subsequent hardening caused one log cycle reduction in the bacterial count of the ice cream fermented with *L. bulgaricus and S. thermophilus*.

Christiansen *et al*. (1996) reported that the number of *L. acidophilus* and *B. bifidum* were either slightly decreased or unchanged during ageing of ice cream mix for 24 hours at 4°C. At freezing or shortly after freezing the number of viable bacteria decreased by 0.6 -1 log unit and the numbers for the frozen ice cream were in the range of 1.2×10^7 cfu/ ml for *L. acidophilus* and about 6×10^7 cfu/ml for *B.bifidum*.

Hagen and Narvhus (1999) explained that decrease in viable numbers of probiotic micro-organisms during ice cream preparation are usually caused by freezing, mechanical stress due to beating and oxygen incorporation. There was a reduction of 0.7 – 0.8 log cfu/g in probiotic cultures after freezing the ice cream mix.

Freezing process causes a thermal shock and consequently an osmotic shock that inevitably affects the viability of the organisms (Ordonez *et al*., 2000).

Heenan *et al*. (2004) reported that freezing did not affect the probiotic count of non-fermented frozen vegetarian dessert containing *B*. *lactis* BDBB2, *B.lactis* Bb-12, *L. acidophilus* and *L. paracasei*.

Salem *et al*. (2005) showed that initial freezing of ice cream mix in the batch freezer followed by hardening at -26 °C caused a reduction of less than one log cycle in total colony count of probiotics. The count in the frozen ice cream was found to be in the range of 7.48 log cfu per g for *L. acidophilus*.

Trindade *et al*. (2006) reported that there was no significant difference in probiotic count before and after ice cream preparation and concluded that the probiotic cultures used (*Bifidobacterium* spp and lactic acid bacteria) were resistant to freezing, churning and air incorporation.

Magarinos *et al*. (2007) observed that the survivability of *L. acidophilus* and *B. lactis* during freezing when added individually in the probiotic ice cream was 91.3 and 90.1 per cent respectively and 89.1 per cent when both bacteria added together. The reduction in the survivability of probiotic bacteria in the cream during freezing was attributed to overrun and freezing process.

According to Christiansen *et al*. (1996), Alamprese *et al*. (2002), Heenan *et al*. (2004) and Trindade *et al*. (2007) resistance to freezing, beating and air incorporation depends on different probiotic microorganisms and conditions of ice cream production.

According to Heenan *et al*. (2004) and Trindade *et al*. (2007) there is slight increase in cell concentration in probiotic ice cream after freezing. This effect could be due to the break up of *Lactobacillus chains* caused by beating the ice cream during freezing.

Akalin and Erisir (2008) explained that the decline in bacterial counts, as a result of freezing is most likely due to the freeze injury of cells leading

eventually the death of cells. During freezing of the mix, the counts of both *L. acidophilus* LA-5 and *B. animalis* Bb-12 decreased by 1.5 to 2.0 log units and their numbers in the frozen ice cream were found to be in range of 5.96 to 6.60 log cfu/g for *B. animalis* Bb-12 and 5.98 to 6.21 log cfu/g for *L. acidophilus* LA-5.

2.7.2.2 Probiotic Count of Ice Cream During storage

Modler *et al*. (1990) described that there was a little change in Bifidobacterium counts from the point of freezing until termination of 70 days of storage studies. The maximum decline in bacterial number did not exceed one log cycle. The addition of fructo oligosaccharides did not appear to have any effect on bacterial numbers.

Studies of Holcomb and Frank (1991) opined that ice cream mix act as a cryoprotector medium due to the presence of casein, sucrose and lactose in it.

Hekmat and McMahon (1992) monitored the survivability of the *L. acidophilus* and *B. bifidum* in the fermented probiotic ice cream during 17 weeks of frozen storage at –29°C. At the end of the storage period, the counts for *L. acidophilus* and *B. bifidum* decreased to 3×10^6 and 1×10^7cfu/ml respectively.

Jay (1992) described that during frozen storage, death of micro organisms probably occur due to incomplete conversion of product's water into ice and presence of highly concentrated residual solution. The composition and concentration of this residual solution can change during the course of storage and ice crystals can enlarge, especially due to temperature fluctuations. After freezing, the death rate of microorganisms is higher at the beginning of the storage period and gradually diminishes thereafter, until the number of surviving microorganisms is stabilized. The cells damaged during freezing die gradually during storage. In addition, those cells that have escaped death by freezing are

later exposed to osmotic effects, which can cause mortality during the melting of the ice cream.

Sheu *et al*. (1993) reported that 80 - 90 per cent of lactic acid bacteria survived in ice cream mix after the mix had been stored at -20°C for 20 weeks.

Christiansen *et al*. (1996) reported that during storage of probiotic ice cream at - 20°C for 16 weeks the number of viable bacteria (*B. bifidum* and *L. acidophilus*) decreased by 0.1 – 0.7 log units.

Inoue *et al*. (1998) investigated the survivability of lactic acid bacteria (*L. bulgaricus and S. thermophilus)* in ice cream type frozen yoghurt prepared with a fat content of 10.6 per cent and with varying pH values and stored at -35°C for six months. The number of bacteria in the ice cream from well fermented mix (pH 4.5) decreased to about one half of the original level during the first weeks storage and then the numbers gradually decreased during the following four months, followed by a further significant decrease in the numbers of bacteria during the next two months. On the other hand, the numbers of lactic acid bacteria in the ice cream prepared from intermediate fermented mix (pH 5.0 - 5.5) initially decreased to about 13 per cent of the original level and then remained constant during the following six months storage. The numbers of lactic acid bacteria in the ice cream prepared from poorly fermented mix pH (6.5) were small numbers (less than 10^4 cfu /g) after freezing and during frozen storage the number reduced to zero.

Lopez *et al*. (1998) showed that the lactic acid bacteria in the yoghurt ice cream are stable during storage at -23°C for one year and do not decrease significantly. Low temperatures, such as -20°C, causes the death of the microorganisms at a slower rate. The opposite occurs when the maintenance temperature approaches the melting point.

Ravula and Shah (1998) assessed the effect of acid casein hydrolysate and cysteine on the viability of probiotic bacteria (*L. acidophilus* and *Bifidobacterium BB* BDBB2) in fermented frozen dairy desserts during a storage period of 12 weeks at –18°C. The counts of *L. acidophilus and Bifidobacterium* BB BDBB2 decreased to less than 10^2 cfu/g in the control sample, whereas the counts were greater than 10^5 cfu/g in the samples supplemented with acid casein hydrolysate or cysteine.

Hagen and Narvhus (1999) observed that the viable count of *B. bifidum*, *L. acidophilus, L. reuteri* and *L. rhamnosus* did not change significantly during 52 weeks of frozen storage in ice cream and remained above the recommended minimum limit of 10^6 cfu/g.

Alamprese *et al.* (2002) reported that after freezing the probiotic (*L. johnsonii*) count decreased by around 0.2 to 0.3 logarithmic units in the ice cream mixes with ten per cent fat and 22 per cent sugar and ice cream mixes with five per cent fat and 15 per cent sugar. Regardless of the formulation, after 240 days of storage counts of *L. johnsonii* did not change significantly in the ice cream.

Kailasapathy and Sultana (2003) analysed the viability of free and encapsulated *L. acidophilus* and *B. lactis* in the probiotic ice cream stored at -20 °C for 24 weeks. The *L. acidophilus* count showed an average of 2.52 log reduction for free cells at the end of the storage period, while the encapsulated state of the same strain showed a decrease of 2.06 log and 2.27 log in the non-fermented and fermented ice creams, respectively. *B. lactis* showed 2.80 log and 2.42 log decrease in the free and encapsulated state of cultures, respectively in non- fermented ice cream. There was a decrease of 2.02 log for the same strain in fermented ice cream.

Heenan *et al*. (2004) observed that there was no marked reduction in the initial population (10^7 - 10^8 cfu/g) of *Lactobacillus* spp and *Bifidobacterium* spp

(expect *L. paracasei*) in probiotic ice cream throughout the storage period of 28 weeks at -20°C. But there was a reduction of 50 per cent in the initial population of *L. paracasei* in the probiotic ice cream during the end of the same storage condition. The level of freeze injury sustained during frozen storage, as indicated by bile sensitivity, was dependent on the strain of micro organism. Bile tolerant sub population remained more stable than the total population during storage. For *L. acidophilus* MJLA1, *L. rhamnosus* 100-C and *B. lactis* Bb-12, the bile tolerant populations decreased significantly slower than the total viable population.

Salem *et al*. (2005) showed that during 12 weeks of storage of probiotic ice cream at -26°C, count of *L. acidophilus* decreased by 2.23 log cfu/g. They reasoned out that the decline in bacterial number was due to freezing of all cells resulting in the death of some cells, mechanical stresses of mixing and freezing process and also incorporation of oxygen into the mix.

According to Basyigit *et al*. (2006) the initial number of lactic acid bacteria in the probiotic ice cream made by addition of fermented milk was 6.0 x 10^8 cfu/ml (sucrose as sweetener) and 8.1 x 10^8 cfu/ml (aspartame as the sweetener). After 180 days of storage, the number of bacteria decreased to 4.2 x 10^8 cfu/ml and 3.9 x 10^8 cfu/ml respectively. The initial number of lactic acid bacteria in the probiotic ice cream made by the direct addition of pregrown culture was 5.3 x 10^7 cfu/ml (sucrose as sweetener) and 2.5 x 10^8 cfu/ml (aspartame as the sweetener). After 180 days of storage, the number of bacteria decreased to 3.5 x 10^7 cfu/ml and 3.9 x 10^7 cfu/ml respectively. The number of lactic acid bacteria in probiotic ice cream made by fermentation method was high initially when compared to the method of direct addition of pre grown cultures. But the decrease was same in ice cream prepared by both the methods during the 180 days of storage. Probiotic cultures remained unchanged in ice cream stored for up to six months regardless of the sweeteners used. The characteristic of the product was not altered due to incorporation of probiotic culture.

Trindade *et al*. (2006) reported that, after 15 weeks of storage of probiotic fermented acerola ice cream at -18°C with pH 4.5 and 5.0, the population of *B. longum* and *B. lactis* showed little decrease (0.1 to 0.6 log unit). But these variations were not statistically significant for both the culture, except for *B. longum* at pH 5.0. The number of viable bifidobacteria in all the probiotic fermented acerola ice cream remained above 10^6 cfu /g during storage period.

Akin *et al.* (2007) studied the effect of inulin and different sugar levels on viability of probiotic bacteria and the physical and sensory characters of probiotic fermented ice cream. Inulin was added at one and two per cent levels. In spite of decrease in the count of *L. acidophilus* by 1.5 to 3.0 log units on storage up to 90 days, the count was found to be above the therapeutic threshold of 10^6 to 10^7 cfu/g.

Haroldo *et al.* (2007) inoculated four per cent culture of *L. acidophilus* into ice cream and stored the ice cream at −25°C for 60 days. It had a final concentration of 2×10^6 cfu/g and the survival rate was 87 per cent.

Magarinos *et al.* (2007) concluded that there was no significant difference in the counts of *L. acidophilus* and *B. lactis* when added individually and in combined mixture in the ice cream, during the storage period of 60 days at -25 °C. In the probiotic ice cream the survival rate of *L. acidophilus* LA-5 was 87 per cent with final concentration of 2×10^6 cfu/ g and that of *B. lactis* Bb-12 was 87 per cent with final concentration of 9×10^6 cfu/ g and when both the organisms were inoculated together the survival rate was 86 per cent at the end of the study.

Trindade *et al.* (2007) produced the ice cream with different starter cultures (*L. acidophilus* 74-2*, L. acidophilus* LAC4 and yoghurt starter culture), after fermenting up to a final pH 4.5 and 5 with concentrations of added cream five and ten per cent. Even though the probiotic counts decreased during storage,

it was still higher than 10^6 cfu/g after 105 days at -18 °C, in all products even in the probiotic ice creams with pH of 4.5.

Akalin and Erisir (2008) showed that the probiotic count significantly decreased (0.3 to 9 log cfu/g) throughout the storage of ice cream at -18°C for 90 days.

Haynes and Playne (2002) and Trindade *et al*. (2007) showed that higher concentration of fat in the ice cream did not provide greater protection to the probiotic microorganisms. Full fat ice cream (ten per cent fat) offered no extra protection to probiotic microorganisms during storage, when compared to those prepared with 3.8 per cent and five per cent fat.

Davidson *et al.* (2000) and Alamprese *et al.* (2002) reported that starter culture bacteria in low fat ice cream did not change significantly during storage.

2.7.2.3 Coliform Count of Ice Cream during Storage

Ice cream is widely consumed in our country and may be subjected to contamination at various stages of preparation, packaging and handling. According to Bureau of Indian Standard specifications (IS: 2802-1964) ice cream should contain not more than 90 per gram for coliform count.

Arora and Sudarsanam (1986) found that the bacteria in ice cream come from two sources (i) ingredients used (ii) conditions of manufacture, handling, storage and transportation.

Shrestha and Sinha (1987) studied the occurrence of coliform bacteria in dairy products and found that 77 per cent of ice cream contained unsatisfactory levels of coliforms on the basis of Indian standards.

Arslan *et al.* (1996) studied the microbial quality of ice cream samples marketed in Elazig and detected Listeria, Salmonella, E.coli type1, and *Klebsiella*

pneumonia indicating that above samples do not meet microbiological quality standards required for consumer health.

Kumari *et al.* (1996) detected organisms such as *Staphylococcus aureus*, *Staphylococcus epidemidis, Streptococcus pyogenes, Klebsiella aerogenes* and *Enterobacter aerogenes* as well as coliforms and fungi such as *Aspergillus* spp, *Pencillium* spp and *Mucor* spp in ice cream samples, which was due to imperfect sanitary conditions followed during handling, production and storage of ice cream in Mumbai region.

Erol *et al.* (1998) found that the pathogenic microorganisms in ice cream predisposed it to a poor hygienic quality, which was responsible for food infections and intoxications and thereby posed potential risk to public health. This can be evaded by following hygienic precautions by producers.

Avramidis *et al.* (2004) incorporated *E. coli* 0157:H7 (food-borne pathogen) in the two types of yoghurt ice cream mix, one made with guar gum and the other one made with xanthan gum. Probiotic bacteria (*L. acidophilus* and *B. bifidum*) were also added to the mix and the ice cream was kept at -20°C for 60 days. The initial count of *E. coli* in the guar-gum and xanthan-gum ice cream mix was 6.15 ± 0.46 log cfu/g and 6.19 ± 0.13 log cfu/g respectively. The ice creams were examined at 1, 5, 10, 15, 30 and 60 days of storage. *E. coli* decreased significantly from one to five days of storage in both the guar-gum and xanthan-gum ice creams and thereafter the count was at rather the same levels, being 2.26 ± 0.49 log cfu/g and 1.73 ± 0.78 log cfu/g respectively at 60 days. The count of *L. acidophilus* and *B. bifidum* was 5.95 ± 0.05 log cfu/g and 3.59 ± 0.81 log cfu/g in the xanthan-gum ice creams, respectively and 6.75 ± 1.64 log cfu/g and 3.57 ± 0.74 log cfu/g in the guar-gum ice creams respectively at 60 days.

2.7.3 Sensory Evaluation of Ice Cream

Arbuckle (1966) reported that texture in ice cream is attributed to the grain or fine structure, which is dependent upon the size, shape and arrangement of the ice crystals. Ice cream with ideal texture will have crystals too small to be detected in the mouth. He also reported that the ideal body is produced by the correct proportion of solids (both butter fat and milk solids- not- fat) and the proper overrun.

Modler *et al*. (1990) showed that probiotic ice cream prepared with *Bifidobacterium* spp and one per cent synthetic fructooligosaccharide (neosugar) was similar in all respects to the control and no significant differences were noted for any of the sensory characteristics (greyness, sweetness, off-flavour, creaminess and firmness). Ice cream with two per cent synthetic fructooligosaccharide is creamier than the control with same sweetness. Probiotic Ice cream with one per cent and two per cent synthetic fructooligosaccharide was slightly icy. Ice cream with two per cent natural fructooligosaccharide (Jerusalem artichoke flour) differed significantly from both the control and probiotic cream with synthetic fructooligosaccharide in off flavour and greyness. Although Jerusalem artichoke flour is an excellent source of neosugar, it is unsuitable in its present form for ice cream.

Hekmat and McMahon (1992) prepared probiotic ice cream with a pH of 5.0, 5.5 and 6.0 by mixing fermented mix with unfermented mix to determine consumer preferences and to compare with standard ice cream (pH 6.5). The preferred pH of probiotic ice cream, based on overall acceptance was 5.5. They demonstrated that probiotic ice cream is a suitable vehicle for delivering beneficial microorganisms such as *L. acidophilus* and *B. bifidum* to consumers.

Inoue *et al*. (1998) reported that the sensory character of ice cream type frozen yoghurt was much richer than that of normal frozen yoghurt. Panelists who consume yoghurt frequently are more sensitive to the difference in sensory

properties of frozen yoghurt than those who do not eat yoghurt. Ice cream type frozen yoghurt containing 0.33 per cent of lactic acid with pH 5.5 was the most favoured one among other ice cream type frozen yoghurt with pH 4.5, 5 and 6.5. The physical properties of ice cream type frozen yoghurt such as smoothness was also dependent on the pH value. There is difference in the characteristic feature of ice cream type frozen yoghurt with varying pH values.

Hagen and Narvhus (1999) prepared probiotic ice cream by adding fermented milk to the regular ice cream mix. The results for the sensory evaluation of the probiotic ice cream were considered satisfactory without any probiotic flavour.

Davidson *et al*. (2000) reported that in general, ice cream fermented with probiotic cultures had presented less aroma and taste of yoghurt than those produced with the traditional culture and that the characteristics of yoghurt were favoured by lower pH.

Heenan *et al.* (2004) opined that from consumer point of view, the non-fermented frozen probiotic soy dessert is suitable product for probiotic delivery. The sensory panel could not distinguish between fresh product inoculated with *L. acidophilus* MJLA1 and the control product containing no probiotics. There were no distinguished sensory differences between fresh and the stored (four months and seven months) product containing *L. acidophilus* MJLA1.

Salem *et al*. (2005) prepared probiotic ice creams with *L. acidophilus*, *L. reuteri*, *L. rhamnosus*, *L. gasseri* and *B. bifidum*. Probiotic ice cream containing *L. acidophilus* was less acidic with good body and texture. But, its flavour was less preferred than the other probiotic ice creams. All the probiotic ice creams scored slightly lower values in melting quality and colour attributes than the control ice cream. This could be due to higher acidity and heating process of the milk needed for fermentation. None of the probiotic ice cream

judged to be icy in texture. All the ice cream supplemented with the probiotic strains were acceptable and gave a good total impression with marked flavour.

Trindade *et al*. (2006) concluded that the reduction in pH caused by fermentation process can result in structural alterations of proteins. This influenced positively the development of a pleasant texture on probiotic fermented acerola ice cream. The acerola ice creams fermented with traditional culture of yoghurt were better accepted in terms of aroma, taste and global acceptance than those produced with probiotic cultures. The fermented acerola ice cream with pH 4.5 resulted in significantly superior sensory acceptance for texture. The pH 4.5 is below the isoelectric point of casein, which probably provoked bigger alterations in the protein structures which resulted in a more pleasant texture for the consumers.

Akin *et al*. (2007) described that the points allocated for colour, body, texture and taste showed that an increase in sugar content brought about an improvement in the structure, creaminess, flavour and aroma of the probiotic ice cream. The addition of inulin had no effect on sensory properties of probiotic ice cream. There was no acidic or probiotic flavour in the ice cream. One reason for this could be high pH of the ice cream. All the samples were medium sour and gave a good total impression, without any marked off flavour during the storage period. None of the ice creams were judged to be crumbly, weak, fluffy or sandy.

Trindade *et al*. (2007) showed that there was a good acceptance for the probiotic fermented yellow mombin ice creams in all attributes. However texture and aroma were the most and least accepted attributes respectively. There were no significant differences amongst the means attributed to taste and overall acceptance. The panelists considered the taste to be the most important attributes, even when evaluating the ice cream as a whole. The estimated means for ice cream produced with *L. acidophilus* were not different from those estimated for ice cream produced with the yoghurt culture except for the attribute

of texture. So it is very difficult to manufacture probiotic fermented milk products with the same acceptance as those produced with yoghurt culture. The panelists reported no off flavours or aroma in the ice cream. With respect to sensory analysis, the results suggested that it is possible to work with a higher pH, thus reducing the energy costs and fermentation time and with a lower cream percentage for reasons of economy and in an attempt to obtain a healthier product.

2.8 COST ANALYSIS OF ICE CREAM MIX

Rao *et al.* (1988) prepared soft serve frozen dessert using varied amounts of sweetened fermented milk and standard plain ice cream mix and estimated the cost of the product based on the existing market prices of ingredients which indicated that a cost reduction of 9.6, 13.0 and 19.3 per cent was possible when different ratios were used.

Materials and Methods

3. MATERIALS AND METHODS

The main objective of the study was to assess the efficiency of micro encapsulation to improve the survivability of probiotic bacteria *L. acidophilus* LA-5 in the synbiotic ice cream. The work was carried out in the Department of Dairy Science, College of Veterinary and Animal Sciences, Mannuthy utilizing the facilities available in the Kerala Agricultural University (KAU) Dairy Plant. The ice cream prepared was analyzed for physico–chemical properties, probiotic survivability and organoleptic qualities. Analytical grade reagents and food grade ingredients were used throughout the study. Six replications were done and the data were analyzed by statistical methods (Snedecor and Cochran, 1989).

3.1 MATERIALS

These are divided into dairy ingredients and non- dairy ingredients.

3.1.1 Dairy Ingredients

3.1.1.1 Milk

Fresh cow milk was procured from the KAU Dairy Plant**,** Mannuthy.

3.1.1.2 Skim Milk and Cream

Skim milk and cream were prepared using fresh cow milk with the help of a centrifugal cream separator.

3.1.1.3 Skim Milk Powder

Spray dried skim milk powder (Amul) was obtained as sample from Gujarat Co-operative Milk Marketing Federation, Cochin.

3.1.1.4 Dairy Whitener

Spray dried Dairy Whitener powder (Nestle) was procured from local market.

3.1.2 Non-Dairy Ingredients

3.1.2.1 Probiotic Culture

Lactobacillus acidophilus LA-5 ® freeze - dried probiotic culture for Direct Vat Set (DVS) was provided as sample from Chr Hansen, Denmark.

3.1.2.2 Sugar

Good quality cane sugar purchased from local market was used in the experiment.

3.1.2.3 Stabilizer and Emulsifier

Cremordan samporana of Danisco supplied by Bharath Marketing, Palakkad, Kerala – 678 014 as free sample was used. It is a mixture containing stabilizer (guar gum and carrageenan) and emulsifier (mono and diglycerides of fatty acids and sorbitan esters of fatty acids).

3.1.2.4 Flavour

Vanilla Flavour was procured, from Givaudan, Jigani, Karnataka – 562106.

3.1.2.5 Colour

Natural annatto was gifted as sample by Aarkay Food Products Ltd, Ahmedabad, Gujarat–380 009. The colour solution was prepared by mixing three gram of powder in 100 ml of distilled water and autoclaved.

3.1.2.6 Lactobacillus MRS Agar

Lactobacillus MRS Agar (Himedia Laboratories Ltd. Mumbai – 400 086) was used for the enumeration of probiotic bacteria *L. acidophilus* LA-5 in ice cream.

3.1.2.7 Violet Red Bile Agar

Violet red bile agar (Himedia Laboratories Ltd. Mumbai – 400 086, India) was used for the enumeration of coliforms in ice cream.

3.1.2.8 Peptone Water

Peptone water (Sisco Research Laboratories, Mumbai – 400 049) was used as a diluent.

3.1.2.9 Whey Protein Concentrate

Microparticulated whey protein concentrate (Simplesse ®100) containing 53 per cent protein, was received as a sample from Cp Kelco, San Diego, USA.

3.1.2.10 Wheat Dextrin

Wheat dextrin (Nutriose FB 06) was obtained as a free sample from Roquette Freres, Lestrem, France.

3.1.2.11 Oligofructose

Beneo P95 Oligofructose a prebiotic powder manufactured by Orfati, Belgium was received as a free sample from, DPO Food Specialties Private Limited, Thane, Maharastra – 400 607.

3.1.2.12 Sucralose

Sucralose powder (Sugar Free Natura), was purchased from the local market.

3.1.2.13 Tween 80

Tween 80 gifted by D.V. Deo Industries, Kalamassery, Kerala – 683 109 was used as an emulsifier in micro encapsulation technique.

3.1.2.14 Plastic Containers

Food grade plastic containers made up of polypropylene was supplied by Riya Luster, Perambavoor, Kerala - 683 546.

3.1.2.15 Polydextrose

Polydextrose (Litesse 11 powder of Danisco) received as a sample from S.A. Pharmachem Pvt. Ltd., Mumbai, Maharastra – 400 063 was used as a bulking agent in the experiment.

3.1.2.16 Sunflower Oil

Sunflower oil (Gold Winner) was procured from local market.

3.1.2.17 Sodium Alginate

Food grade sodium alginate was procured as a free sample from Snap Naturals and Alginate Products Limited, Ranipet, Tamilnadu – 632 403 .

3.1.2.18 Calcium Chloride

Calcium chloride (food grade) powder procured from Ambrish Metchem, Mumbai, Maharastra - 400 009 was used for microencapsulation.

3.2 METHODS

3.2.1 Analysis of Dairy Ingredients

3.2.1.1 Analysis of Fat in Whole Milk, Cream, Skim Milk, Dairy Whitener and Skim Milk Powder

The fat content of whole milk, cream, skim milk, dairy whitener and skim milk powder were estimated by the procedure laid out in IS - SP: 18 Part IX, (1981).

3.2.1.2 Analysis of Total Solids in Whole Milk, Cream, Skim Milk, Dairy Whitener and Skim Milk Powder

The total solids content of whole milk, cream, skim milk, dairy whitener and skim milk powder were estimated by the procedure laid out in IS - SP: 18 Part IX, (1981).

3.2.2 *L. acidophilus* LA-5 Count of Direct Vat Set (DVS) Culture

One gram of direct vat set (DVS) culture containing *L. acidophilus* LA-5 was dissolved in nine ml of sterilized peptone water and it was serially diluted. One ml of samples each from dilutions of 10^{-9} to 10^{-11} was used for *L. acidophilus* LA-5 count using MRS agar. The petriplates were incubated at 37°C for 48 hours.

3.3 PROCEDURE FOR THE PREPARATION OF ICE CREAM

A modified procedure of Hekmat and McMahon (1992) was followed in the preparation of ice cream.

3.3.1 Selection of Ingredients

The ingredients such as dairy whitener, cream, raw milk, sugar, stabilizer, emulsifier, vanilla flavour and annatto powder for colour were selected.

3.3.2 Figuring the Mix

The proportionate quantity of different ingredients to meet the minimum standard for fat (ten per cent) and total solids (36 per cent) as per Prevention of Food Adulteration Act (1954) for the preparation of ice cream was calculated.

3.3.3 Making the Mix

Ingredients selected for the preparation of ice cream were weighed. Solid ingredients (dairy whitener, sugar, stabilizer and emulsifier) were mixed together and kept separately. The liquid ingredients (milk and cream) were taken in milk cooker and heated with stirring. The thoroughly mixed solid ingredients were added into the milk cooker when the temperature of the liquid content reached around 42°C. Heating was continued further with frequent stirring until the temperature of the mix reached 65°C.

3.3.4 Homogenizing the Mix

Homogenization of the mix was done at a temperature of 65°C by using a pressure of 150 kg/cm^2 at first stage and 30 kg/cm^2 at the second stage.

3.3.5 Pasteurizing the Mix

The mix was pasteurized at a temperature of 82°C for 30 minutes.

3.3.6 Cooling and Ageing the Mix

The mix after pasteurization was immediately cooled to 4°C and later transferred to a cold storage maintained at a temperature of 4 ± 1°C and kept for overnight.

3.3.7 Addition of Flavour and Colour

Vanilla flavour at the rate of 5 ml per kg of the mix and three per cent annatto colour were added to the mix and mixed well.

3.3.8 Separation of the Ice Cream Mix

Then the ice cream mix was divided into three groups:-

I. **Group A** separated into treatments T1 and T2

T1 - probiotic ice cream with non – encapsulated *L. acidophilus* LA-5

T2 - synbiotic ice cream with non – encapsulated *L. acidophilus* LA-5 and oligofructose

II. Group B separated into treatments T3, T4 and T5.

T3 - probiotic ice cream with encapsulated *L. acidophilus* LA-5

T4 - synbiotic ice cream with encapsulated *L. acidophilus* LA-5 and oligofructose

T5 - low fat synbiotic ice cream with encapsulated *L. acidophilus* LA-5 and oligofructose

III. Group C as control.

Group A

3.3.8.1 Probiotic Ice Cream Mix with Non-encapsulated L. acidophilus LA-5 (T1)

One per cent of non encapsulated *L. acidophilus* LA-5 (dissolved in 100 ml of sterilized skim milk) was added to ice cream mix before freezing.

3.3.8.2 Synbiotic Ice Cream Mix with Non-encapsulated L. acidophilus LA-5 (T2)

One per cent of non encapsulated *L. acidophilus* LA-5 (dissolved in 100 ml of sterilized skim milk) and two per cent of oligofructose was added to ice cream mix before freezing.

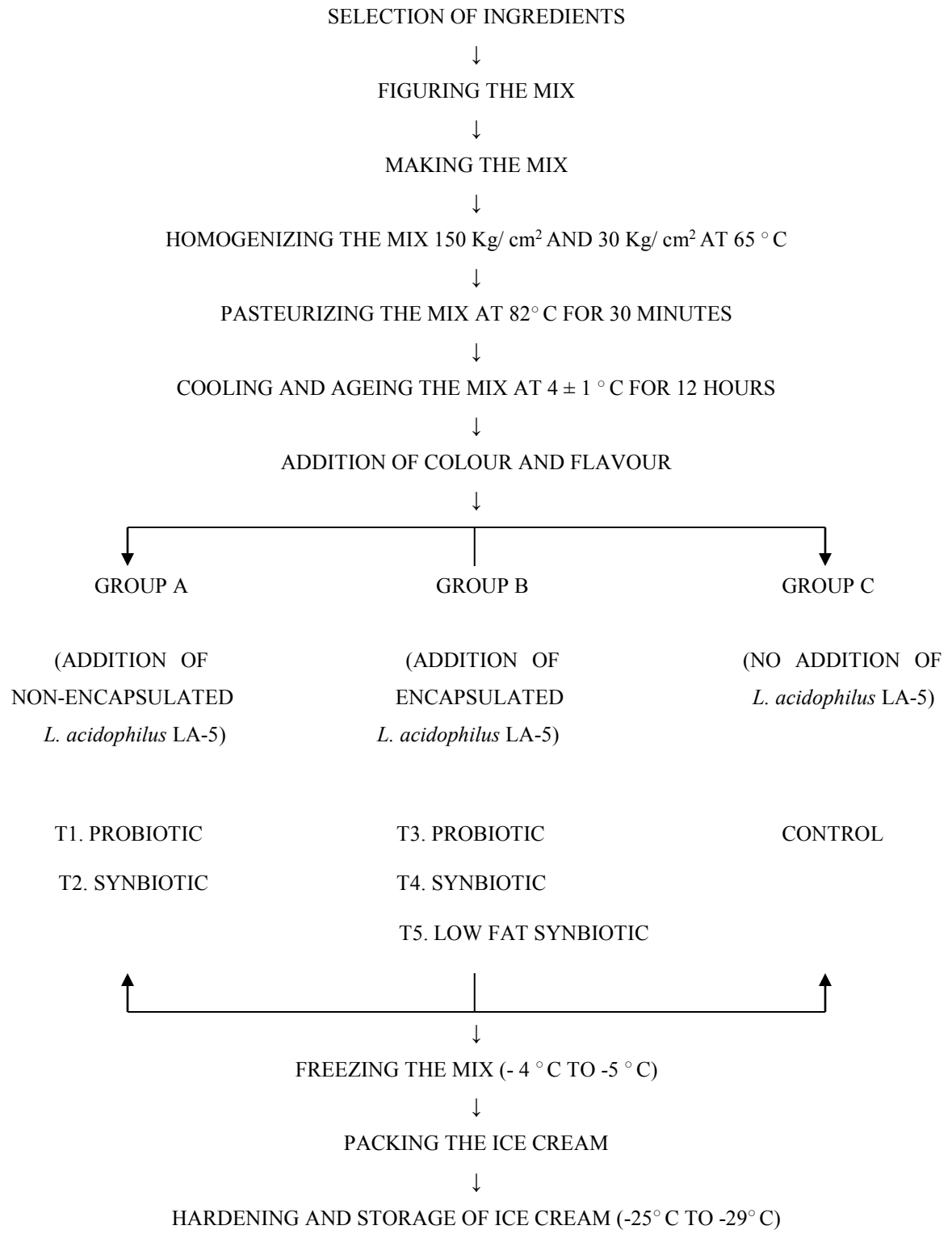

Fig. 1 Flow Chart for Ice Cream Preparation

Group B

3.3.8.3 Probiotic Ice Cream Mix with Encapsulated L. acidophilus LA-5 (T3)

3.3.8.3.1 Procedure for Encapsulation

All the glass wares and solutions used in the protocol were sterilized at 121°C for 15 minutes. Direct Vat Set (DVS) culture of *L. acidophilus* LA-5 with 10^{10} to 10^{11} colony forming units per gram was used. Alginate beads were produced using a modified encapsulation method originally reported by Sheu and Marshall (1993) and Sultana *et al.* (2000). One per cent of DVS culture was used in the ice cream mix. One part of DVS culture was mixed with four parts of one per cent of sodium alginate and four per cent of whey protein solution for encapsulated probiotic ice cream. One part of DVS culture was mixed with four parts of one per cent of sodium alginate, one per cent of whey protein and three per cent of oligofructose P95 solution for encapsulated synbiotic ice cream and encapsulated synbiotic low fat ice cream.

One part of alginate cell mixture was then added drop wise to five parts of vegetable oil (edible sunflower oil) containing Tween 80 (0.2 per cent), which was magnetically stirred at 200 rpm. Within ten minutes, a uniformly turbid emulsion was obtained. Calcium chloride (0.1M) was added quickly but gently (20 ml/s) until the water in oil emulsion was broken. Calcium alginate beads were formed within ten minutes. The beads were allowed to harden in calcium chloride (0.1M) for 30 minutes and collected by filtering through Whatman No 41 filter paper. The residual oil was removed by washing the beads with sterile saline solution. Then beads finally stored in sterilized skim milk broth at 5 ^{o}C and used within a day of preparation.

3.3.8.3.2 Measurement of Bead Size

The calcium alginate beads (containing *L.acidophilus* LA-5), were stained with safranin and its diameter was measured with an eyepiece micrometer on an optical microscope at a magnification of 40X. At least 120 randomly selected beads were measured for each sample. Scanning electron microscopy (SEM) was employed to examine the structure of the calcium alginate beads. The facility in Sophisticated Test & Instrumentation Centre, Cochin University of Science and Technology, Cochin-682022 was utilized for taking the electron photomicrograph of microencapsulated beads. The bead samples were dried and coated with platinum to make it conductive and then mounted on an aluminum stub. Microscopy was performed under scanning electron microscope at an accelerated voltage of 15 kV and 20 kV

One per cent of encapsulated *L. acidophilus* LA-5 (dissolved in 100 ml of sterilized skim milk) was added to ice cream mix before freezing. The same procedure outlined for control ice cream was used for preparation of probiotic ice cream with encapsulated *L. acidophilus* LA-5.

3.3.8.4 Synbiotic Ice Cream Mix with Encapsulated L. acidophilus LA -5 (T4)

One per cent of encapsulated *L. acidophilus* LA-5 (dissolved in 100 ml of sterilized skim milk) and two per cent of oligofructose was added to ice cream mix before freezing.

3.3.8.5 Low Fat Synbiotic Ice Cream Mix with Encapsulated L. acidophilus LA-5 (T5)

According to Prevention of Food Adulteration Rules (1955) the minimum standards for low fat ice cream are total solids not less than 26 per cent and fat not more than 2.5 per cent. The low fat synbiotic ice cream mix was formulated to make up the total solids to 36 per cent (in order to compare with the control ice cream) and fat less than 2.5 per cent. The synbiotic low fat ice cream mix was

prepared with fat and sugar replacement. Whey protein concentrate (six per cent), polydextrose (five per cent) and wheat dextrin (three per cent) was incorporated into the mix in order to reduce the fat percentage and to act as a bulking agent. Sugar was replaced with sucralose (2.25 per cent) which is an artificial sweetener. Skim milk and skim milk powder was used to replace the whole milk.

One per cent of encapsulated *L. acidophilus* LA-5 (dissolved in 100 ml of sterilized skim milk) and two per cent of oligofructose was added to ice cream mix before freezing.

Group C

3.3.8.6 Control Ice Cream Mix

Control Ice cream mix was prepared with out addition of *L. acidophilus* LA – 5 and oligofructose.

3.3.9 Freezing of Ice Cream Mix

The separated ice cream mixes in each group were frozen individually using a softy ice cream freezer.

3.3.10 Packaging of Ice Cream

The frozen product was collected in 500 ml ice cream containers (polypropylene –food grade).

3.3.11 Hardening and Storage of Ice Cream

The frozen ice cream was placed in deep freezer at a temperature of -25 to -29 ^{o}C for hardening and subsequent storage.

3.4 ANALYSIS OF ICE CREAM MIXES

3.4.1 Physico-chemical Properties of Ice Cream Mix

3.4.1.1 pH

The pH of control and treatment ice cream mixes was determined using a digital pH meter (ScientificTech).

3.4.1.2 Titratable Acidity

The titratable acidity of control and treatment mixes were determined as per the procedure outlined by IS - SP: 18 Part IX, (1981).

3.4.1.3 Specific Gravity

The specific gravity of the ice cream mixes after ageing for four hours was determined using standard specific gravity bottle. The mix was weighed at a temperature of 20°C. Weight of equivalent amount of water was recorded at the same temperature. Specific gravity was calculated using the formula (Rajor, 1980).

$$\text{Specific Gravity} = \frac{\text{Weight in gram of the sample}}{\text{Weight in gram of water}}$$

3.4.1.4 Fat

The percentage of fat in control and treatment ice cream mixes was determined using the procedure outlined by Arbuckle (1966).

3.4.2 Microbial Analysis of Ice Cream Mix

3.4.2.1 Preparation of Diluents

Peptone water was used for serial dilution of samples. Eleven grams of ice cream samples were transferred aseptically into 99 ml of peptone water. One milliliter of samples each from dilutions of 10^{-7} to 10^{-9} was used for probiotic

count and one milliliter of sample from dilution of 10^{-1} was used for coliform count.

3.4.2.2 Solubilization of Microencapsulated L. acidophilus LA-5 Beads

For quantitative measurements of cell viability by the plate count method, it was necessary to solubilize the calcium alginate polymer beads to release the entrapped probiotic cells. Eleven grams of ice cream mix samples containing alginate beads was suspended aseptically into 99 ml of phosphate buffer (1 M, pH 7.5), followed by gentle shaking at room temperature for ten minutes in order to release probiotic bacteria from the bead.

3.4.2.3 Probiotic Count

The probiotic count of *L.acidophilus* LA 5 in the ice cream mix (after solubilising the bead for treatments containing encapsulated *L. acidophilus* LA-5) was performed using MRS agar as per Inoue *et al*. (1998). The petriplates were incubated at 37°C for 48 hours.

3.4.2.4 Coliform Count

The coliform count of ice cream mix was determined by using the procedure outlined in IS - SP: 18, Part IX (1981).

3.5 ANALYSIS OF ICE CREAM

3.5.1 Physico-chemical Properties of Ice Cream

3.5.1.1 pH

The pH of control and treatment ice cream mixes was determined using a digital pH meter (ScientificTech).

3.5.1.2 Fat

The percentage of fat in control and treatment ice creams was determined using the procedure outlined by Arbuckle (1966).

3.5.1.3 Overrun

The over run percentage obtained in the control and experimental ice cream were calculated using the formula suggested by De (1980).

$$\text{Percentage overrun} = \frac{\left[\begin{array}{l}\text{Weight of unit}\\ \text{volume of mix}\end{array}\right] - \left[\begin{array}{l}\text{Weight of unit}\\ \text{volume of ice cream}\end{array}\right]}{\text{Weight of unit volume of ice cream}} \times 100$$

3.5.1.4 Whipping ability

The whipping ability of the product was determined by the procedure outlined by Rajor (1980). While the mix was being frozen in a softy ice cream freezer, a certain volume of the mix was drawn at five minutes intervals up to ten minutes and weighed. The loss of weight of the mix due to air incorporation was recorded.

3.5.1.5 Meltdown Time

The meltdown time was estimated following the procedure outlined by Rajor (1980). Hundred grams of ice cream was carefully placed on a four square inch glass plate rested on the brim of five inches glass funnel, fitted on a metal stand with its tail end leading into a 100ml graduated cylinder. The time taken for complete meltdown was recorded.

3.5.1.6 Weight Per Litre

Weight per litre of ice cream was estimated using the procedure outlined in IS - SP: 18 Part IX, (1981).

3.5.2 Microbial Analysis of Ice Cream

3.5.2.1 Probiotic Count

The probiotic count of ice cream after freezing and during 0, 15 and 30 days of storage was performed as that of ice cream mix.

3.5.2.2 Coliform Count

The coliform count of ice cream during 0, 15 and 30 day of storage was performed as that of ice cream mix.

3.5.3 Sensory Evaluation

Organoleptic evaluation was carried out by a panel of selected judges. The frozen ice cream was served in 50 ml cups for sensory evaluation. The evaluation was done by using the score card as per Homayouni *et al*. (2008[b]).

3.6 COST ANALYSIS OF ICE CREAM MIX

The cost of preparation of 1kg of ice cream mix was calculated based on ingredient cost. The prevailing market rate of ingredients was taken into accounts for calculating the cost of ice cream.

3.7 STATISTICAL ANALYSIS

The research was carried out with 6 replications. The data obtained were subjected to statistical analysis (Snedecor and Cochran, 1989).

Results

4. RESULTS

A study was conducted in detail to assess survivability of microencapsulated *L. acidophilus* LA-5 in synbiotic ice cream. The results of the experiments in six replications for each group are presented in the following section.

4.1 ANALYSIS OF THE DAIRY INGREDIENTS

The dairy ingredients used in the preparation were analysed for fat and total solids. The mean fat (per cent) and total solids (per cent) presented in Table.1 were 3.80 and 12.20 for milk, 57.00 and 60.25 for cream, 0.11 and 8.72 for skim milk, 19.00 and 97.00 for dairy whitener, 1.00 and 96. 50 for skim milk powder respectively (Table 1).

Table 1. Mean Fat and Total Solids of Dairy Ingredients (per cent)

Ingredients	**Fat**	**Total Solids**
Milk	3.80	12.20
Cream	57.00	60.25
Skim Milk	0.11	8.72
Dairy Whitener	19.00	97.00
Skim Milk Powder	1.00	96. 50

4.2 PROBIOTIC COUNT OF DVS CULTURE *L. acidophilus* LA- 5

The mean probiotic count of *L. acidophilus* LA-5 DVS culture was 4.93 ± 0.312 × 10^{11} cfu per gram or 11.693 log cfu per gram (Table 2 and Fig. 21).

Table 2. *L. acidophilus* LA-5 Count of Direct Vat Set Culture (DVS)

S.No	Count*
1	5.5
2	4.7
3	3.9
4	6.1
5	4.6
6	4.8
Mean ± S.E	4.93 ± 0.312

* -- cfu per gram × 10^{11}

4.3 MICROENCAPSULATED BEAD SIZE

The mean diameter of bead used in the probiotic ice cream mix (T3) was 220.64 ± 61.83µm. (Table 3a and Fig. 22 & 23).

The mean diameter of bead used in the synbiotic ice cream mix (T4 and T5) was 116.03 ± 27.57µm (Table 3b and Fig. 24 & 25).

Table 3a. Mean Diameter of Bead used in Probiotic Ice Cream Mix (T3)

S.No	Diameter in µm*
1	230.36
2	109.65
3	480.36
4	300.65
5	102.35
6	100.48
Mean ± S.E.	220.64 ± 61.83

* Mean diameter of 120 randomly selected beads.

Table 3b. Mean diameter of bead used in synbiotic ice cream mix (T4 and T5)

S.No	Diameter in µm*
1	105.48
2	54.63
3	231.14
4	78.65
5	159.36
6	66.93
Mean ± S.E.	116.03 ± 27.57

* Mean diameter of 120 randomly selected beads.

4.4 ANALYSIS OF ICE CREAM MIX

4.4.1 Physico- chemical Properties of Ice Cream Mix

4.4.1.1 *pH of Ice Cream Mix*

The mean pH for control and treatments ((T1, T2, T3, T4 and T5) mixes were 6.37 ± 0.016, 6.34 ± 0.010, 6.33 ± 0.010, 6.36 ± 0.011, 6.35 ± 0.012 and 6.35 ± 0.017 respectively. Statistical analyses revealed that there was no significant difference in the mean pH value between control and all the treatment ice cream mixes and among the treatment mixes (Table 4 and Fig.2).

Table 4. pH of Ice Cream Mix

	Control	T1	T2	T3	T4	T5
Mean ± SE	6.37 ± 0.016	6.34 ± 0.010	6.33 ± 0.010	6.36 ± 0.011	6.35 ± 0.012	6.35 ± 0.017
Range	6.30-6.42	6.30-6.36	6.30-6.36	6.33-6.40	6.30-6.41	630-6.42

No significant difference among ice cream mixes (P>0.05)

4.4.1.2 *Titratable Acidity of Ice Cream Mix*

Analysis of the data with regard to titratable acidity (per cent lactic acid) of ice cream mixes presented in (Table 5 and Fig.3) revealed that incorporation of *L. acidophilus* LA-5 in ice cream did not caused any significant increase in the titratable acidity. The mean titratable acidity for control and treatment (T1, T2, T3, T4 and T5) mixes were 0.211 ± 0.001, 0.215 ± 0.001, 0.217 ± 0.003, 0.212 ± 0.001, 0.213 ± 0.001 and 0.214± 0.001 respectively.

Table 5. Titratable Acidity of Ice Cream Mix (per cent of lactic acid)

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	0.211± 0.001	0.215 ± 0.001	0.217 ± 0.003	0.212± 0.001	0.213± 0.001	0.214± 0.001
Range	0.207-0.217	0.212-0.216	0.211-0.230	0.203-0.220	0.211-0.216	0.212-0.216

No significant difference among ice cream mixes (P>0.05)

4.4.1.3 Specific Gravity of Ice Cream Mix

Analyses of the data with regard to specific gravity of control and treatment mixes are presented in Table.6 and Fig 4. The mean of specific gravity for control and treatment (T1, T2, T3, T4 and T5) mixes were 1.101 ± 0.02, 1.115 ± 0.01, 1.124 ± 0.01, 1.119 ± 0.01, 1.130 ± 0.01 and 1.138 ± 0.02 respectively. Except for treatment T1, all treatments had significantly higher specific gravity (T2, T4 and T5 ($P<0.01$) and T3 ($P<0.05$)) than control ice cream mix. The specific gravity of T4 was significantly higher ($P<0.05$) from T1 treatment ice cream mix. The mean specific gravity of T5 treatment ice cream mix was significantly higher than that of T2, T3 ($P<0.05$) and T1 ($P< 0.01$) treatment ice cream mixes.

Table 6. Specific Gravity of Ice Cream Mix

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	1.101± 0.02^{A}	1.115± 0.01^{AB}	1.124± 0.01^{BC}	1.119± 0.01^{B}	1.130± 0.01^{BC}	1.138± 0.02^{C}
Range	1.062-1.118	1.097-1.125	1.110-1.131	1.103-1.128	1.122-1.135	1.114-1.147

(Means bearing different superscripts differ significantly at P<0.01)

4.4.1.4 Fat Content in Ice Cream Mix

The mean fat values for control and treatment mixes (T1, T2, T3, T4 and T5) were 10.20 ± 0.100, 10.13 ± 0.115, 10.06 ± 0.042, 10.13 ± 0.088, 10.13 ± 0.080 and 0.47± 0.033 per cent respectively. The mean fat value of treatment mixes T1, T2, T3 and T4 was not significantly different from the mean fat value of control ice cream mix. The mean fat value of T5 was significantly lower (P<0.01) from control and other treatment mixes (Table 7 and Fig.5).

Table 7. Fat Content in Ice Cream Mix (per cent)

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	10.20± 0.100^{A}	10.13± 0.115^{A}	10.06± 0.042^{A}	10.13± 0.088^{A}	10.13± 0.080^{A}	0.47± 0.033^{B}
Range	10.00-10.50	10.00-10.70	10.00-10.20	10.00-10.50	10.00-10.50	0.40-0.60

(Means bearing different superscripts differ significantly at P<0.01)

4.4.2 Microbial Properties of Ice Cream Mix

4.4.2.1 Probiotic Count of Ice Cream Mix

The probiotic count of treatment ice cream mixes were presented in Table 8 and Fig. 6. The mean probiotic count for treatment mixes (T1, T2, T3, T4 and T5) expressed in 10^{9} cfu per gram were 45.00 ± 0.58, 43.17 ± 1.25, 45.67 ± 1.87,

45.14 ± 1.08 and 43.83 ± 1.89 respectively. There was no significant differences (P>0.05) among the treatment ice cream mixes in the probiotic count.

Table 8. Probiotic Count in Ice Cream Mix (10^9 cfu per gram)

	T1	T2	T3	T4	T5
Mean ± SE	45.00 ± 0.58	43.17 ± 1.25	45.67 ± 1.87	45.14 ± 1.08	43.83 ± 1.89
Range	43-47	38-47	40-51	41-49	38-50

(No significant differences among treatments P>0.05)

4.4.2.2 Coliform Count of Ice Cream Mix

The mean with respect to coliform count per g for control and treatment (T1, T2, T3, T4 and T5) mixes were 33.17 ± 1.19, 32.17 ± 1.14, 30.50 ± 1.12, 32.67±1.73, 33.83 ± 1.33 and 32.17 ± 1.80 respectively (Table.9 and Fig. 7). There was no significant difference in coliform count between control and treatment ice cream mixes and among treatment ice cream mixes (P>0.05).

Table.9. Coliform Count of Ice Cream Mix (cfu per gram)

	Control	T1	T2	T3	T4	T5
Mean ± SE	33.17± 1.19	32.17 ± 1.14	30.50± 1.12	32.67 ± 1.73	33.83± 1.33	32.17± 1.80
Range	29-36	29-36	26-33	28-40	29-37	25-38

(No significant differences among the control and treatments (P>0.05))

4.5 ANALYSIS OF ICE CREAM

4.5.1 Physico-chemical Properties of Ice Cream

4.5.1.1 pH of Ice Cream during Storage

The mean pH for control and treatment (T1, T2, T3, T4 and T5) ice creams for 0^{th} day of storage were 6.36 ± 0.027, 6.19 ± 0.004, 6.18 ± 0.007, 6.29

± 0.007, 6.27 ± 0.026 and 6.28 ± 0.006 respectively, for 15th day of storage were 6.33 ± 0.025, 6.17 ± 0.004, 6.15 ± 0.006, 6.27 ± 0.007, 6.24 ± 0.011 and 6.25 ± 0.011 respectively and for 30th day 6.29 ± 0.017, 6.15 ± 0.004, 6.13 ± 0.004, 6.25 ± 0.005, 6.23 ± 0.013 and 6.23 ± 0.008 respectively (Table10a, 10b & 10c and Fig. 8). Statistical analyses showed that throughout the storage period mean pH value of all treatment ice creams were significantly lower ($P<0.01$) from that of the control ice cream. The mean pH value of the treatment ice creams containing non-encapsulated *L. acidophilus* LA-5 (T1 and T2) were significantly lower ($P<0.01$) from treatment ice creams containing encapsulated *L. acidophilus* LA-5 (T3, T4 and T5).

Table 10a. pH of Ice Cream during 0th day of Storage

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	6.36 ± 0.027^{A}	6.19 ± 0.004^{C}	6.18 ± 0.007^{C}	6.29 ± 0.007^{B}	6.27 ± 0.026^{B}	6.28 ± 0.006^{B}
Range	6.30-6.45	6.18-6.21	6.16-6.20	6.27-6.32	6.20-6.38	6.26-6.30

(Means bearing different superscripts differ significantly at $P<0.01$)

Table 10b. pH of Ice Cream during 15th day of Storage

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	6.33 ± 0.025^{A}	6.17 ± 0.004^{C}	6.15 ± 0.006^{C}	6.27 ± 0.007^{B}	6.24 ± 0.011^{B}	6.25 ± 0.011^{B}
Range	6.26-6.40	6.16-6.19	6.14-6.18	6.25-6.30	6.20-6.28	6.21-6.29

(Means bearing different superscripts differ significantly at $P<0.01$)

Table 10c. pH of Ice Cream during 30th day of Storage

	Control	T1	T2	T3	T4	T5
Mean ± SE	6.29 ± 0.017^{A}	6.15 ± 0.004^{C}	6.13 ± 0.004^{C}	6.25 ± 0.005^{B}	6.23 ± 0.013^{B}	6.23 ± 0.008^{B}
Range	6.24-6.35	6.13-6.16	6.12-6.15	6.24-6.28	6.19-6.28	6.19-6.25

(Means bearing different superscripts differ significantly at P<0.01)

4.5.1.2 Fat Content in Ice Cream during Storage

Analyses of the data with respect to fat content of control and treatment ice creams (expressed in per cent) were presented in Table 11a, 11b & 11c and Fig.9. The mean value of fat content for control and treatment ice creams (T1, T2, T3, T4 and T5) were 10.18 ± 0.10, 10.10 ± 0.08, 10.08 ± 0.04, 10.08 ± 0.05, 10.10 ± 0.05 and 0.45 ± 0.02 per cent respectively for 0th day of storage, 10.18 ± 0.10, 10.10 ± 0.08, 10.08 ± 0.04, 10.08 ± 0.05, 10.10 ± 0.05 and 0.45 ± 0.02 per cent respectively for 15th day of storage and 10.18 ± 0.10, 10.10 ± 0.08, 10.08 ± 0.04,10.08 ± 0.05, 10.10 ± 0.05 and 0.45 ± 0.02 respectively for 30th day of storage. The mean fat content of T1, T2, T3 and T4 treatment ice cream mixes had no significant difference(P>0.05) from that of control ice cream mix. The T5 treatment ice cream mix was significantly lower (P<0.01) in the mean fat content from that of control and other treatment ice cream mixes.

Table 11a. Fat of the Ice Cream during 0th day of Storage (per cent)

	Control	T1	T2	T3	T4	T5
Mean ± SE	10.18± 0.10^{A}	10.10± 0.08^{A}	10.08± 0.04^{A}	10.08± 0.05^{A}	10.10± 0.05^{A}	0.45± 0.02^{B}
Range	10.00-10.50	10.00-10.50	10.00-10.20	10.00-10.30	10.00-10.30	0.40-0.50

(Means bearing different superscripts differ significantly at P<0.01)

Table 11b. Fat Content of Ice Cream during 15th day of Storage (per cent)

	Control	T1	T2	T3	T4	T5
Mean ± SE	10.18± 0.10^{A}	10.10± 0.08^{A}	10.08± 0.04^{A}	10.08± 0.05^{A}	10.10± 0.05^{A}	0.45± 0.02^{B}
Range	10.00-10.50	10.00-10.50	10.00-10.20	10.00-10.30	10.00-10.30	0.40-0.50

(Means bearing different superscripts differ significantly at P<0.01)

Table 11c. Fat Content of Ice Cream during 30th day of Storage (per cent)

	Control	T1	T2	T3	T4	T5
Mean ± SE	10.18± 0.10^{A}	10.10± 0.08^{A}	10.08± 0.04^{A}	10.08± 0.05^{A}	10.10± 0.05^{A}	0.45± 0.02^{B}
Range	10.00-10.50	10.00-10.50	10.00-10.20	10.00-10.30	10.00-10.30	0.40-0.50

(Means bearing different superscripts differ significantly at P<0.01)

4.5.1.3 Overrun of Ice Cream

The mean values of overrun (expressed in per cent) for control and treatment ice creams (T1, T2, T3, T4 and T5) were 36.21 ± 1.12, 32.54 ± 0.649, 36.57 ± 1.69, 33.82 ± 2.87, 36.39 ± 2.42 and 30.00 ± 0.790 respectively. There was no significant differences between control and treatment ice creams and also among the treatment ice creams (P>0.05) in the mean value of the overrun (Table 12 and Fig.10).

Table 12. Overrun of Ice Cream (per cent)

	Control	T1	T2	T3	T4	T5
Mean ± SE	36.21± 1.12	32.54± 0.649	36.57± 1.69	33.82± 2.87	36.39± 2.42	30.00± 0.790
Range	32.6-38.83	30.23-34.67	33.32-44.86	22.54-40.39	30.27-45.39	27.51-31.76

(No significant differences among control and treatments (P>0.05))

4.5.1.4 Whipping Ability of Ice Cream

The mean values of whipping ability for control and treatment ice creams (T1, T2, T3, T4 and T5) were 47.40 ± 1.15, 43.84 ± 1.08, 47.79 ± 1.09, 44.13 ± 1.50, 47.19 ± 1.63and 31.01 ± 0.774 respectively. Statistical analysis revealed that the mean value of whipping ability of T5 treatment was significantly lower ($P<0.01$) from that of the control and other treatment ice creams (Table 13 and Fig.10).

Table 13. Whipping ability of Ice cream (per cent)

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	47.40± 1.15^{A}	43.84± 1.08^{A}	47.79± 1.09^{A}	44.13± 1.50^{A}	47.19± 1.63^{A}	31.01± 0.774^{B}
Range	44.38-50.56	40.91-47.47	44.27-50.87	40.11-50.23	43.89-54.53	28.16-54.53

(Means bearing different superscripts differ significantly at $P<0.01$)

4.5.1.5 Meltdown Time of Ice Cream during Storage

The mean meltdown time (in minutes) for control and treatment (T1, T2, T3, T4 and T5) ice creams were 55.00 ± 0.40, 62.17 ± 0.90, 66.34 ± 0.61, 56.38 ± 0.78, 60.84 ± 0.68 and 50.01 ± 0.57 respectively for 0^{th} day, 57.49 ± 0.51, 64.07 ± 0.91, 68.69 ± 0.52, 58.58 ± 0.53, 63.29 ± 0.60 and 52.52 ± 0.58 respectively for 15^{th} day of storage and 61.48 ± 0.75, 66.75 ± 1.13, 71.65 ± 1.33, 62.60 ± 0.44, 65.50 ± 0.49 and 55.73 ± 0.56 respectively for 30^{th} day of storage. Statistical analysis throughout the storage period showed the mean meltdown time of control and all the treatments are significantly lower ($P<0.01$) to that of T2 ice cream and significantly higher than that of T5 ice cream. Treatment T1 and T4 had meltdown time significantly higher ($P<0.01$) than that of control and T3 ice cream (Table 14a, 14b & 14c and Fig. 11).

Table 14a. Meltdown Time for Ice Cream 0th day of Storage (minutes)

	Control	T1	T2	T3	T4	T5
Mean ± SE	55.00± 0.40^{A}	62.17± 0.90^{B}	66.34± 0.61^{C}	56.38± 0.78^{A}	60.84± 0.68^{B}	50.01± 0.57^{D}
Range	53.33-56.05	60.13-66.11	64.14-68.05	55.46-57.34	58.23-63.40	48.17-52.30

(Means bearing different superscripts differ significantly at P<0.01)

Table 14b. Meltdown Time for Ice Cream 15th day of Storage (minutes)

	Control	T1	T2	T3	T4	T5
Mean ± SE	57.49± 0.51^{A}	64.07± 0.91^{B}	68.69± 0.52^{C}	58.58± 0.53^{A}	63.29± 0.60^{B}	52.52± 0.58^{D}
Range	55.57-59.00	62.09-68.01	67.23-70.56	56.57-60.54	61.26-65.50	50.54-54.43

(Means bearing different superscripts differ significantly at P<0.01)

Table 14c. Meltdown Time for Ice Cream 30th day of Storage (minutes)

	Control	T1	T2	T3	T4	T5
Mean ± SE	61.48± 0.75^{A}	66.75± 1.13^{B}	71.65± 1.33^{C}	62.60± 0.44^{A}	65.50± 0.49^{B}	55.73± 0.56^{D}
Range	59.67-64.13	63.25-70.20	67.45-75.28	61.25-64.23	64.06-67.24	54.03-57.14

(Means bearing different superscripts differ significantly at P<0.01)

4.5.1.6 Weight per Litre of Ice Cream during Storage

The mean values of weight per litre(expressed in gram per litre) for control and treatment (T1, T2, T3, T4 and T5) ice creams were 678.32 ± 2.67, 697.02 ± 3.01, 724.29 ± 3.97, 705.97 ± 4.18, 729.97 ± 5.46 and 793.51 ± 6.85 respectively for 0th day of storage, 662.33 ± 3.23, 684.20 ± 6.19, 709.47 ± 2.99, 691.74 ± 6.74, 720.40 ± 3.05 and 772.72 ± 6.88 respectively for 15th day of storage and 647.17 ± 2.13, 675.09 ± 5.63, 698.65 ± 3.39, 679.28 ± 7.88, 708.67 ± 3.04 and 761.95 ± 7.64 respectively for 30th day of storage (Table 15a,15b & 15c

and Fig.12). Statistical analysis of weight per litre on 0th day of storage, revealed that all treatment ice creams were significantly higher (P<0.01) from that of control ice cream. Probiotic ice creams T1 and T3 had weight per litre significantly lower (P<0.01) than that of synbiotic ice creams T2 and T4. Low fat ice cream T5 had the significantly higher weight per litre among all treatment ice cream. The statistical analysis of 15th and 30th day of storage gave similar result to that of 0th day with reduction in the values of weight per litre.

Table 15a. Weight per Litre during 0th day of Storage (gram per litre)

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	678.32± 2.67^{A}	697.02± 3.01^{B}	724.29± 3.97^{C}	705.97± 4.18^{B}	729.97± 5.46^{C}	793.51± 6.85^{D}
Range	668.45-687.50	688.75-707.80	712.56-735.25	696.72-723.00	715.25-750.35	769.28-818.75

(Means bearing different superscripts differ significantly at P<0.01)

Table 15b. Weight per Litre during 15th day of storage (gram per litre)

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	662.33± 3.23^{A}	684.20± 6.19^{B}	709.47± 2.99^{C}	691.74± 6.74^{B}	720.40± 3.05^{C}	772.72± 6.88^{D}
Range	653.69-675.37	670.64-709.90	699.00-718.70	673.29-714.95	709.65-728.58	750.60-792.47

(Means bearing different superscripts differ significantly at P<0.01)

Table 15c. Weight per Litre during 30th day of storage (gram per litre)

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	647.17± 2.13^{A}	675.09± 5.63^{B}	698.65± 3.39^{C}	679.29± 7.88^{B}	708.67± 3.04^{C}	761.95± 7.64^{D}
Range	640.56-653.65	664.52-700.38	685.20-709.58	658.35-705.48	698.65-717.25	735.60-782.10

(Means bearing different superscripts differ significantly at P<0.01)

4.5.2 Microbial Properties of Ice Cream

4.5.2.1 Probiotic Count of Ice Cream After Freezing

The mean values of probiotic count after freezing the ice cream mix of the treatments T1, T2, T3, T4 and T5 (expressed in 10^8 cfu per gram of ice cream) were 11.50 ± 0.56, 19.17 ± 0.70, 23.83 ± 1.70, 27.83 ± 0.75 and 25.50 ± 2.09 respectively (Table 16 and Fig.13). Statistical analyses of probiotic count after freezing revealed that treatment ice creams T3, T4 and T5 with microencapsulated *L. acidophilus* LA-5 had significantly higher (P<0.01) mean probiotic count than T1 and T2 treatment ice creams. The probiotic count of T2 was significantly higher (P<0.01) than that of T1 treatment ice cream.

Table 16. Probiotic Count of Ice Cream after Freezing (10^8 cfu per gram)

	T1	**T2**	**T3**	**T4**	**T5**
Mean ± SE	11.50 ± 0.56^{A}	19.17 ± 0.70^{B}	23.83 ± 1.70^{C}	27.83 ± 0.75^{C}	25.50 ± 2.09^{C}
Range	10-13	17-22	20-30	26-31	21-35

(Means bearing different superscripts differ significantly at P<0.01)

4.5.2.2 Probiotic Count of Ice Cream during Storage

The mean values of probiotic count (expressed in 10^8 cfu per gram of ice cream) for treatment ice creams T1, T2, T3, T4 and T5 were 10.50 ± 0.50, 18.17 ± 1.40, 23.00 ± 1.65, 26.67 ± 0.92 and 24.50 ± 1.98 respectively for 0^{th} day of storage, 9.83 ± 0.31, 17.33 ± 1.26, 22.67 ± 1.61, 26.17 ± 1.08 and 23.83 ± 1.80 respectively for 15^{th} day of storage and 9.67 ± 0.33, 16.33 ± 1.09, 21.50 ± 1.63, 25.33 ± 1.05 and 23.00 ± 1.71 respectively for 30^{th} day of storage (Table17a, 17b & 17c and Fig.14 and Fig 26). Statistical analyses of probiotic count throughout the storage period revealed that treatment ice creams T3, T4 and T5 with microencapsulated *L. acidophilus* LA-5 had significantly higher (P<0.01) mean

probiotic count than T1 and T2 treatment ice creams. The probiotic count of T2 was significantly higher (P<0.01) than that of T1 treatment ice cream.

Table.17a. Probiotic Count of Ice Cream during 0th day of Storage (10^8 cfu per gram)

	T1	**T2**	**T3**	**T4**	**T5**
Mean ± SE	10.50 ± 0.50^{A}	18.17 ± 1.40^{B}	23.00 ± 1.65^{C}	26.67 ± 0.92^{C}	24.50 ± 1.98^{C}
Range	9-12	15-23	19-29	25-30	20-33

(Means bearing different superscripts differ significantly at P<0.01)

Table.17b. Probiotic Count of Ice Cream during 15th day of Storage (10^8 cfu per gram)

	T1	**T2**	**T3**	**T4**	**T5**
Mean ± SE	9.83 ± 0.31^{A}	17.33 ± 1.26^{B}	22.67 ± 1.61^{C}	26.17 ± 1.08^{C}	23.83 ± 1.80^{C}
Range	9-11	14-22	19-28	24-30	19-31

(Means bearing different superscripts differ significantly at P<0.01)

Table.17c. Probiotic Count of Ice Cream during 30th day of Storage (10^8 cfu per gram)

	T1	**T2**	**T3**	**T4**	**T5**
Mean ± SE	9.67 ± 0.33^{A}	16.33 ± 1.09^{B}	21.50 ± 1.63^{C}	25.33 ± 1.05^{C}	23.00 ± 1.71^{C}
Range	9-11	13-20	16-27	23-29	19-30

(Means bearing different superscripts differ significantly at P<0.01)

4.5.2.3 Coliform Count of Ice Cream during Storage

The mean values of coliform count (expressed in cfu per gram) of control, T1, T2, T3, T4 and T5 treatment ice creams were 29.50 ± 0.62, 23.00 ± 0.73, 21.83 ± 1.56, 28.33 ± 1.56, 27.50 ± 1.98 and 28.83 ± 1.76 respectively for 0^{th} day of storage, 26.67 ± 0.42, 18.17 ± 0.95, 17.67 ± 1.54, 23.33 ± 2.32, 24.17 ± 2.54 and 24.83 ± 1.30 respectively for 15^{th} day of storage and 25.50 ± 0.89, 15.83 ± 0.95, 14.67 ± 1.28, 21.83 ± 1.99, 22.17 ± 2.40 and 22.17 ± 1.25 respectively for 30^{th} day of storage (Table 18a,18b & 18c and Fig.15). Statistical analysis throughout the storage period showed that the coliform count of treatment ice creams containing non encapsulated *L. acidophilus* LA-5 (T1 and T2) were significantly lower ($P<0.01$) than that of control and treatment ice creams with encapsulated *L. acidophilus* LA-5 (T3, T4 and T5).

Table 18a. Coliform Count of Ice Cream 0^{th} day of Storage (cfu per gram**)**

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	29.50 ± 0.62^{A}	23.00 ± 0.73^{B}	21.83 ± 1.56^{B}	28.33 ± 1.56^{A}	27.50 ± 1.98^{A}	28.83 ± 1.76^{A}
Range	27-31	21-36	17-25	24-35	19-32	22-34

(Means bearing different superscripts differ significantly at $P<0.01$)

Table 18b. Coliform Count of Ice Cream 15^{th} day of Storage (cfu per gram**)**

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	26.67 ± 0.42^{A}	18.17 ± 0.95^{B}	17.67 ± 1.54^{B}	23.33 ± 2.32^{A}	24.17 ± 2.54^{A}	24.83 ± 1.30^{A}
Range	25-28	15-21	11-22	15-32	14-30	21-30

(Means bearing different superscripts differ significantly at $P<0.01$)

Table 18c. Coliform Count of Ice Cream 30th day of Storage (cfu per gram)

	Control	T1	T2	T3	T4	T5
Mean ± SE	25.50± 0.89^{A}	15.83± 0.95^{B}	14.67± 1.28^{B}	21.83± 1.99^{A}	22.17± 2.40^{A}	22.17± 1.25^{A}
Range	23-28	13-19	9-18	15-29	13-29	19-27

(Means bearing different superscripts differ significantly at P<0.01)

4.5.3 Sensory Evaluation of Ice Cream during Storage

4.5.3.1 Flavour and Taste Score

The mean flavour and taste score for control and treatment (T1, T2, T3, T4 and T5) ice creams on 0th day of storage were 8.75 ± 0.195, 7.57 ± 0.138, 7.78 ± 0.151, 8.65 ± 0.050, 8.65 ± 0.081 and 6.73 ± 0.320 respectively, on 15th day of storage were 9.12 ± 0.054, 7.87 ± 0.163, 8.12 ± 0.119, 8.83 ± 0.080, 8.95 ± 0.072 and 6.82 ± 0.183 respectively and on 30th day of storage were 8.93 ± 0.242, 7.76 ± 0.176, 7.87 ± 0.115, 8.82 ± 0.114, 8.82 ± 0.201 and 6.82 ± 0.299 respectively (Table 19a, 19b & 19c and Fig.16). Statistical analyses throughout the storage period revealed that control and treatment ice creams T3 and T4 were significantly higher (P<0.01) in the mean flavour and taste scores from that of other treatment ice creams. The mean flavour and taste score of treatment ice cream T5 was significantly lower (P<0.01) from that of other ice creams.

Table 19a. Flavour and Taste Score of Ice Cream during 0th day of Storage

	Control	T1	T2	T3	T4	T5
Mean ± SE	8.75± 0.195^{A}	7.57± 0.138^{B}	7.78± 0.151^{B}	8.65± 0.050^{A}	8.65± 0.081^{A}	6.73± 0.320^{C}
Range	8.2-9.3	7.3-8.0	7.2-8.3	8.5-8.8	8.5-9.0	5.6-7.5

(Means bearing different superscripts differ significantly at P<0.01)

Table 19b. Flavour and Taste Score of Ice Cream during 15th day of Storage

	Control	T1	T2	T3	T4	T5
Mean ± SE	9.12± 0.054^{A}	7.87± 0.163^{B}	8.12± 0.119^{B}	8.83± 0.080^{A}	8.95± 0.072^{A}	6.82± 0.183^{C}
Range	9.0-9.3	7.3-8.2	7.6-8.5	8.6-9.2	8.7-9.2	6.4-7.7

(Means bearing different superscripts differ significantly at P<0.01)

Table 19c. Flavour and Taste Score of Ice Cream during 30th day of Storage

	Control	T1	T2	T3	T4	T5
Mean ± SE	8.93± 0.242^{A}	7.76± 0.176^{B}	7.87± 0.115^{B}	8.82± 0.114^{A}	8.82± 0.201^{A}	6.82± 0.299^{C}
Range	7.8-9.5	7.3-8.3	7.6-8.3	8.3-9.0	8.0-9.2	6.8-7.5

(Means bearing different superscripts differ significantly at P<0.01)

4.5.3.2 Body and Texture Score

The mean data with respect to body and texture score of control and treatment (T1, T2, T3, T4 and T5) ice creams were 4.35 ± 0.150, 4.27 ± 0.109, 4.18 ± 0.087, 4.37 ± 0.131, 4.30 ± 0.232 and 4.05 ± 0.169 respectively for 0th day of storage, 4.51 ± 0.074, 4.48 ± 0.054, 4.48 ± 0.065, 4.46 ± 0.123, 4.50 ± 0.113 and 4.30 ± 0.100 respectively for 15th day of storage and 4.48 ± 0.106, 4.43 ± 0.152, 4.41 ± 0.147, 4.55 ± 0.095, 4.55 ± 0.115 and 4.30 ± 0.077 respectively for 30th day of storage (Table.20a, 20b & 20c and Fig. 17). Statistical analysis revealed no significant differences in the mean values of body and texture score among the control and treatment groups and within the treatment groups throughout the storage period.

Table 20a. Body and Texture Score of Ice Cream during 0th day of Storage

	Control	T1	T2	T3	T4	T5
Mean ± SE	4.35± 0.150	4.27± 0.109	4.18± 0.087	4.37± 0.131	4.30± 0.232	4.05± 0.169
Range	3.9-4.7	3.9-4.5	3.9-4.5	3.9-4.7	3.2-4.7	3.4-4.5

No significant difference among ice creams (P>0.05)

Table 20b. Body and Texture Score of Ice Cream during 15th day of Storage

	Control	T1	T2	T3	T4	T5
Mean ± SE	4.51± 0.074	4.48± 0.054	4.48± 0.065	4.46± 0.123	4.50± 0.113	4.30± 0.100
Range	4.2-4.7	4.3-4.7	4.2-4.7	4.0-4.7	4.0-4.7	4.0-4.5

No significant difference among ice creams (P>0.05)

Table 20c. Body and Texture Score of Ice Cream during 30th day of Storage

	Control	T1	T2	T3	T4	T5
Mean ± SE	4.48± 0.106	4.43± 0.152	4.41± 0.147	4.55± 0.095	4.55± 0.115	4.30± 0.077
Range	4.0-4.7	3.7-4.7	3.8-4.7	4.-4.7	4.0-4.7	4.0-4.5

No significant difference (P>0.05)

4.5.3.3 Colour and Appearance Score

The mean scores for colour and appearance for control and treatment (T1, T2, T3, T4 and T5) ice creams for 0th day of storage were 4.53 ± 0.117, 4.40 ± 0.115, 4.35 ± 0.134, 4.45 ± 0.109, 4.45 ± 0.106 and 4.15 ± 0.159 respectively for 15th day of storage were 4.58 ± 0.094, 4.45 ± 0.092, 4.52 ± 0.090, 4.58 ± 0.083, 4.58 ± 0.083 and 4.45 ± 0.050 respectively and for 30th day of storage were 4.55 ± 0.106, 4.45 ± 0.134, 4.42 ± 0.125, 4.55 ± 0.088, 4.60 ± 0.010 and 4.30 ± 0.077

respectively (Table. 21a, 21b & 21c and Fig. 18). Statistical analysis revealed no significant differences in the mean values of colour and appearance score among the control and treatment groups and within the treatment groups throughout the storage period.

Table 21a. Colour and Appearance Score of Ice Cream during 0th day of Storage

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	4.53± 0.117	4.40± 0.115	4.35± 0.134	4.45± 0.109	4.45± 0.106	4.15± 0.159
Range	4.1-4.8	4.1-4.8	4.0-4.8	4.0-4.7	4.2-4.8	3.4-4.5

No significant difference (P>0.05)

Table 21b. Colour and Appearance Score of Ice Cream during 15th day of Storage

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	4.58± 0.094	4.45± 0.092	4.52± 0.090	4.58± 0.083	4.58± 0.083	4.45± 0.050
Range	4.3-4.8	4.2-4.8	4.2-4.7	4.4-4.8	4.2-4.7	4.2-4.5

No significant difference (P>0.05)

Table 21c. Colour and Appearance Score of Ice cream during 30th day of Storage

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	4.55± 0.106	4.45± 0.134	4.42± 0.125	4.55± 0.088	4.60± 0.010	4.30± 0.077
Range	4.2-4.8	4.0-4.8	4.0-4.8	4.3-4.8	4.2-4.8	4.0-4.5

No significant difference (P>0.05)

4.5.3.4 Total Score

The mean total score obtained by sensory evaluation considering the above mentioned parameters (flavour, body, texture, colour and appearance) for

control and treatment (T1, T2, T3, T4 and T5) ice creams for 0th day were 17.63 ± 0.424, 16.23 ± 0.244, 16.28 ± 0.349, 17.46 ± 0.256, 17.43 ± 0.376 and 14.93 ± 0.554 respectively, for 15th day were 18.22 ± 0.162, 16.80 ± 0.159, 17.12 ± 0.182, 17.88 ± 0.244, 18.03 ± 0.180 and 15.57 ± 0.184 respectively and for 30th day were 17.97 ± 0.420, 16.65 ± 0.385, 16.70 ± 0.313, 17.92 ± 0.287, 17.97 ± 0.406 and 15.37 ± 0.406 respectively (Table. 22a, 22b & 22c and Fig. 19). Statistical analysis throughout the storage period revealed that control and treatment ice creams T3 and T4 were significantly higher ($P<0.01$) for total scores from that of other treatments. The mean total score of T5 was significantly lower ($P<0.01$) from that of all other ice creams.

Table 22a. Total Score of Ice Cream during 0th day of Storage

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	17.63 ± 0.424^{A}	16.23 ± 0.244^{B}	16.28 ± 0.349^{B}	17.46 ± 0.256^{A}	17.43 ± 0.376^{A}	14.93 ± 0.554^{C}
Range	16.4-18.7	15.3-16.8	14.9-17.2	16.7-18.2	15.9-18.4	12.8-16.3

(Means bearing different superscripts differ significantly at $P<0.01$)

Table 22b. Total Score of Ice Cream during 15th day of Storage

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	18.22 ± 0.162^{A}	16.80 ± 0.159^{B}	17.12 ± 0.182^{B}	17.88 ± 0.244^{A}	18.03 ± 0.180^{A}	15.57 ± 0.184^{C}
Range	17.8-18.7	16.3-17.3	16.5-17.6	17.2-18.7	17.4-18.6	15.2-16.4

(Means bearing different superscripts differ significantly at $P<0.01$)

Table 22c. Total Score of Ice cream during 30th day of Storage

	Control	**T1**	**T2**	**T3**	**T4**	**T5**
Mean ± SE	17.97± 0.420^{A}	16.65± 0.385^{B}	16.70± 0.313^{B}	17.92± 0.287^{A}	17.97± 0.406^{A}	15.37± 0.406^{C}
Range	16.0-18.8	15.0-17.7	15.4-17.4	16.8-18.5	16.2-18.7	13.8-16.2

(Means bearing different superscripts differ significantly at P<0.01)

4.6 COST ANALYSIS OF ICE CREAM MIX

The cost of preparation of one kg of ice cream mix was calculated based on the cost of ingredients. The prevailing market rate of ingredients was taken into account for calculating the cost of ice cream. The cost of ingredients for one kg of control ice cream mix (in Rupees) was 55.9, where as for treatment ice cream mixes T1, T2, T3, T4 and T5 were 71.9, 80.3, 98.0, 106.4 and 156.2 respectively (Table 23 and Fig. 20).

Table 23. Cost analysis of Ice cream mix

Quantity of Ingredient per 1 kg	Treatments											
	Control		T1		T2		T3		T4		T5	
	Qty (g)	Cost (Rs)	Qty (g)	Cost (Rs)	Qty (g)	Cost (Rs)	Qty (g)	Cost (Rs)	Qty (g)	Cost (Rs)	Qty (g)	Cost (Rs)
Milk	677.5	13.8	677.5	13.8	677.5	13.8	677.5	13.8	677.5	13.8	-	-
Skim Milk	-	-	-	-	-	-	-	-	-	-	693.5	5.3
Skim milk powder											139	27.8
Dairy whitener	75	15.4	75	15.4	75	15.4	75	15.4	75	15.4	-	-
Sugar	136.5	2.7	136.5	2.7	136.5	2.7	136.5	2.7	136.5	2.7		
Sucralose	-	-	-	-	-	-	-	-	-	-	22.5	24.7
Stabilizer & Emulsifier	5	1.6	5	1.65	5	1.6	5	1.6	5	1.6		
Cream	106	18.7	106	18.7	106	18.7	106	18.7	106	18.7		
Culture	-	-	10	16	10	16	10	16	10	16	10	16
Micro encap sulation								26.1		26.1		26.1
Oligo Fructose	-	-	-	-	20	8.4	-	-	20	8.4	20	8.4
Whey Protein	-	-	-	-	-	-	-	-	-	-	60	24.9
Poly Dextrose											50	13.5
Wheat Dextrin											30	6
Flavour & Colour		2.5		2.5		2.5		2.5		2.5		2.5
Total		**55. 9**		**71. 9**		**80. 3**		**98. 0**		**106. 4**		**156. 2**

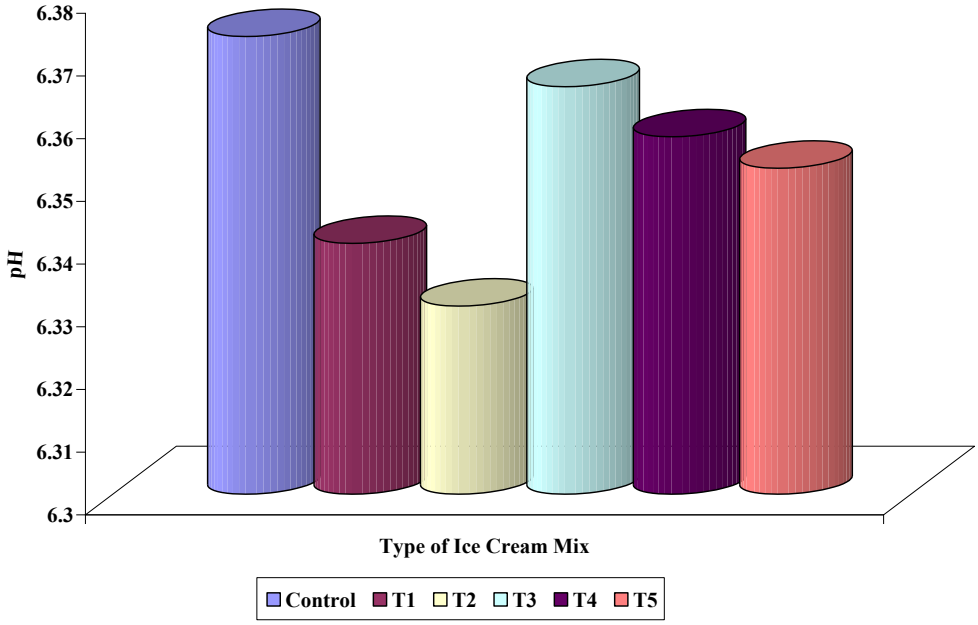

Fig. 2. pH of Ice Cream Mix

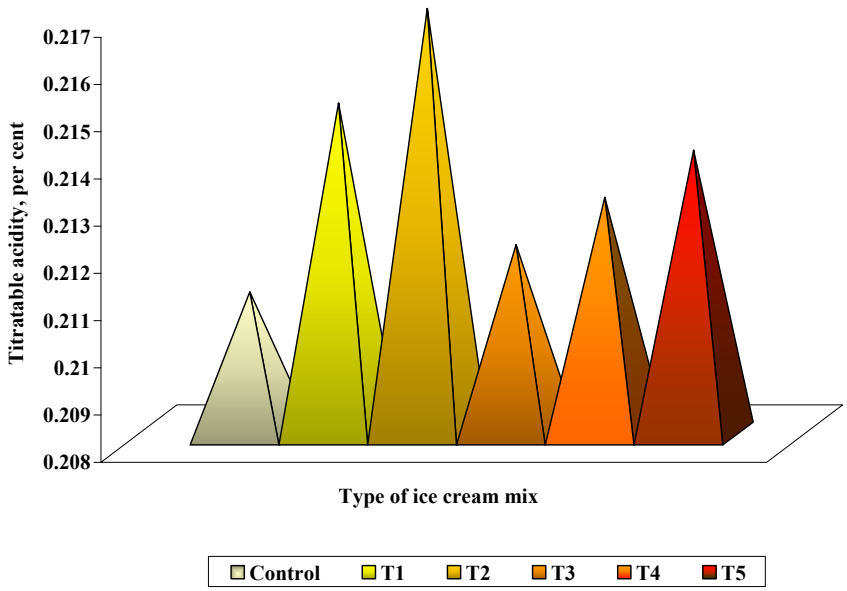

Fig. 3. Titratable Acidity of Ice Cream Mix

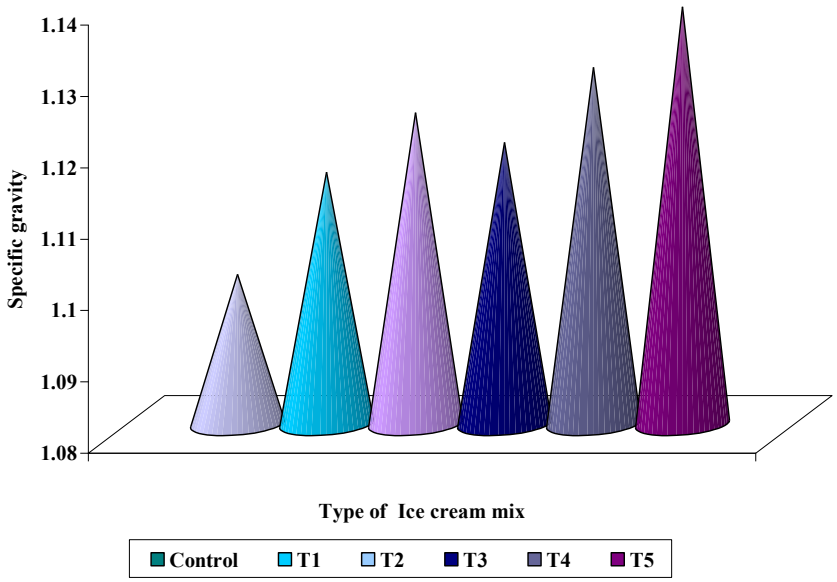

Fig. 4. Specific Gravity of Ice Cream Mix

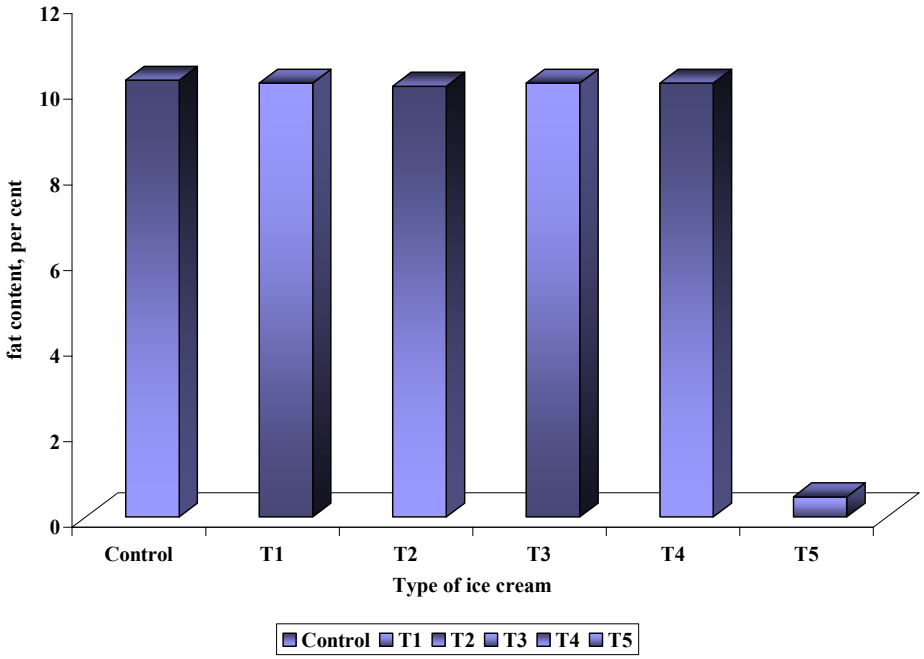

Fig. 5. Fat Content in Ice Cream Mix

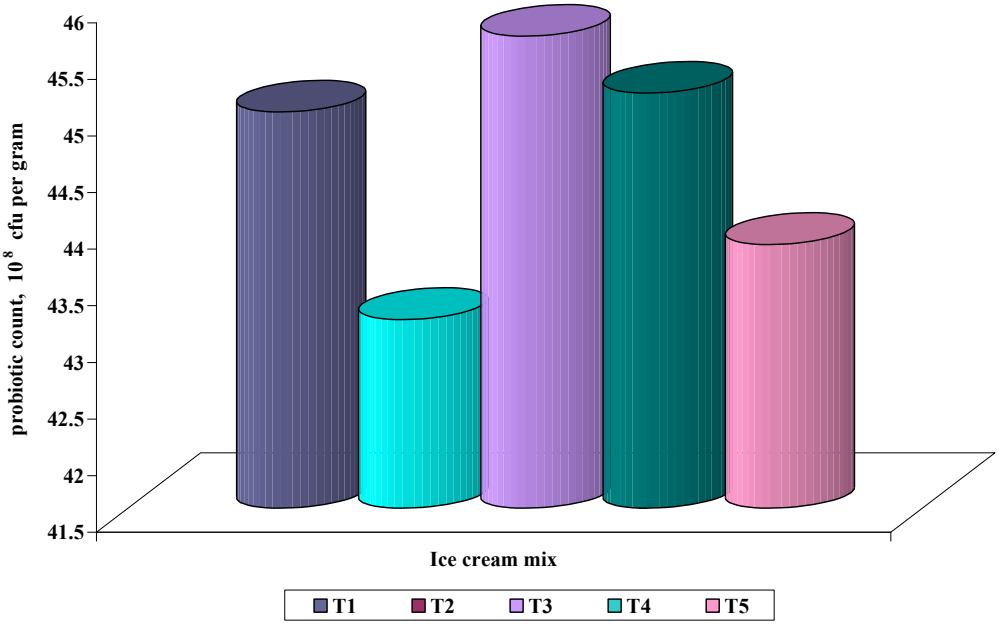

Fig. 6. Probiotic Count of Ice Cream Mix

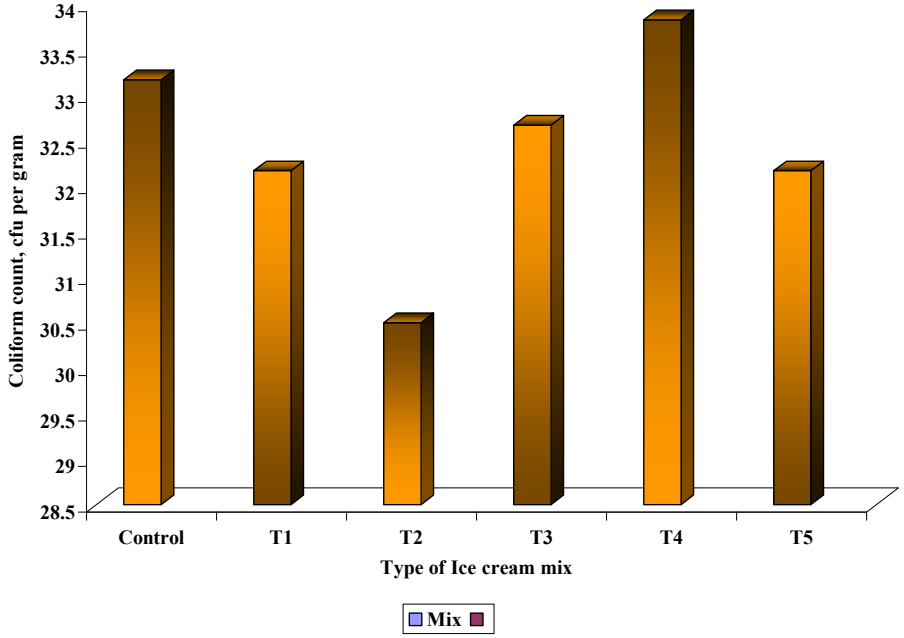

Fig. 7. Coliform Count of Ice Cream Mix

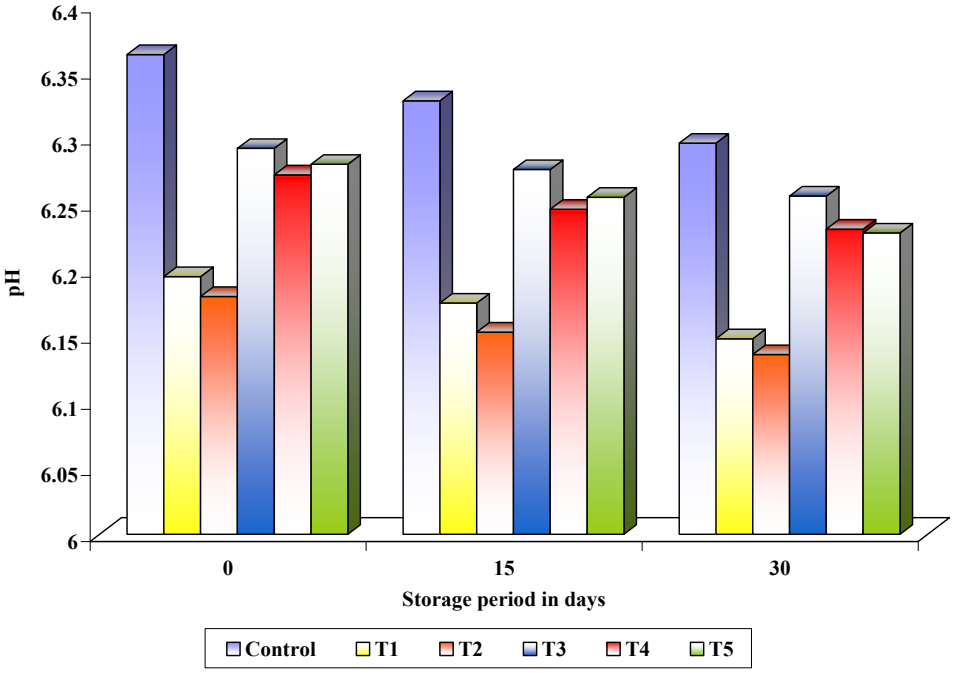

Fig. 8. pH of Ice Cream during Storage

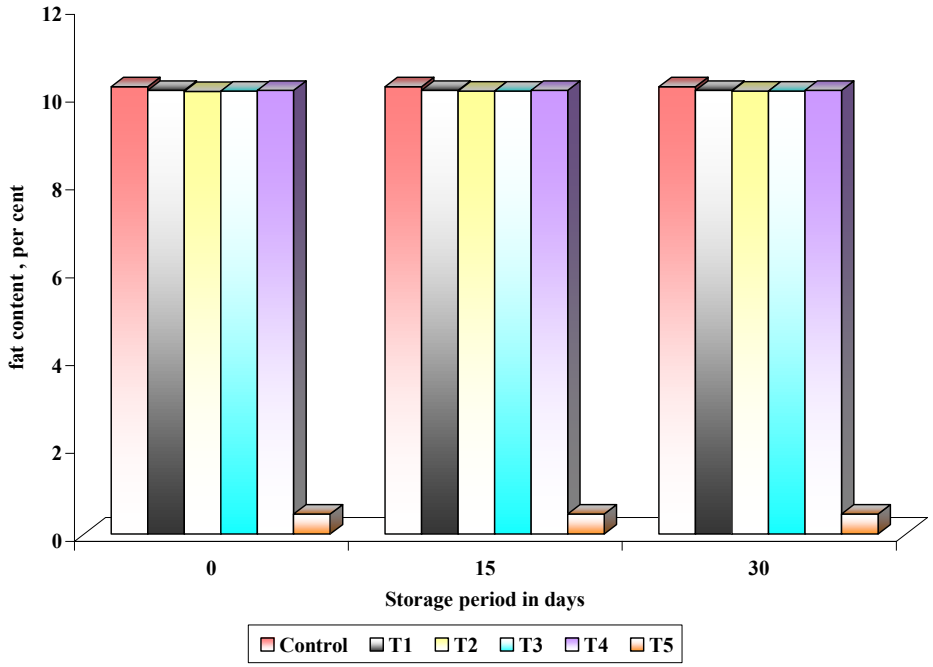

Fig. 9. Fat Content in Ice Cream during Storage

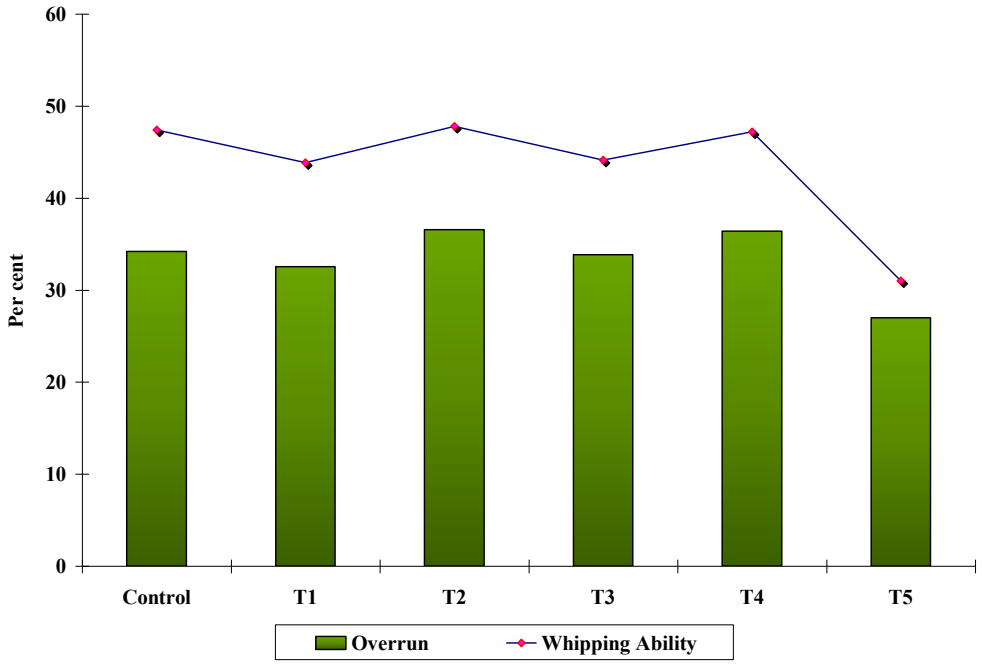

Fig. 10. Overrun and Whipping Ability of Ice Cream

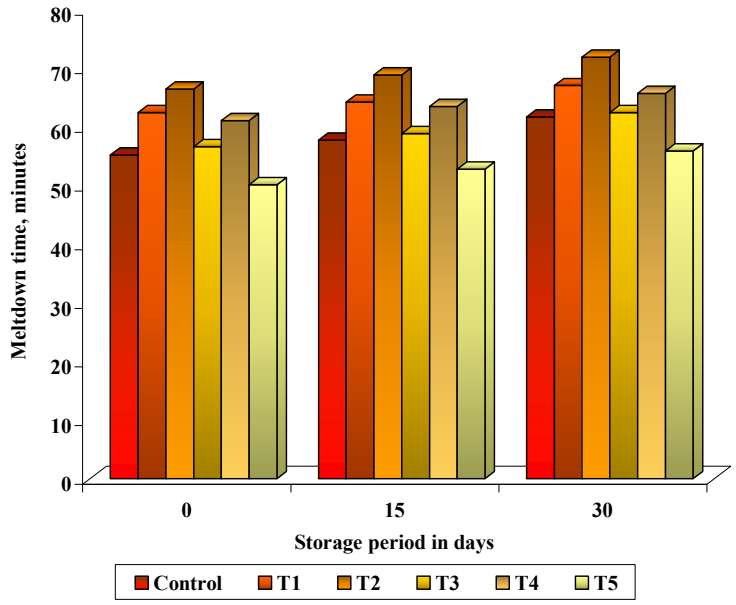

Fig. 11. Meltdown Time of Ice Cream during Storage

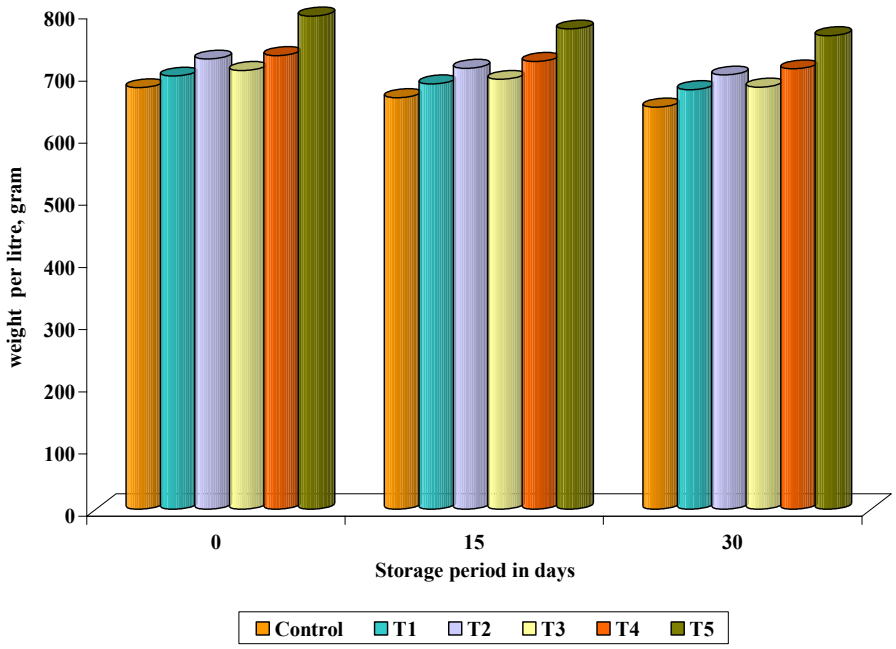

Fig. 12. Weight per Litre of Ice Cream during Storage

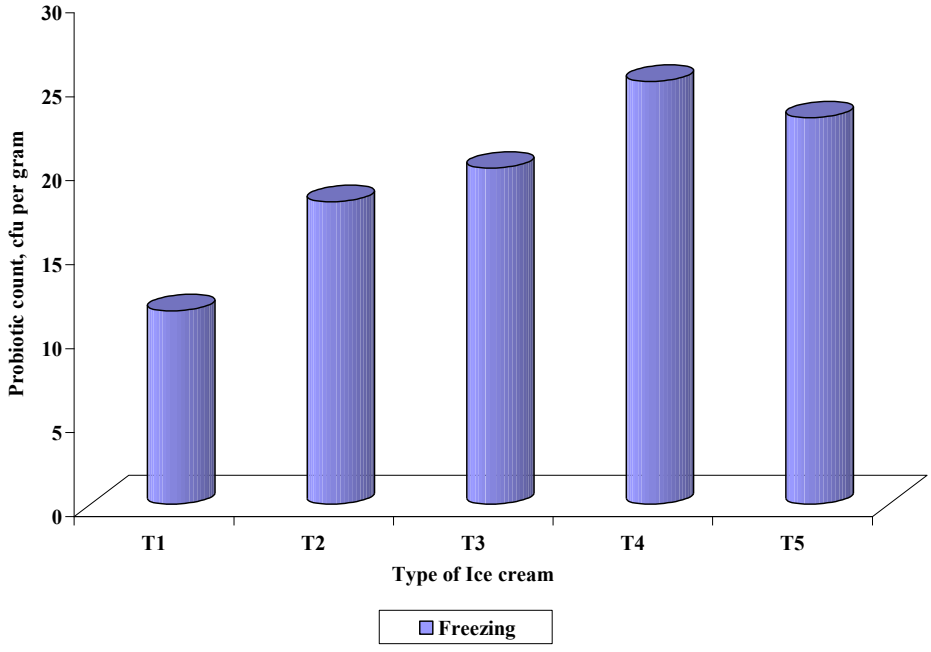

Fig. 13. Probiotic Count of Ice Cream after Freezing

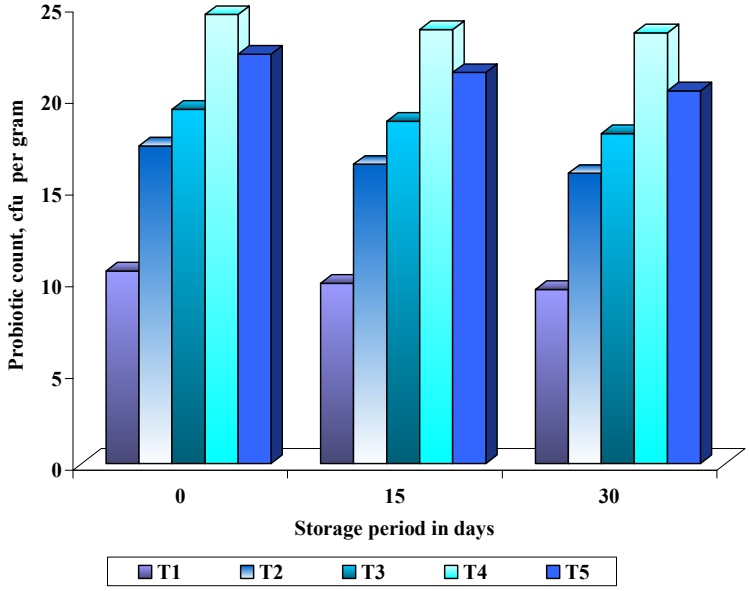

Fig. 14. Probiotic Count of Ice Cream during Storage

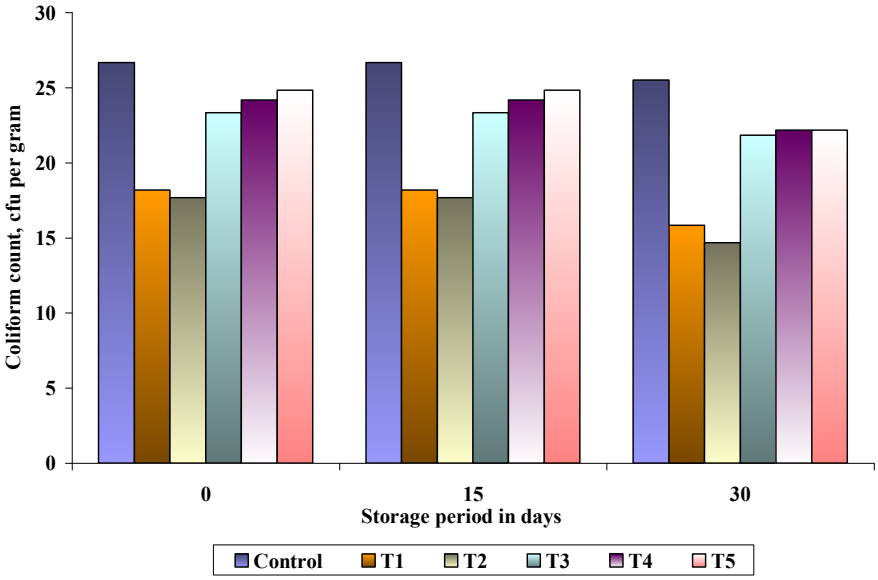

Fig. 15. Coliform Count of Ice Cream during Storage

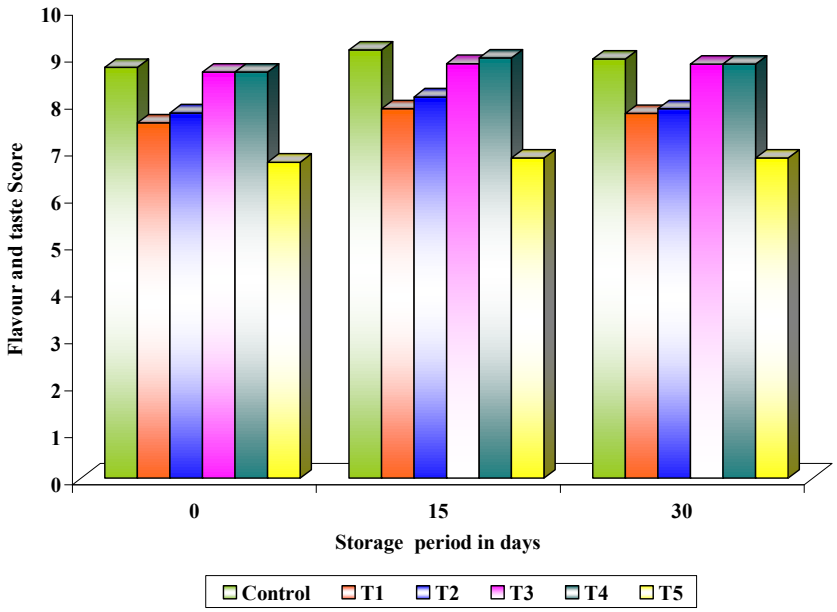

Fig.16. Flavour and Taste Score of Ice Cream during Storage

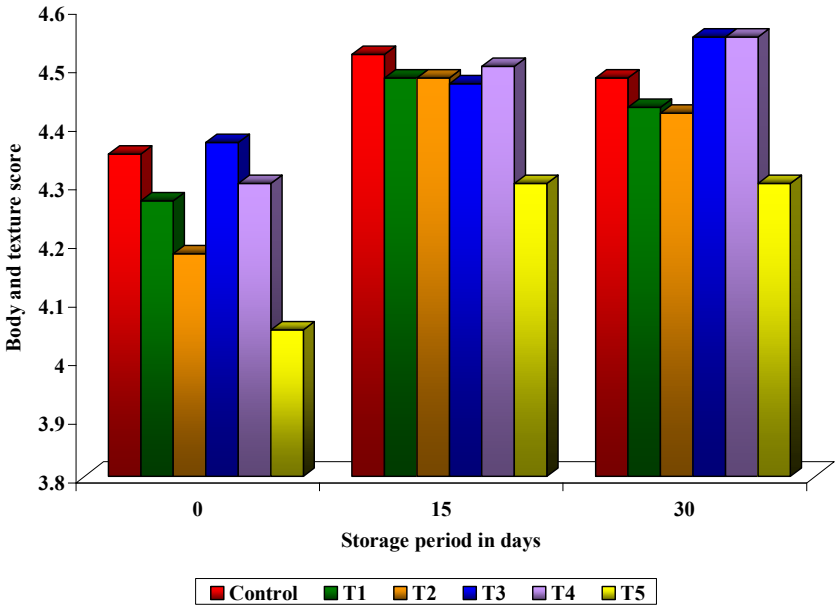

Fig. 17. Body and Texture Score of Ice Cream during Storage

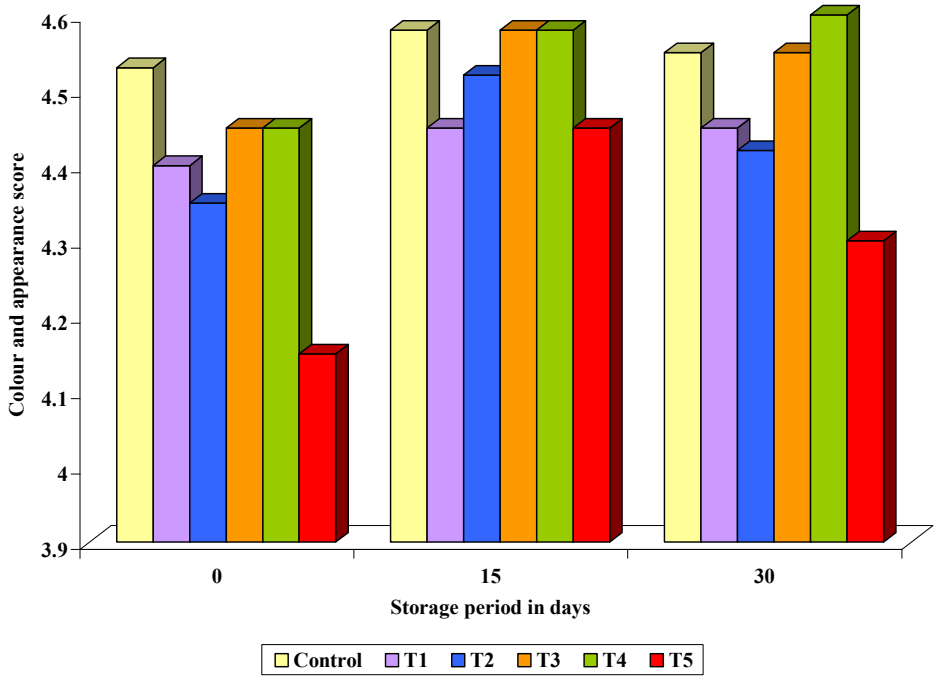

Fig. 18. Colour and Appearance Score of Ice Cream during Storage

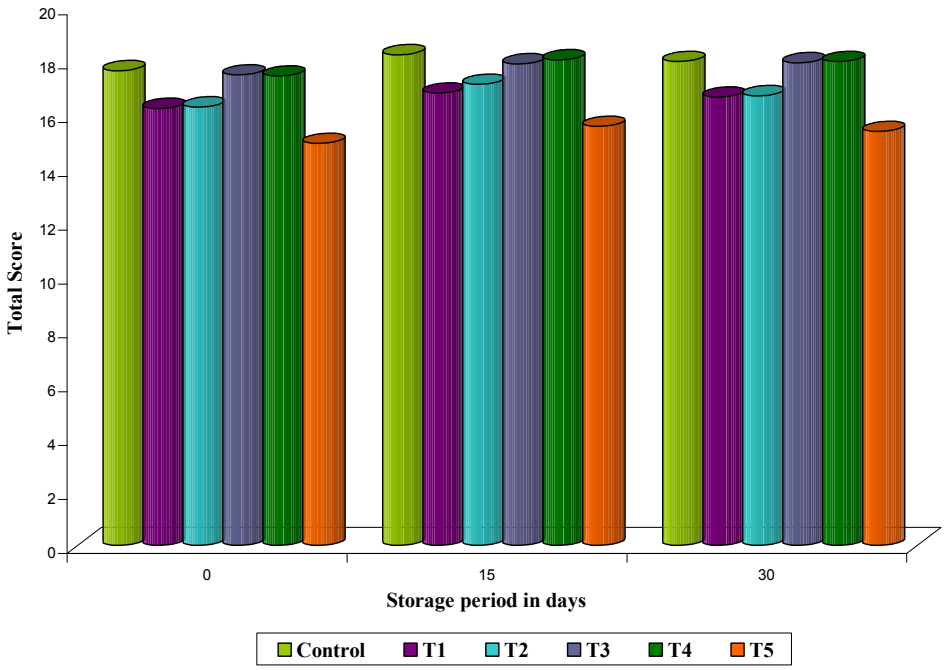

Fig.19. Total Score of Ice Cream during Storage

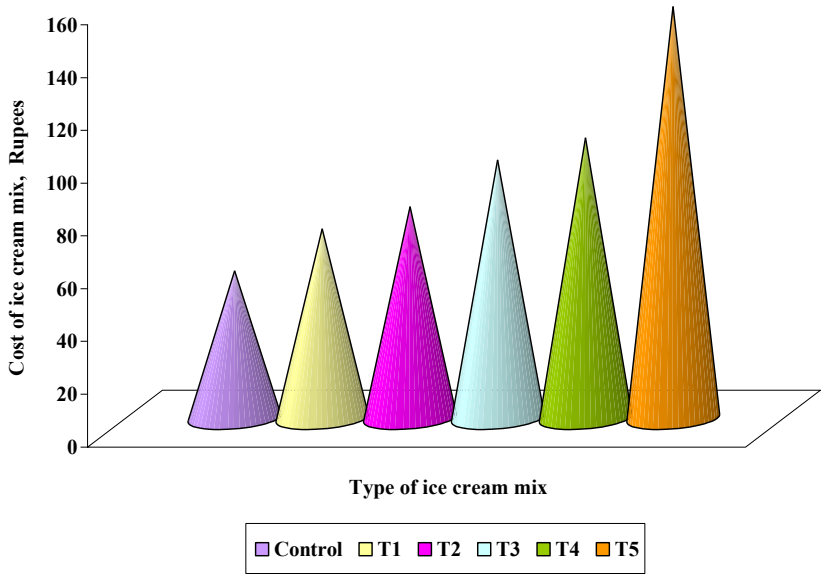

Fig. 20. Cost Analysis of Ice Cream Mix

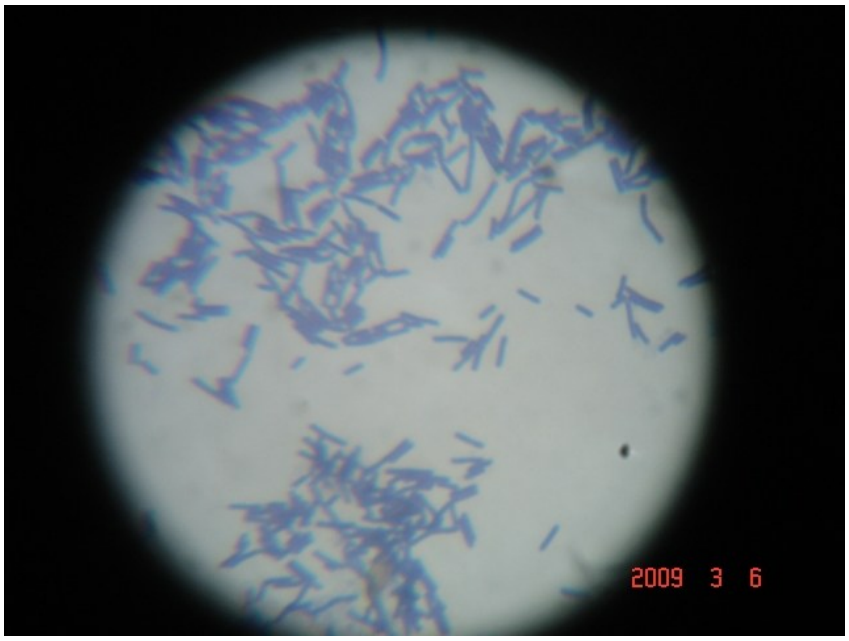

Fig .21. Microscopic Structure of *L. acidophilus* LA-5 of Direct Vat Set Culture

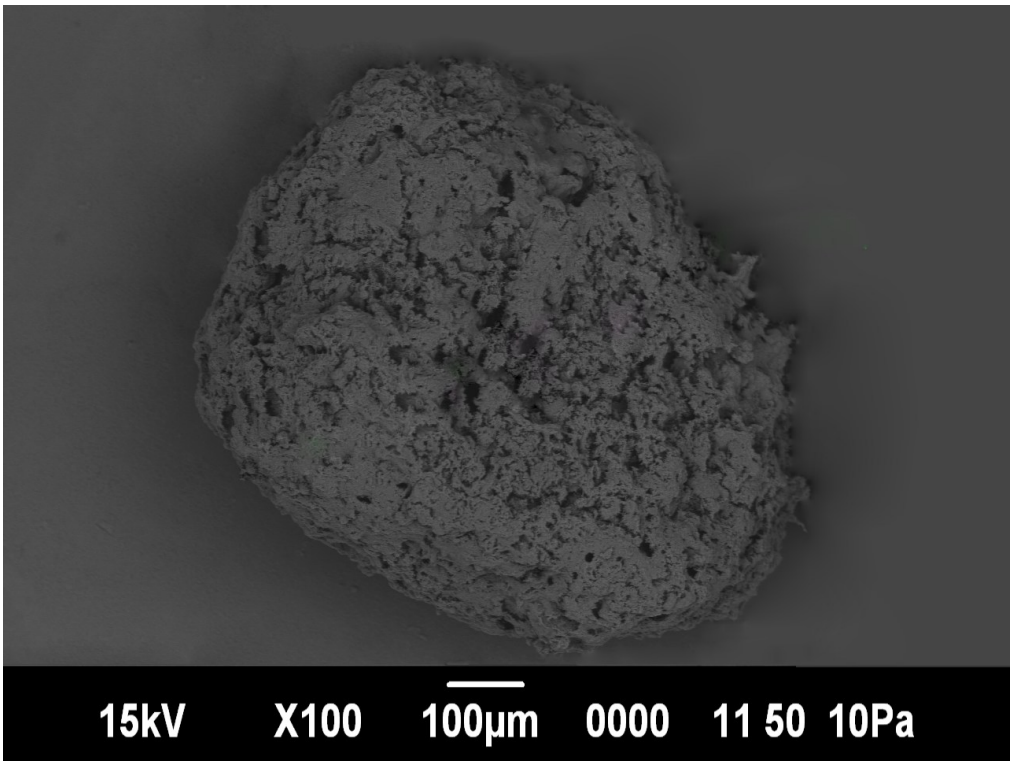

Fig. 22. Electron Photograph of a Microencapsulated Probiotic Bead

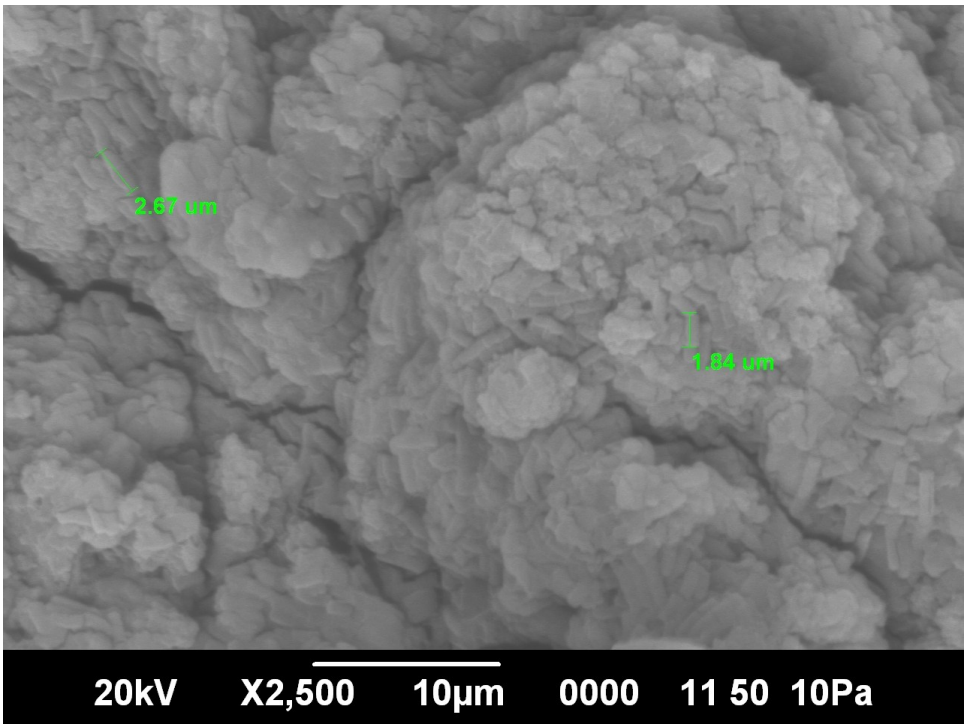

Fig. 23. Electron Photograph of *L. acidophilus* LA-5 in the Microencapsulated Probiotic Bead

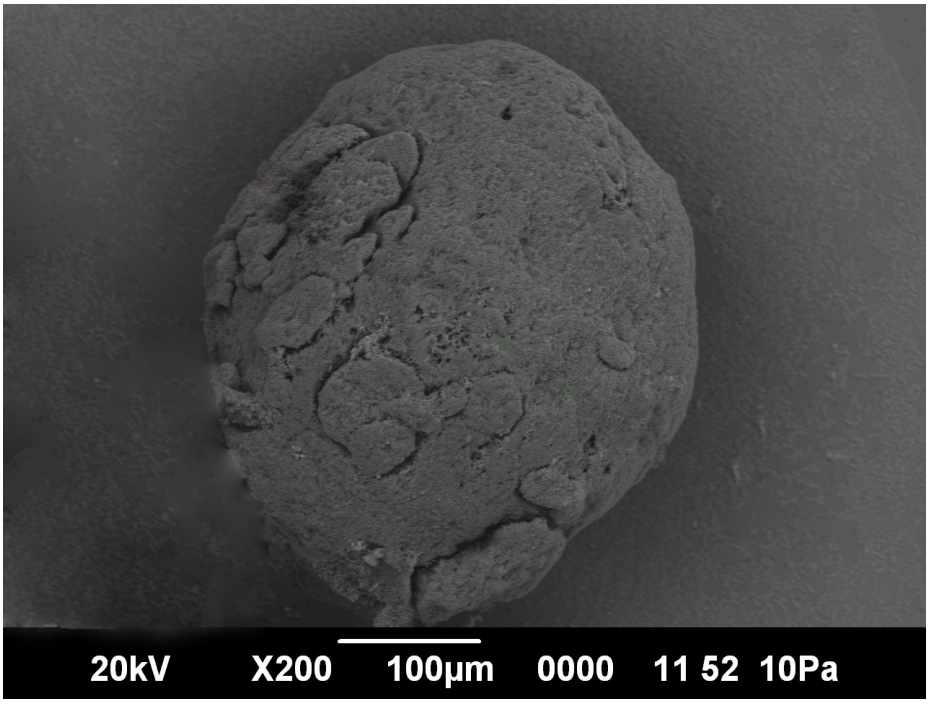

Fig. 24. Electron Photograph of a Microencapsulated Synbiotic Bead

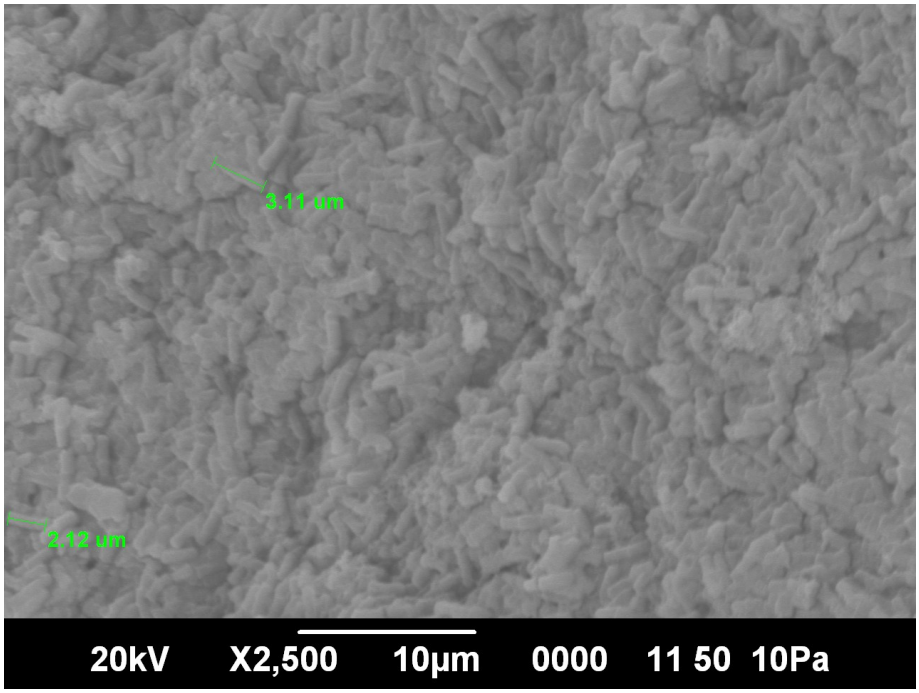

Fig. 25. Electron Photograph of *L. acidophilus* LA-5 in the Microencapsulated Synbiotic Bead

Fig.26. Colonies of *L. acidophilus* LA-5 from Ice Cream on 30th Day of Storage (10^8 cfu per gram)

Discussion

5. DISCUSSION

5.1 ANALYSIS OF DAIRY INGREDIENTS

The dairy ingredients used in the preparation were analysed for fat and total solids. The mean fat (per cent) and total solids (per cent) presented in Table.1. The values were 3.80 and 12.20 for milk, 57.00 and 60.25 for cream, 0.11 and 8.72 for skim milk, 19.00 and 97.00 for dairy whitener, 1.00 and 96. 50 for skim milk powder respectively.

The values obtained for milk, cream, skim milk, dairy whitener and skim milk powder were with in the minimum limit prescribed by Prevention of Food Adulteration Act (1954) and in close agreement with the report of De (1980).

5.2 MICROENCAPSULATED BEAD SIZE

The mean diameter of bead used in the probiotic ice cream mix (T3) was 220.64 ± 61.83 µm. (Table 3a). The mean diameter of bead used in the synbiotic ice cream mix (T4 and T5) was 116.03 ± 27.57 µm (Table 3b).

The mean diameter of bead used in probiotic ice cream mix was slightly larger than that of bead used in the synbiotic ice cream mix. This could be attributed to the compositional difference used in producing both types of beads since all other parameters were kept same for performing the microencapsulation. The beads used for probiotic ice cream mix contained four per cent whey protein while that of synbiotic ice cream mix contained the bead with one per cent whey protein and three per cent oligofructose. Sodium alginate in both types of beads was added uniformly at one per cent level.

Scanning electron microscope was used to examine the structure of the beads. The beads were in spherical shapes with group of entrapped bacteria evident in the internal voids and surrounded by the matrix. The external surface

of the beads used in synbiotic ice cream were smoother than the beads used in the probiotic ice cream which can also be attributed to the compositional difference among the bead structure (Fig. 22, 23, 24 & 25)

5.3 ANALYSIS OF ICE CREAM MIX

5.3.1 Physico – chemical Properties of Ice Cream Mix

5.3.1.1 pH of Ice cream mix

The mean pH for control and treatments ((T1, T2, T3, T4 and T5) mixes were 6.37 ± 0.016, 6.34 ± 0.010, 6.33 ± 0.010, 6.36 ± 0.011, 6.35 ± 0.012 and 6.35 ± 0.017 respectively. Statistical analyses revealed that there was no significant difference in the mean pH value between control and all the treatment ice cream mixes and among the treatment mixes (Table 4 and Fig.2).

During the preparation of ice cream mix, fermentation was not allowed to occur as the required concentration of *L. acidophilus* LA-5 was added into the ice cream mix in the form of direct vat set culture just before freezing.

In T5 ice cream mix, fat and sugar replacers did not significantly decline the pH as reported by Khillari *et al.* (2007) that substitution of fat up to 60 per cent with whey protein concentrate did not affect the pH of the ice cream mix.

Arbuckle (1966), De (1980) and Marshall *et al*. (2003) reported that the normal pH of an ice cream mix is about 6.3. Similarly, in this trial, overall mean pH of control and all treatment ice cream mixes was in the range of 6.34 to 6.37.

5.3.1.2 Titratable Acidity of Ice Cream Mix

The mean values of titratable acidity for control and treatment (T1, T2, T3, T4 and T5) ice cream mixes were 0.211 ± 0.001, 0.215 ± 0.001, 0.217 ± 0.003, 0.212 ± 0.001, 0.213 ± 0.001 and 0.214 ± 0.001 respectively (Table 5 and Fig.3). There was no significant difference in mean value of titratable acidity

between the control and treatment ice cream mixes and among the treatment groups. Statistical analyses revealed that direct addition of culture *L. acidophilus* LA-5 into the ice cream mixes in a concentrated form (Direct vat set culture containing $4.93 \pm 0.312 \times 10^{11}$ cfu per gram) either in free or encapsulated state did not bring any significant increase in the acidity of the treatment ice cream mixes. Similar observations of non significant influence on acidity of the ice cream mix by addition of concentrated form of probiotic culture have been reported by Haynes and Playne (2002) and Basyigit *et al*. (2006).

In low fat synbiotic ice cream mix, fat and sugar replacers did not bring any significant change in the titratable acidity of the mix in similar manner as it did not affected pH of the mix.

In this present experiment, the titratable acidity of all the ice cream mixes was inversely proportional to their pH. The titratable acidity for control and all treatment ice cream mixes were with in the limits prescribed by Bureau of Indian Standard (IS: 2802 – 1964) specification and De (1980).

5.3.1.3 Specific Gravity of Ice Cream Mix

The mean of specific gravity for control and treatment (T1, T2, T3, T4 and T5) ice cream mixes were 1.101 ± 0.02, 1.115 ± 0.01, 1.124 ± 0.01, 1.119 ± 0.01, 1.130 ± 0.01 and 1.138 ± 0.02 respectively (Table 6 and Fig 4). Except for treatment T1, all treatments had significantly higher specific gravity (T2, T4 and T5 ($P<0.01$) and T3 ($P<0.05$)) than control ice cream mixes. The mean specific gravity of T4 was significantly higher ($P<0.05$) from T1 treatment ice cream mix. The mean specific gravity of T5 treatment ice cream mix was significantly higher than that of T2, T3 ($P<0.05$) and T1 ($P< 0.01$) treatment ice cream mixes.

Addition of non encapsulated *L. acidophilus* LA-5 into the ice cream mix (T1) did not bring any significant change in the specific gravity. Similar observations of no significant increase in specific gravity of the ice cream mix on addition of culture at a level of ten per cent (Salem *et al.,* 2005), 25 per cent and

even 50 per cent (Christiansen *et al.*, 1996) have been reported. But on contrary, increase in specific gravity of ice cream mix due to incorporation to fermented milk in ice cream mixes was reported by Rao *et al*. (1988).

The significant increase in the specific gravity of T2 treatment mix than that of the control could be attributed to the addition of the oligofructose along with non encapsulated *L. acidophilus* LA-5.

Incorporation of microencapsulated beads of probiotic bacteria *L. acidophilus* LA-5 significantly increased the specific gravity of the treatment ice cream mixes T3, T4 and T5 than the control mix. This increase in specific gravity of the mix could be attributed to the presence of sodium alginate, whey protein and oligofructose in the microencapsulated bead structure.

The highest mean value of specific gravity of synbiotic low fat (T5) ice cream mix can be due to incorporation of whey protein, polydextrose and wheat dextrin along with addition of encapsulated *L. acidophilus* LA-5 and oligofructose. This result was in agreement with Khillari *et al*. (2007) who reported that the specific gravity of ice cream prepared by substituting 20 per cent milk fat with whey protein concentrate was 1.080 which is significantly higher than control ice cream (1.068).

De (1980) and Marshall *et al.* (2003) showed that specific gravity of all ice cream mixes depends upon the composition and may range from 1.054 to 1.123. In this experiment the specific gravity of all the ice cream mixes ranged between 1.101 and 1.138.

5.3.1.4 Fat Content in Ice Cream Mix

The mean fat content of control and treatment mixes (T1, T2, T3, T4 and T5) were 10.20 ± 0.100, 10.13 ± 0.115, 10.06 ± 0.042, 10.13 ± 0.088, 10.13 ± 0.080 and 0.47 ± 0.033 per cent respectively. The mean fat content value of treatment mixes T1, T2, T3 and T4 was not significantly different ($P>0.05$) from

that of control ice cream mix. The mean fat content value of T5 was significantly different ($P<0.01$) from that of control and other treatment mixes (Table 7 and Fig.5).

According to Prevention of Adulteration Act (1954) and Bureau of Indian Standards (IS: 2802-1964) specifications, normal full fat ice cream should contain minimum ten per cent of fat and low fat ice cream should contain not more than 2.5 per cent of fat. In the present study, control and T1, T2, T3 and T4 treatment ice cream mixes were formulated to have a minimum of ten per cent of fat and T5 ice cream mix was formulated to have a fat content less than 2.5 per cent in order to meet the legal standards of full fat and low fat ice cream respectively.

5.3.2 Microbial Properties of Ice Cream Mix

5.3.2.1 Probiotic Count of the Ice Cream Mix

The amount of *L. acidophilus* LA-5 DVS culture was added into the ice cream mix, both in encapsulated and non-encapsulated form at the level of one per cent (i.e.,10 gram of *L. acidophilus* LA-5 DVS culture in one kg of ice cream). The mean probiotic count for treatment mixes (T1, T2, T3, T4 and T5) expressed in 10^9 cfu per gram was 45.00 ± 0.58, 43.17 ± 1.25, 45.67 ± 1.87, 45.14 ± 1.08 and 43.83 ± 1.89 respectively (Table 8 and Fig.6). Among the treatment ice cream mixes, probiotic counts did not show significant difference ($P>0.05$) such that the ice cream mixes had a comparable count of *L. acidophilus* LA-5 in them before freezing.

There was an average decrease of 1.2 log unit in the probiotic count of the culture due to the dilution in the ice cream mix. This experimental finding is in agreement with the result of Salem *et al.* (2005) who reported that when fermented milk was added to ice cream mix at the rate of ten per cent ((v/v), the number of viable count of ice cream mix decreased by one log unit. A similar

decrease of *L. acidophilus* LA-5 by 1.9 log unit has been reported by Magarinos *et al.* (2007).

5.3.2.2 Coliform Count of Ice Cream Mix

The mean values of coliform count per gram for control and treatments (T1, T2, T3, T4 and T5) ice cream mixes were 33.17 ± 1.19, 32.17 ± 1.14, 30.50 ± 1.12, 32.67 ± 1.73, 33.83 ± 1.33 and 32.17 ± 1.80 respectively (expressed in cfu per gram) and presented in (Table 9 and Fig. 7).

There was no significant difference (P>0.05) among the control and treatment ice cream mixes with respect to coliform count. This may be because all the treatment ice creams were prepared from a common ice cream mix. The additional steps like addition of probiotic and prebiotics were done in a hygienic way which did not contribute to any increase in the coliform count. The findings indicates the values of coliform count are far below than what is permitted by BIS specification indicating good hygienic conditions followed in the preparation of ice cream mix.

5.4 ANALYSIS OF ICE CREAM

5.4.1 Physico – chemical Properties of Ice Cream

5.4.1.2 pH of Ice Cream during Storage

The mean pH for control and treatment (T1, T2, T3, T4 and T5) ice creams for 0^{th} day of storage were 6.36 ± 0.027, 6.19 ± 0.004, 6.18 ± 0.007, 6.29 ± 0.007, 6.27 ± 0.026 and 6.28 ± 0.006 respectively, for 15^{th} day of storage were 6.33 ± 0.025, 6.17 ± 0.004, 6.15 ± 0.006, 6.27 ± 0.007, 6.24 ± 0.011 and 6.25 ± 0.011 respectively and for 30^{th} day 6.29 ± 0.017, 6.15 ± 0.004, 6.13 ± 0.004, 6.25 ± 0.005, 6.23 ± 0.013 and 6.23 ± 0.008 respectively (Table 10a, 10b & 10c and Fig.8.).

Statistical analysis throughout the storage period showed that all treatment ice creams had a significantly lower pH ($P<0.01$) than that of the control ice cream. Treatment ice creams containing non-encapsulated *L. acidophilus* LA-5 (T1 and T2) possessed a significantly lower mean pH ($P<0.01$) value than the treatment ice creams containing encapsulated *L. acidophilus* LA-5 (T3, T4 and T5).

Incorporation of *L. acidophilus* LA-5 in free from (non encapsulated) contributed to the decrease of mean pH value of T1 treatment ice cream. This result is in agreement with reports of Christiansen *et al.* (1996), Alamprese *et al.* (2002), Salem *et al*. (2005), Basyigit *et al.* (2006) and Trindade *et al.* (2006) that pH of the ice cream decreased with addition of probiotic bacteria.

The reduction in the mean pH value of T2 treatment ice cream was associated with the addition of non-encapsulated LA- 5 along with prebiotic oligofructose. Similar result in reduction of pH in the ice cream due to incorporation of prebiotics into the probiotic ice cream was observed by Akin *et al*. (2007) and Akalin and Erisir (2008) by adding inulin and oligofructose into the probiotic ice cream.

Treatment ice creams with microencapsulated *L. acidophilus* LA-5 (T3, T4 and T5) had significantly higher ($P<0.01$) pH than that of treatments which contain free (non encapsulated) *L. acidophilus* LA-5 (T1 and T2). This result is in agreement with Homayouni *et al.* (2007) who observed that microencapsulation delays the reduction in pH of the ice cream by slackening the metabolic activity of the bacteria immobilized inside the bead. Similarly, Larisch *et al.* (1994) and Zhou *et al.* (1998) observed during the fermentation of milk with *Lactococcus lactis* ssp. *cremoris* that there was a reduction in the rate of lactic acid production with immobilized cells, in comparison to non encapsulated cells.

In T5 ice cream mix, fat and sugar replacers does not significantly reduced the pH as reported by Khillari *et al.* (2007) that substitution of fat up to 60 per cent with whey protein concentrate does not affect the pH of the ice cream mix thereby the ice cream .

Inoue *et al*. (1998) reported that changes in pH of the ice cream type frozen yoghurt during storage were small and it was inversely related to the lactic acid content of the products. Haynes and Playne (2002) found that pH of full fat and low fat probiotic ice cream remained virtually unchanged over the storage period of 12 months. Kailasapathy and Sultana (2003) also reported that the pH of the fermented ice cream (pH- 4.5) remained virtually unchanged over the 24 weeks storage period.

In this trial also, storage period of 30 days did not influence the pH of the ice creams significantly.

5.4.1.2 Fat Content in Ice Cream during Storage

The mean value for control and treatment ice creams(T1, T2, T3 and T4) were 10.18 ± 0.10, 10.10 ± 0.08, 10.08 ± 0.04, 10.08 ± 0.05, 10.10 ± 0.05 and 0.45 ± 0.02 per cent respectively for 0^{th} day of storage, 10.18 ± 0.10, 10.10 ± 0.08, 10.08 ± 0.04, 10.08 ± 0.05, 10.10 ± 0.05 and 0.45 ± 0.02 per cent respectively for 15^{th} day of storage respectively and 10.18 ± 0.10, 10.10 ± 0.08, 10.08 ± 0.04, 10.08 ± 0.05, 10.10 ± 0.05 and 0.45 ± 0.02 respectively for 30^{th} day of storage (Table 11a, 11b & 11c and Fig.9.). Statistically analyzed data revealed that all treatments except T5 had no significant difference ($P>0.05$) when compared to control. The fat per cent of T5 was significantly lowest ($P<0.01$) from control and all other treatments because the ice cream mix of T5 was formulated as such with fat per cent lower than 2.5 per cent to meet the legal standards of low fat ice cream.

Marshall *et al.* (2003) opined that fat component of the ice cream increases the richness of flavour, produce a characteristic smooth texture and good body and aids in producing desirable melting properties.

This study also revealed a similar observation with regard to role of fat in improving the appeal of ice cream. Low fat ice cream (T5) had the lowest score during sensory evaluation.

5.4.1.3 Overrun of Ice cream

Data with respect to overrun per cent for control and treatments (T1, T2, T3, T4, T5) ice cream are presented in Table 12 and Fig.10. The mean values of overrun for control and treatments (T1, T2, T3, T4 and T5) were 36.21 ± 1.12, 32.54 ± 0.649, 36.57 ± 1.69, 33.82 ± 2.87, 36.39 ± 2.42 and 30.00 ± 0.790 respectively.

Statistical analysis of the data revealed that there was no significant difference ($P>0.05$) between control and all treatments and also among the treatments indicating addition of probiotic organisms have no significant influence on overrun. Similar findings have been reported by Alamprese *et al.* (2002) and Akin and Erisir (2008).

The overrun values of T2 and T4 treatment ice creams were slightly higher (not significantly) than control, T1 and T3 ice creams. This is attributed to incorporation of oligofructose. Similar increase in overrun of the ice cream mix was obtained by Akin and Erisir (2008) by addition of four per cent of oligofructose into probiotic ice cream mix.

Overrun of T5 treatment ice cream containing fat replacers was lowest but not significantly different from the other treatments and control ice cream. Similar result was shown by Yilsay *et al.* (2006) and Khillari *et al*. (2007) that incorporation of fat replacers decreases the overrun value.

A higher overrun value was not achievable in this experiment since a softy freezer that did not have a provision to incorporate higher amount of air, was used for freezing the ice cream mix.

5.4.1.4 Whipping Ability of Ice Cream

Data with regard to whipping ability of control and treatment ice cream five minutes after the commencement of freezing are presented in Table 13 and Fig.10. The mean values for control and treatments (T1, T2, T3, T4 and T5) were 47.40 ± 1.15, 43.84 ± 1.08, 47.79 ±1.09, 44.13 ± 1.50, 47.19 ± 1.63 and 31.01 ± 0.774 respectively. Statistical analysis revealed that there was no significant difference ($P>0.05$) in whipping ability between control and treatments T1, T2, T3, T4 and among these treatments.

The whipping ability of T5 was significantly lower ($P<0.01$) than control and other treatments due to incorporation of whey protein, polydextrose and wheat dextrin. This finding is in agreement with Schmidt *et al*. (1993) who reported that the use of maltodextrin based fat replacer in low fat ice creams resulted in mixes, with low whipping ability. Adapa *et al.* (2000) reported that protein and carbohydrate based fat replacers do enhance or decrease the whipping ability of the ice cream by exhibiting a viscous behaviour. Marshall *et al*. (2003) reported that solution of polydextrose have higher viscosities than sucrose or sorbitol at equivalent concentrations. This higher viscosity contributes for lower whipping ability of the ice cream.

5.4.1.5 Meltdown Time of Ice Cream during Storage

The data with respect to meltdown time (minutes) revealed that the time required for meltdown increased in the treatments with incorporation of *L. acidophilus* LA-5 than the control (Table 14a, 14b & 14c and Fig 11). The mean meltdown time (in minutes) for control and treatment (T1, T2, T3, T4 and T5) ice creams were 55.00 ± 0.40, 62.17 ± 0.90, 66.34 ± 0.61, 56.38 ± 0.78, 60.84 ± 0.68, and 50.01 ± 0.57 respectively for 0^{th} day, 57.49 ± 0.51, 64.07 ±

0.91, 68.69 ± 0.52, 58.58 ± 0.53, 63.29 ± 0.60 and 52.52 ± 0.58 respectively for 15th day of storage and 61.48 ± 0.75, 66.75 ± 1.13, 71.65 ± 1.33, 62.60 ± 0.44, 65.50 ± 0.49 and 55.73 ± 0.56 respectively for 30th day of storage.

Statistical analysis throughout the storage period showed the mean meltdown time of control and all the treatments are significantly lower ($P<0.01$) to that of T2 ice cream and significantly higher than that of T5 ice cream. Treatment T1 and T4 had meltdown time significantly higher ($P<0.01$) than that of control and T3 ice cream.

The meltdown time of T1 is attributed to the incorporation of *L. acidophilus* LA-5. This observation was similar to that of Christiansen *et al.* (1996), Rao *et al.* (1988) and Trindade *et al.* (2007) who reported that the melting resistance of ice cream increased due to addition of fermented milk.

The meltdown time of T2 is due to the addition of *L. acidophilus* LA-5 along with the prebiotic oligofructose. This result is similar to the reports of Akin *et al.* (2007) and Akalin and Erisir (2008) who observed the increase in meltdown time due to the incorporation of prebiotics inulin and oligofructose respectively in the ice cream.

The meltdown time of T3 was similar to that of the control. It may be due to the presence of encapsulated *L. acidophilus* LA-5 cells which do not establish a marked decrease in pH of the ice cream and thereby no significant increase in the meltdown time of the ice cream. This finding is in agreement with Trindade *et al.* (2007) who opined the role of low pH value in increasing the meltdown time of the ice cream.

The meltdown time of T4 ice cream is determined by oligofructose as presence of microencapsulated *L. acidophilus* LA-5 has no contribution to it. Similarly El-Nagar *et al.* (2002) reported that addition of prebiotic inulin improved the meltdown time of the ice cream.

Reddy *et al*. (1987), Tirumalesha *et al.* (1998) and Khillari *et al*. (2007) stated that melting resistance of ice cream decreased significantly with incorporation of whey protein concentrate. In this experiment also meltdown time of the T5 ice cream was reduced due to the addition of whey protein concentrate.

The trend in the meltdown time observed between the control and treatment ice creams and among the treatment groups on the 0^{th}, 15^{th} and 30^{th} day of storage was same. But as the storage time increased, meltdown time increased proportionately for control as well as for all the treatment ice creams. This result is in an agreement with Akalin and Erisir (2008) where the meltdown time increased consequently in all ice cream samples as the storage period increased from 0^{th}, 30^{th}, 60th to 90th day of storage.

5.4.1.6 Weight per litre of Ice Cream during Storage

The mean values of weight per litre in gram for ice cream are presented in Table 15a, 15b & 15c and Fig.12. The mean values of weight per litre(expressed in gram) for control and treatment (T1, T2, T3, T4 and T5) ice creams were 678.32 ± 2.67, 697.02 ± 3.01, 724.27 ± 3.97, 705.97 ± 4.18, 729.97 ± 5.46 and 793.51 ± 6.85 respectively for 0^{th} day of storage, 662.33 ± 3.23, 684.20 ± 6.19, 709.47 ± 2.99, 691.74 ± 6.74, 720.40 ± 3.05 and 772.72 ± 6.88 respectively for 15^{th} day of storage and 647.17 ± 2.13, 675.09 ± 5.63, 698.65 ± 3.39, 679.28 ± 7.88, 708.67 ± 3.04 and 761.95 ± 7.64 respectively for 30^{th} day of storage.

Statistical analysis of weight per litre on 0^{th} day of storage, revealed that all treatment ice creams were significantly higher ($P<0.01$) from that of control ice cream. Probiotic ice creams T1 and T3 had weight per litre significantly lower ($P<0.01$) than that of synbiotic ice creams T2 and T4. Low fat ice cream T5 had the significantly higher weight per litre among all treatment ice creams.

Addition of *L .acidophilus* LA-5 in both the forms (non encapsulated and encapsulated) in the ice cream increased the weight per litre in T1 and T3 without

any significant difference (P>0.05) among them. Similar result was reported by Salem *et al*. (2005) that addition of probiotic culture increases the weight per litre of the ice cream.

The increase in weight per litre of synbiotic ice creams (T2 and T4) than the probiotic ice creams (T1 and T3) can be attributed to the addition of oligofructose along with *L. acidophilus* LA-5.

The mean weight per litre of low fat ice cream T5 was highest among all ice creams which may be due to incorporation of whey protein concentrate, wheat dextrin and polydextrose along with addition of encapsulated *L. acidophilus* LA-5 and oligofructose.

The statistical analysis of 15^{th} and 30^{th} day of storage gave similar result to that of 0^{th} day with reduction in the values of weight per litre.

As per Bureau of Indian Standards (IS: 2802 -1964) specifications, minimum weight in gram per litre for plain ice cream is 525. In the present study, weight per litre of control and all treatment ice creams throughout the storage period was above the recommended specification.

5.4.2 Microbial Properties of Ice Cream

5.4.2.1 *Probiotic Count of Ice Cream after Freezing*

The mean values of probiotic count after freezing the ice cream mixes of the treatments T1, T2, T3, T4 and T5 (Table 16 and Fig.13) expressed in 10^8 cfu per gram were 11.50 ± 0.56, 19.17 ± 0.70, 23.83 ± 1.70, 27.83 ± 0.75 and 25.50 ± 2.09 respectively. After freezing, there was a reduction of 1.50 log units, 1.37 log units, 1.35 log units, 1.25 log units and 1.28 log units for T1, T2, T3, T4 and T5 respectively in the probiotic count. The survivability of *L. acidophilus* LA-5 after freezing in T2, T3, T4 and T5 (expressed in per cent log) was 1.974, 2.203, 3.179 and 2.92 respectively, more than that of T1 ice cream. Statistical analyses after freezing revealed that T3, T4 and T5 treatment ice creams, containing

microencapsulated *L. acidophilus* LA-5 had significantly higher ($P<0.01$) probiotic count than T1 and T2 ice creams containing free (non encapsulated) *L. acidophilus* LA-5 . The probiotic count of T2 ice cream was significantly higher ($P<0.01$) than that of T1 ice cream.

The reduction in the count of *L. acidophilus* LA-5 during freezing can be attributed to injury due to freezing temperature, mechanical stress due to beating and oxygen incorporation, thermal and osmotic shock which were similarly reported by Akalin and Erisir (2008), Ordonez *et al*. (2000), Hagen and Narvus (1999). On contrary, slight increase in cell concentration in probiotic ice cream after freezing due to break up of *Lactobacillus* chains was reported by Heenan *et al.* (2004) and Trindade *et al.* (2007).

According to Christiansen *et al*. (1996), Alamprese *et al*. (2002), Heenan *et al*. (2004) and Trindade *et al*. (2007) resistant to freezing temperature, beating and air incorporation during freezing depends on different probiotic micro-organisms and conditions of ice cream production.

Hekmat and McMahon (1992) and Salem *et al.* (2005) reported that freezing process caused a reduction of atleast one log cycle and less than one log cycle respectively in the *L. acidophilus* count, in the probiotic ice cream. Akalin and Erisir (2008) observed that the count of *L. acidophilus* LA-5 was decreased about two log units during freezing of the probiotic ice cream mix. In this present experiment there was a reduction of 1.5 log units in the probiotic count while freezing the T1 ice cream mix containing non encapsulated *L. acidophilus* LA-5.

The reduction in survivability of *L. acidophilus* LA-5 after freezing T2 ice cream was 1.37 log units. The *L. acidophilus* LA-5 survivability in T2 was 1.974 per cent of log more than in T1 due to addition of oligofructose. Similarly Akalin and Erisir (2008) observed that the count of *L. acidophilus* LA-5 along with oligofructose was decreased by 1.5 to 2 log units during freezing of the probiotic ice cream mix. Akin *et al.* (2007) reported a decrease of 1.09-1.12 log unit of

L. acidophilus during freezing the probiotic ice cream added with ten per cent fermented milk supplemented with one to two per cent inulin.

Sheu *et al.* (1993) observed that survivability of *L. bulgaricus* during freezing the ice milk was 85 per cent without microencapsulation and 90 per cent with microencapsulation. Thereby confirming that microencapsulation improves the survivability of microorganisms during freezing condition. In this experiment also, microencapsulation improved the survivability of *L. acidophilus* during freezing. The survivability of *L. acidophilus* LA-5 in T1 ice cream during freezing without microencapsulation was 85.05 per cent and in T3 with microencapsulation it was 87.25 per cent. The reduction in survivability of *L. acidophilus* LA-5 after freezing T3 ice cream was 1.35 log units.

Homayouni *et al*. (2008[b]) observed that in synbiotic ice cream containing *L. casei* and prebiotic Hi-maize, micro encapsulation increased 1.82 per cent of log survivability of *L. casei* during freezing when compared to the survivability of free cells of *L. casei*. Similarly in this trial also, microencapsulation and addition of prebiotic oligofructose improved the survivability of *L. acidophilus* LA-5 during freezing, 3.179 per cent of log more in T4 ice cream than in T1 ice cream which contains free non encapsulated *L. acidophilus* LA-5.

The survivability of *L. acidophilus* LA-5 in the low fat ice cream during freezing was 2.92 per cent of log more than T1 due to addition of oligofructose and microencapsulation of *L. acidophilus* LA-5. There was no significant difference in the count of microencapsulated *L. acidophilus* LA-5 after freezing between T4 ice cream (synbiotic full fat ice cream) and T5 ice cream (synbiotic low fat ice cream). This result concluded that full fat ice cream did not offer greater protection to *L. acidophilus* LA-5 during freezing than that of the low fat ice cream. Similar observation was reported by Haynes and Playne (2002) while studying the survivability of probiotic culture (*L. acidophilus*, *B. lactis* and *L. paracasei*) in low fat ice cream prepared with prebiotic resistant starch Hi-maize. The survivability *L. acidophilus* LA-5 in low fat ice cream during freezing

could be attributed to the cryoprotective property of whey protein (containing 33 per cent lactose) and polydextrose. Similar findings of cryoprotective property of whey protein and polydextrose was reported by Marth (1973) and Sultanbawa and Li-Chan (1998).

5.4.2.2 Probiotic Count of Ice Cream During Storage

The mean values for probiotic count. expressed in 10^8 cfu per gram of T1, T2, T3, T4 and T5 were 10.50 ± 0.50, 18.17 ± 1.40, 23.00 ± 1.65, 26.67 ± 0.92 and 24.50 ± 1.98 respectively for 0^{th} day of storage, 9.83 ± 0.31, 17.33 ± 1.26, 22.67 ± 1.61, 26.17 ± 1.08 and 23.83 ± 1.80 respectively for 15^{th} day and 9.67 ± 0.33, 16.33 ± 1.09, 21.50 ± 1.63, 25.33 ± 1.05 and 23.00 ± 1.71 respectively for 30^{th} day (Table 17a, 17b & 17c and Fig 14 & 26).

Statistical analyses throughout the storage period revealed that T3, T4 and T5 treatment ice creams, containing microencapsulated *L. acidophilus* LA-5 had significantly higher ($P<0.01$) probiotic count than T1 and T2 ice creams containing free (non encapsulated) *L. acidophilus* LA-5. The probiotic count of T2 ice cream was significantly higher ($P<0.01$) than that of T1 ice cream.

From the point of freezing to the end of 30 days of the storage, the reduction of *L. acidophilus* LA-5 count in T1, T2, T3, T4 and T5 were 0.083, 0.055, 0.047, 0.029 and 0.0535 (expressed in log cycle) respectively. Thus there is no marked decrease in the probiotic count in all the treatment ice creams from the point of freezing to the end of 30 days of the storage. Similarly Lopez *et al.* (1998), Hagen and Narvhus (1999), Alamprese *et al.* (2002) and Basyigit *et al.* (2006) showed that probiotic culture bacteria in ice cream did not change significantly during storage.

In this study, freezing and mixing involved in converting the mix into ice cream had a greater effect on culture survivability than hardening and storage in ice cream. A similar finding was also reported by Modler *et al.* (1990), Hagen and Narvhus (1999), Magarinos *et al.* (2007) and Akalin and Erisir (2008).

The overall survivability of *L. acidophilus* LA-5 in ice cream (from ice cream mix to the end the 30 days of storage period) for T1, T2, T3, T4 and T5 were (expressed in per cent log) 84.27, 86.50, 86.82, 87.65 and 87.47 respectively and overall reduction expressed in log cycle for T1, T2, T3, T4 and T5 were 1.67, 1.43, 1.40, 1.28 and 1.33 respectively.

The overall reduction of free *L. acidophilus* LA-5 in T1 ice cream during the end of 30 days of storage period was 1.67 log cycle or overall survivability of 84.27 per cent. Similar studies of Heenan *et al.* (2004) showed that the overall survivability of *L. acidophilus* MJLA1 in soy based frozen dessert at end of 35 days of storage was 78.4 per cent. Akalin and Erisir (2008) showed that the overall decrease in the *L. acidophilus* LA-5 count in the probiotic ice cream after 30 days of storage was 2.21 log cycle. In the study done by Alamprese *et al.* (2002), the survival rate of *L. johnsonii* La1 in the probiotic ice cream at the end of 30 days of frozen storage was 90 per cent.

In T2 treatment ice cream, the survivability of *L. acidophilus* LA-5 was 86.50 per cent at the end of 30 day of storage. The overall reduction in *L. acidophilus* LA-5 count was 1.43 log cycles and it was 0.240 log cycle less than T1 ice cream due to addition of oligofructose. Similar increase in the overall probiotic count by addition of prebiotics in the ice cream was shown by Akin *et al.* (2007) reported that the at the end of 30 day of storage, overall survival rate of *L. acidophilus* in the probiotic ice cream was 77.78 per cent and in synbiotic ice cream containing two per cent inulin was 80.61 per cent. Modler *et al.* (1990) and Akalin and Erisir (2008) also showed similar findings of increase in probiotic count by addition prebiotics in ice cream.

The overall survivability of microencapsulated *L. acidophilus* LA-5 in T3 ice cream, at the end of 30 day of storage was 86.82 per cent log and the reduction in *L. acidophilus* LA-5 was 1.40 log cycle. There was 0.272 log cycle less reduction in probiotic count in T3 than T1 ice cream at the end of 30 day of storage which can be attributed due to microencapsulation of

L. acidophilus LA-5. Similar reports of improving the overall survivability of probiotic bacteria in frozen ice milk and ice cream by microencapsulation was confirmed by Sheu *et al.* (1993), Kebary *et al.* (1998) and Shah and Ravula (2000).

The overall reduction in probiotic count of T4 ice cream containing microencapsulated *L. acidophilus* LA-5 at the end of 30 days of storage was 1.28 log units. The overall survivability of *L. acidophilus* LA-5 (from day of preparation to end of storage period of 30 days) in T4 ice cream was 87.65 per cent. The reduction in *L. acidophilus* LA-5 count in T4 ice cream at the end of storage period was 0.392 log units less than that of in T1 ice cream. This can be attributed to addition of oligofructose and microencapsulation of *L. acidophilus* LA-5. This experimental findings were similar to the report of Homayouni *et al*. (2008[b]), that microencapsulation along with addition of prebiotic Hi-maize increased the survivability of microencapsulated encapsulated *L. casei*, 0.359 log units higher than that of non-encapsulated *L. casei* at the end of 30 days of frozen storage in the synbiotic ice cream.

The overall reduction in the *L. acidophilus* LA-5 count in T5 low fat ice cream at the end of 30 days of storage is 1.33 log units with 0.342 log units less reduction than T1 ice cream. In the T5 low fat synbiotic ice cream, the overall survivability of microencapsulated *L. acidophilus* LA-5 was 87.46 per cent which was similar to that of T4 full fat synbiotic ice cream. Therefore it can be concluded that fat content present in the full fat ice cream did not resulted in any significant improvement in the overall survivability of *L. acidophilus* LA-5. This finding is similar to that of Haynes and Playne (2002) who observed that there was no significant difference in the overall probiotic count in the low fat synbiotic ice cream made with addition of Hi-maize. Davidson *et al.* (2000), Alamprese *et al*. (2002) and Trindade *et al.* (2007) also reported that probiotic culture bacteria in low fat ice cream did not change significantly during storage. The survivability *L. acidophilus* LA-5 in low fat ice cream during storage could be due to the cryoprotective activity of whey protein (containing 33 per cent

lactose) and polydextrose. Similarly, Marth (1973) and Sultanbawa and Li-Chan (1998) reported about the cryoprotective property of whey protein and polydextrose.

The standard for any food sold with health claims from the addition of probiotics is that it must contain per gram at least 10^6 to 10^7 cfu of viable probiotic bacteria (FAO/WHO, 2001). In the trial all the experimental ice creams throughout the storage period contained probiotic count of *L. acidophilus* LA-5 above the recommended level.

5.4.2.3 Coliform Count of Ice Cream during Storage

The mean values for coliform count during 0^{th}, 15^{th} and 30^{th} day of storage were presented in Table.18a, 18b & 18c and Fig.15. Mean values (expressed in cfu/g) of control, T1, T2, T3, T4 and T5 were 29.50 ± 0.62, 23.00 ± 0.73, 21.83 ± 1.56, 28.33 ± 1.56, 27.50 ± 1.98 and 28.83 ± 1.76 respectively for 0^{th} day of storage, 26.67 ± 0.42, 18.17 ± 0.95, 17.67 ± 1.54, 23.33 ± 2.32, 24.17 ± 2.54 and 24.83 ± 1.30 respectively for 15^{th} day of storage and 25.50 ± 0.89, 15.83 ± 0.95, 14.67 ± 1.28, 21.83 ± 1.99, 22.17 ± 2.40 and 22.17 ± 1.25 respectively for 30^{th} day of storage

Statistical analysis throughout the storage period showed that the coliform count of treatment ice creams containing free (non encapsulated) *L. acidophilus* LA-5 (T1 and T2) were significantly lower ($P<0.01$) than control and treatment containing microencapsulated (T3, T4 and T5). The reduction in coli form count when compared to control in T1, T2, T3, T4 and T5 expressed in percent of log were 12.92, 13.91, 4.05, 4.49 and 3.22.

Shrestha and Sinha (1987) studied the occurrence of coliform bacteria in dairy products and found that 77 percent of ice cream contained unsatisfactory levels of coliforms on the basis of Indian standards. Arora and Sudarsanan (1986) found that the bacteria in ice cream come from two sources (i) ingredients used (ii) conditions during manufacture, handling, storage and transportation. The

microbial quality of any food product is an index of the hygienic practices followed during the various stages of the processing. Specifications of Bureau of Indian Standard allows maximum of 90 coliform per gram. In this trial, all the ice cream the mean coliform counts were below the permissible level of BIS specification.

There was significant reduction ($P<0.01$) in coliform count during 30 days of frozen storage of treatment ice creams (T1 and T2) containing free (non encapsulated) *L. acidophilus* LA-5 than that of T3, T4 and T5 treatment ice creams containing microencapsulated *L. acidophilus* LA-5 and control ice cream. Similar reduction of coliform count during frozen storage in the ice cream containing free (non encapsulated) probiotic bacteria was reported by Avramidis *et al*. (2004).

During storage period of 30 days the coliform count of treatment ice creams T3, T4 and T5 containing encapsulated *L. acidophilus* LA-5 was similar to that of control ice cream. This can be concluded that encapsulated form of *L. acidophilus* LA-5 bacteria did not had any significant effect on the reduction of coliform count of the ice cream during storage like that of free *L. acidophilus* LA-5.

5.4.3 Sensory Evaluation of Ice Cream during Storage

5.4.3.1 Flavour and Taste Score

The mean flavour and taste score for control and treatment T1, T2, T3, T4 and T5 on 0^{th} day of storage were 8.75 ± 0.195, 7.57 ± 0.138, 7.78 ± 0.151, 8.65 ± 0.050, 8.65 ± 0.081 and 6.73 ± 0.320 respectively, on 15^{th} day of storage were 9.12 ± 0.054, 7.87 ± 0.163, 8.12 ± 0.119, 8.83 ± 0.080, 8.95 ± 0.072 and 6.82 ± 0.183 respectively and on 30^{th} day of storage were 8.93 ± 0.242, 7.76 ± 0.176, 7.87 ± 0.115, 8.82 ± 0.114, 8.82 ± 0.201 and 6.82 ± 0.299 respectively (Table 19a, 19b & 19c and Fig 16).

Statistical analyses on the 0^{th} day of storage revealed that control, T3 and T4 ice creams were significantly higher in mean flavour and taste score ($P<0.01$) than that of T1 and T2 ice creams. The mean flavour and taste score of T5 ice cream was significantly lower ($P<0.01$) than that of all other ice creams in the experiment. The statistical result of mean flavour and taste score on 15^{th} and 30^{th} day of storage was similar to that of 0^{th} day.

Mortazavian *et al.* (2007) and Krasaekoopt *et al.* (2003) reported that microencapsulation fixes and improves the sensory properties and also controls the flavour of probiotic products. In this experiment, the mean flavour score of T3 and T4 ice creams containing microencapsulated *L. acidophilus* LA-5 was similar to that of control ice cream. This may be attributed to the microencapsulation of *L. acidophilus* LA-5 which maintained the pH of T3 and T4 ice cream slightly closer to control ice cream thereby reducing the acidic or sour flavour due to the bacteria in ice cream. Similarly, Homayouni *et al.* (2008^{b}) observed no yoghurt or probiotic flavour in the ice cream containing probiotic in the microencapsulated state.

The mean flavour and taste score of T1 and T2 was significantly lower ($P<0.01$) than that of control and microencapsulated probiotic ice creams (T3 and T4). This may be attributed to lower pH value of T1 (6.04 - 6.11) and T2 (6.01 to 6.05) due to addition of non-encapsulated *L. acidophilus* LA-5 (in concentration of around 10^{9} cfu/g) which resulted in slight acidic or sour flavour. Similarly, Hagen and Narvhus (1999) prepared probiotic ice cream by adding fermented milk to the regular ice cream mix. The results for the sensory evaluation of the probiotic ice cream were considered satisfactory without any probiotic flavour. Salem *et al*. (2005) and Akin *et al*. (2007) opined that when the pH of probiotic ice cream is maintained in higher range (5.8 to 6.4) there is absence of yoghurt or probiotic flavour. The probiotic ice creams had sour or acidic flavour and gave a good total impression without any marked off-flavour during the storage period.

The flavour and taste score of T5 low fat ice cream containing microencapsulated *L. acidophilus* was significantly lower ($P<0.01$) than all treatment and control ice creams due to its low fat percentage. Similarly, Guinard *et al*. (1996) and Li *et al*. (1997) opined about the importance of milk fat content in flavour perception of vanilla ice cream. The less flavour and taste score of T5 could also be due to the slight off flavour caused by incorporation of polydextrose (five per cent) and wheat dextrin (three per cent). Similarly, Goff and Jordan (1984) reported the presence of off flavour with slight burnt aftertaste in the calorie reduced frozen dairy dessert made by incorporating polydextrose at 13.9 per cent.

On whole, there was no probiotic or yoghurt flavour, rancid and oxidized flavour, marked off flavour, or lack of flavour in the control and among experimental ice creams.

5.4.3.2 Body and Texture Score

Data with respect to body and texture of control and treatments (T1, T2, T3, T4 and T5) were 4.35 ± 0.150, 4.27 ± 0.109, 4.18 ± 0.087, 4.37 ± 0.131, 4.30 ± 0.232 and 4.05 ± 0.169 respectively for 0^{th} day of storage, 4.51 ± 0.074, 4.48 ± 0.054, 4.48 ± 0.065, 4.46 ± 0.123, 4.50 ± 0.113 and 4.30 ± 0.100 respectively for 15^{th} day of storage and 4.48 ± 0.106, 4.43 ± 0.152, 4.41 ± 0.147, 4.55 ± 0.095, 4.55 ± 0.115 and 4.30 ± 0.077 respectively for 30^{th} day of storage (Table. 20a, 20b & 20c and Fig. 17).

The mean diameter of bead used in the probiotic ice cream mix (T3) was 220.64 ± 61.83µm (Table 3a). The mean diameter of bead used in the synbiotic ice cream mix (T4 and T5) was 116.03 ± 27.57µm (Table 3b). During sensory evaluation none of the treatment ice creams (T3, T4 and T5) containing microencapsulated *L. acidophilus* LA-5, were reported to be coarse in texture. None of the ice creams were judged to be crumbly, icy, weak or fluffy in the body and texture. Data when statistically analysed revealed no significant

difference (P>0.05) between control and treatments and among treatments indicating that the organoleptic quality with respect to body and texture of was not influenced by addition of non encapsulated or encapsulated *L. acidophilus* LA-5. Similarly, Salem *et al.* (2005) showed that addition of probiotic bacteria in the ice cream does not affect its body and texture. Trindade *et al.* (2006) opined that addition of probiotic bacteria influenced positively on development of a pleasant body and texture by reducing the pH of the ice cream. Homayouni *et al.* (2008[b]) observed that the body and texture is not being affected by addition of probiotic bacteria in ice cream either in non encapsulated or encapsulated state.

5.4.3.3 Colour and Appearance Score

The mean colour and appearance scores presented in the Table 21a, 21b & 21c and Fig. 18 for control and treatments T1, T2, T3, T4 and T5 for 0^{th} day of storage were 4.53 ± 0.117, 4.40 ± 0.115, 4.35 ± 0.134, 4.45 ± 0.109, 4.45 ± 0.106 and 4.15 ± 0.159 respectively, for 15^{th} day of storage were 4.58 ± 0.094, 4.45 ± 0.092, 4.52 ± 0.090, 4.58 ± 0.083, 4.58 ± 0.083 and 4.45 ± 0.050 respectively and for 30^{th} day of storage were 4.55 ± 0.106, 4.45 ± 0.134, 4.42 ± 0.125, 4.55 ± 0.088, 4.60 ± 0.010 and 4.30 ± 0.077 respectively (Table 21a, 21b & 21c and Fig. 14). Statistical analysis with regard to colour and appearance revealed no significant difference (P>0.05) between control and treatments and among treatment for 0^{th}, 15^{th} and 30^{th} day of storage.

Salem *et al.* (2005) reported that probiotic ice cream prepared by mixing milk fermented with probiotic cultures into the ice cream mix received scores slightly lower than in colour attributes than the control ice cream and could be due to heating process of milk needed for fermentation. In this trial, the ice cream mix was added with *L. acidophilus* in the form of direct vat set culture (either as free or microencapsulated state) and so there was no significant difference between the control and treatment ice creams in colour attributes.

5.4.3.4 Total Score

The mean total score obtained by sensory evaluation considering the above mentioned parameters (flavour, body, texture, colour and appearance) for control and treatment ice creams T1, T2, T3, T4 and T5 for 0^{th} day were 17.63 ± 0.424, 16.23 ± 0.244, 16.28 ± 0.349, 17.46 ± 0.256, 17.43 ± 0.376 and 14.93 ± 0.554 respectively, for 15^{th} day were 18.22 ± 0.162, 16.80 ± 0.159, 17.12 ± 0.182, 17.88 ± 0.244, 18.03 ± 0.180 and 15.57 ± 0.184 respectively and for 30^{th} day were 17.97 ± 0.420, 16.65 ± 0.385, 16.70 ± 0.313, 17.92 ± 0.287, 17.97 ± 0.406 and 15.37 ± 0.406 respectively (Table 22a, 22b & 22c and Fig. 19). The statistical analysis for total scores throughout the storage period revealed that control, T3 and T4 had no significant difference ($P > 0.05$) but significantly higher ($P < 0.01$) from T1 and T2. The mean total of T5 was significantly lower ($P<0.01$) than that of all other ice creams. This significant difference among ice creams in total score was mainly contributed by flavour and taste attribute while other parameters (colour, appearance, body and texture) are similar.

Overall acceptance for the probiotic ice creams containing encapsulated *L. acidophilus* LA-5 with or without addition of oligofructose (T3 and T4) was similar to the control. So it can be concluded that microencapsulation had no effect over the sensory attributes of the ice cream. Probiotic ice cream with non-encapsulated *L. acidophilus* LA-5 had overall acceptance slightly lesser than probiotic ice cream with encapsulated *L. acidophilus* LA-5. The overall acceptance of T5 low fat ice cream was lower than that of all other ice creams.

5.5 COST ANALYSIS OF ICE CREAM MIX

The cost of ingredients for one kg of control ice cream mix (Rupees) was 55.9, where as for treatment T1, T2, T3, T4 and T5 were 71.9, 80.3, 98.0, 106.4 and 156.2 respectively (Table 23 and Fig. 20). The cost of all treatment mixes was higher than the control ice cream mix.

Rao *et al.* (1988) showed that cost reduction of 9 to 19 per cent is possible in the preparation of soft serve frozen dessert using varied amounts of sweetened fermented milk and standard plain ice cream mix.

In this experiment, the increase in cost of treatment ice cream mixes was mainly contributed by encapsulation procedure (done by emulsion method using edible oil), then by addition of probiotic culture and oligofructose.

The cost of preparation of T5 low ice cream mix was higher when compared to control and all other treatment ice cream mixes. The bulking agent and replacers of fat and sugar in low fat ice cream along with encapsulation technique increased the cost of T5 ice cream mix. Even though the costs of all the ice cream prepared form all these treatment mixes are higher than the control ice cream, the beneficial and therapeutic effects present in them, makes them a valuable functional food. It is also important to note that no technological parameters in ice cream preparation were negatively affected by the method of addition of probiotic bacteria in microencapsulated form.

Summary

6. SUMMARY

A detailed study was carried out to formulate a synbiotic ice cream using the probiotic culture *L. acidophilus* LA-5 and the prebiotic oligofructose. The efficiency of microencapsulation to improve the survivability of probiotic organisms in ice cream was also studied in detail. The properties of the treatment ice cream mix and ice cream were compared with the control by using standard procedures.

Dairy ingredients used for preparing the ice cream mix were analyzed for fat and total solids and the results were within the normal range. Direct vat set culture containing *L. acidophilus* LA-5 culture ($4.93 \pm 0.312 \times 10^{11}$ cfu per gram) was used as probiotic at one per cent level and oligofructose as prebiotic at two per cent level. Standard procedure was followed for the preparation of treatment ice-cream groups where group A consist of treatments T1 (probiotic) and T2 (synbiotic) with free *L. acidophilus* LA-5, group B consist of treatments T3 (probiotic), T4 (synbiotic) and T5 (low fat synbiotic) with microencapsulated *L. acidophilus* LA-5 and group C consist of control.

As *L. acidophilus* LA-5 culture was added directly into the ice cream mix without fermentation the mean values of pH and titratable acidity of all ice cream mixes were similar. The mean pH values of all the ice cream mixes ranged from 6.33 to 6.37 and the mean acidity was less than 0.25. During storage, the mean pH values of T3, T4 and T5 ice creams with microencapsulated *L. acidophilus* LA-5 were significantly higher than T1 and T2 ice creams, since microencapsulation slackens the metabolic activity of *L. acidophilus* LA-5. All treatments had significantly lower mean pH value than the control. The range of mean pH of control and all treatment ice creams during storage period of 30 days was 6.01 to 6.44.

Addition of microencapsulated *L. acidophilus* LA-5 and oligofructose significantly increased specific gravity of the ice cream mix. The highest mean

specific gravity in low fat synbiotic ice cream was due to addition of sugar and fat replacers (sucralose and whey protein, polydextrose, wheat dextrin). The fat content of all ice cream mixes except T5 were kept at ten per cent and that of T5 treatment ice cream mix was kept at 0.4 to 0.6 per cent to satisfy the legal standards of normal full fat and low fat ice cream respectively. The mean fat content of all the ice creams were similar to that of the ice cream mix without any marked reduction throughout the storage.

Addition of *L. acidophilus* LA-5 (free or encapsulated) with or without addition of oligofructose did not cause any effect on the overrun or whipping ability of the ice cream. Fat and sugar replacers decreased the overrun and whipping ability of T5 ice cream. During the storage period the mean meltdown time of all the ice creams were significantly lower ($P<0.01$) than T2 ice cream and significantly higher than T5 ice cream. Treatment T1 and T4 ice creams had mean meltdown time significantly higher ($P<0.01$) than control and T3 ice creams. Increased mean meltdown time of treatment ice creams was due to addition of oligofructose and low pH caused by addition of free *L. acidophilus* LA-5. The decrease in mean meltdown time of T5 was due to the addition of fat and sugar replacers. Increase in meltdown time of all ice creams was proportionate to the storage period. During the storage period, all treatment ice creams had significantly higher mean weight per litre ($P<0.01$) than that of control ice cream. Synbiotic ice creams T2 and T4 had significantly higher ($P<0.01$) mean weight per litre than that of probiotic ice creams T1 and T3 due to the addition of oligofructose. Low fat ice cream T5 had the highest mean weight per litre value among all treatment ice creams. The mean weight per litre values of control and all the treatment ice creams were above the recommended minimum level of 525 gram per litre according to BIS specification. The above findings also clearly indicated that addition of probiotic culture in microencapsulated form did not affect the major technological parameters in the ice cream production such as overrun, meltdown time, and whipping ability.

All the treatment mixes had comparable mean *L. acidophilus* count (ranging from 43.17 to 45.67 x 10^9 cfu/g) and comparable mean coliform count (33.83 to 30.50 cfu/g) among them before freezing. The mean values of probiotic count after freezing for T1, T2, T3, T4 and T5 mixes (expressed in 10^8 cfu/g) were 11.50 ± 0.56, 19.17 ± 0.70, 23.83 ± 1.70, 27.83 ± 0.75 and 25.50 ± 2.09 respectively. After freezing, there was a reduction of 1.50 log units, 1.37 log units, 1.35 log units, 1.25 log units and 1.28 log units for T1, T2, T3, T4 and T5 respectively in the probiotic count. From the point of freezing to the end of 30 days of the storage, the reduction of *L. acidophilus* LA-5 count in T1, T2, T3, T4 and T5 was 0.083, 0.055, 0.047, 0.029 and 0.0535 (expressed in log cycle) respectively. Thus there was no marked decrease in the probiotic count in all the treatment ice creams from the point of freezing to the end of 30 days of the storage. The overall survivability of *L. acidophilus* LA-5 in ice cream (from mix to the end of the storage period) for T1, T2, T3, T4 and T5 was (expressed in per cent log) 84.27, 86.50, 86.82, 87.65 and 87.47 respectively. After freezing and during storage T3, T4 and T5 treatment ice creams had significantly higher ($P<0.01$) mean *L. acidophilus* LA-5 count due to microencapsulation than that of T1 and T2 ice creams containing free *L. acidophilus* LA-5. The mean probiotic count of T2 ice cream was significantly higher ($P<0.01$) than that of T1 ice cream due to addition of oligofructose. Inspite of lowering the fat percentage in T5 ice cream, the survivability of *L. acidophilus* LA-5 was similar to that of T4 ice cream in which fat percentage was normal, presumably due to the protective action of whey protein, polydextrose and wheat dextrin present in the treatment T5.

During storage T1 and T2 ice creams showed significant reduction ($P<0.01$) in the mean coliform count when compared to all other ice creams. This may be due to the claimed antagonistic effect of *L. acidophilus* against coliforms in free state rather than in encapsulated state. The total coliform count of control and all treatment ice creams remained within legal limits. Overall sensory acceptance of control, T3 and T4 ice creams containing

microencapsulated *L. acidophilus* LA-5 was similar. Slight acidic or sour taste was observed for T1 and T2 ice creams due to addition of free *L. acidophilus* LA-5. Other sensory attributes like body, texture, appearance and colour was not affected by addition of *L. acidophilus* in either state (free or encapsulated) and with or without addition of oligofructose. The lowest sensory acceptance was observed in T5 low fat synbiotic ice cream. The cost of all the treatment ice creams are higher than the control but the dietary beneficial and therapeutic effects present in them, makes them a valuable functional food.

From the results of the experiment conducted, it can be concluded that probiotic ice cream with *L. acidophilus* LA-5 and synbiotic ice cream with addition of *L. acidophilus* LA-5 and oligofructose could be prepared without affecting any technological parameters. It is also conclusively proved that the microencapsulation of probiotic bacteria has tremendously improved the survivability of the organism during freezing and on storage. The consumer acceptability of the probiotic ice cream with microencapsulated organism was observed to be very high.

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* Originals not consulted.

Appendix

Appendix-1

Ice Cream Score Card

Write scores opposite the rating for perfect score. Check criticisms in the space opposite the defects noted and in the proper sample column.

Criticism	Sample No					
	1	2	3	4	5	6
Flavour System Range (1-10**)**						
No criticism						
Cooked Flavour						
Lack of sweetness						
Too Sweet						
Lack of Flavour						
Yogurt /probiotic flavour						
Acidic /sour						
Rancid & Oxidized						
Other						
Average*						
Body & Texture Range (1-5)						
No critism						
Crumbly						
Coarse						
Weak						
Gummy						
Fluffy						
Sandy						
Average*						
Colour & Appearance Range (1-5)						
No Critism						
Pale colour						
Non-uniform Colour						
Unnatural colour						
Average*						

Total Score(out 20) - Name of the judge -

Date - Signature -

* Average of scores if there is more than one defect

SURVIVABILITY OF MICROENCAPSULATED *Lactobacillus acidophilus* LA-5 IN SYNBIOTIC ICE CREAM

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ABSTRACT

An experiment was conducted to study the efficiency of microencapsulation to improve the survivability of *L. acidophilus* LA-5 along with the addition of oligofructose in the formulated synbiotic ice cream. The treatment mixes and ice creams were analyzed for various properties by using standard procedures and compared with the control. It was found that addition of *L. acidophilus* LA-5 either in free or microencapsulated state has not affected the acidity and pH of the ice cream mix. Microencapsulation of *L. acidophilus* LA-5 increased the specific gravity of the ice cream mix along with addition of oligofructose. Fat, probiotic and coliform counts of the all the treatment ice cream mix were similar to that of the control.

The fat content of all treatment ice creams were similar to that of their ice cream mix. Addition of free or encapsulated L. *acidophilus* LA-5 has not affected the overrun or whipping ability of ice cream. The pH of ice cream was significantly reduced by *L. acidophilus* LA-5 in free state rather than in microencapsulated state. Addition of oligofructose and low pH increased the meltdown time of ice cream. The weight per litre of ice cream increased significantly with addition of oligofructose than by addition of *L. acidophilus* LA-5 in both state. Fat and sugar replacers increased the specific gravity of ice cream mix, weight per litre of ice cream and reduced the whipping ability, overrun and meltdown time. Reduction in probiotic count of ice cream was more pronounced during freezing than hardening and storage. The overall probiotic count in ice cream with microencapsulated form of *L. acidophilus* LA-5 was significantly higher than the ice cream with free form of *L. acidophilus* LA-5. Low level of fat content has not affected the survivability of *L. acidophilus* LA-5 in low fat synbiotic ice cream. Overall sensory acceptance of ice cream with microencapsulated *L. acidophilus* LA-5 was similar to that of the control. Free form of *L. acidophilus* LA-5 caused slight acidic or sour flavour in the ice cream. Low fat synbiotic ice cream had lowest sensory acceptability. Cost of

production of production of synbiotic ice cream with microencapsulated *L. acidophilus* LA-5 was more than the control.

Addition of microencapsulated *L. acidophilus* LA-5 did not affect any of the technological parameters of the formulated synbiotic ice cream. Microencapsulation efficiently improved the survivability of *L. acidophilus* LA-5 in the ice cream and ascribed to it the status of a suitable functional food to deliver the recommended level of probiotics with very good sensory attributes to the consumer.