Effect of Inulin on the Survival of Lactic Acid and Probiotic Bacteria in Ice Cream

By

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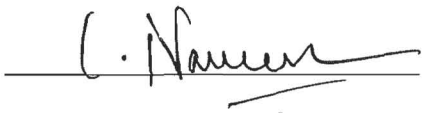

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Abstract

This study investigated the effect of inulin (0% control, 1.5% and 3.0% w/w) on the survival of lactic acid (LAB) and probiotic bacteria in ice cream. A mixed culture of probiotic bacteria was added into a standard formulation ice cream recipe at a level of 10^6 CFU/g prior to freezing the mix using a Lolla Musso Pola 5030 freezer. Inulin levels were tested to determine protective effects on bacterial survival. Ice cream samples were taken before and after freezing, and during storage under steady-state (-20˚C) and repeated thaw-freeze conditions (-20˚C/-5˚C) over 28-days to enumerate for bacterial survival. Yield stress test and viscosity analysis of ice cream samples were conducted at the end of storage period.

LAB and probiotic bacteria had no significant decrease in populations during the storage period. Addition of inulin to ice cream had no effect on bacterial survival at steady-state storage (-20˚C) or during thaw-freeze cycles over 28-days. The freezing phase had no destructive effect on bacterial populations. Difference in yield stress values between ice cream samples was not

statistically significant after 28-days storage period at (-20˚C). Addition of 3.0% inulin had a significant effect on the viscosity of ice cream samples. Inulin, when used at 1.5% and 3.0% had no significant effect (protective or destructive) on LAB and probiotic bacteria in ice cream.

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Chapter I: Introduction

Inulin is a carbohydrate-based fat replacer widely used in many products in the food industry including yogurt, ice cream and many types of salad dressings. In addition, recent studies (Akalin & Erisir, 2008; Haynes & Playne, 2002) have indicated the ability of inulin to increase the survival of probiotic bacteria in ice cream and yogurt. This observation is important because clinical experiments (Dunne et al., 1999; O'Sullivan, 2001) have documented the positive effect of probiotic bacteria on gut health, modulating blood cholesterol, demonstrating antitumor activities and enhancing immune response. With increased consumer awareness of the importance of including probiotics in their daily diet, food manufacturers are responding by introducing probiotics in commercially-sold packaged food.

Two microbial genera of bacterial origin are widely used as probiotics: *Lactobacillus* and *Bifidobacterium*. By definition probiotics food products must contain a sufficient quantity of live probiotics microorganisms (FAO, 2001). Health benefits acquired from consuming probiotics food products are not only correlated to the type of therapeutic effects induced by the probiotic bacteria, but also to their presence in sufficient numbers in the food carrier to stimulate positive results (Klaenhammer & Kullen, 1999). According to Magariños, Selaive, Costa, Flores, and Pizarro (2007), a minimum dose of 10^6 of active *Bifidobacterium*/g per serving of food is essential to promote health benefits.

Statement of the Problem

Bacterial survival rate in food has been shown to decrease when the food is exposed to repeated freeze-thaw fluctuations (Jay, Loessner & Golden, 2005). This effect has also been shown to occur in probiotic bacteria present in dairy products. Bacterial death during frozen storage of food products results in reduced probiotic load at the point of consumption.

Purpose of the Study

The goal of the study was to evaluate the effect of inulin on the survival of probiotic bacteria in ice cream during frozen storage under freeze-thaw conditions. Another objective of the study was to measure the change in textural properties in ice cream due to the addition of inulin.

The experiment took place during Spring 2011 at the research facilities within the Departments of Biology and Food and Nutritional Sciences at the University of Wisconsin-Stout.

Importance of the Study

Product or process development methodologies, including the addition of inulin to enhance the survival of probiotic bacteria in ice cream has significant commercial and health-based applications. In the commercial context, ice cream manufacturers can develop probiotic products to extend their product lines with healthier choices. The study benefits consumers by determining product development strategies that could enhance the survival of LAB in ice cream, thereby delivering the desired levels of LAB at the point of consumption.

Assumptions of the Study

The major assumptions of the study are:

1. The survival of a mixed culture of LAB strains is indicative of survival of probiotic bacteria in ice cream.

2. *Bifidobacterium lactis* will show growth when cultured on De Man, Rogosa and Sharpe media (MRS).

3. Freeze-thaw conditions simulated in the study are a representation of a worst-case commercial retailing scenario.

Objectives

The objectives of this study were to

1. Investigate the protective effect of inulin during the initial freezing phase of the ice cream preparation.

2. Investigate the correlation between the amount of inulin used in ice cream (0 %, 1.5% and 3.0%) and the count of probiotic bacteria during 28-days of storage at -20˚C.

3. Determine the effect of inulin content (0%, 1.5%, and 3.0% w/w) on the survival of probiotic bacteria under extended freeze-thaw conditions.

4. To analyze the effect of adding inulin on the textural structure (yield stress, viscosity) of ice cream.

Definition of Terms

Freeze-Thaw: A cyclical process of product storage under freezing and ambient temperature as a means of testing product stability and its ability to maintain its physical, textural and organoleptic characteristics during shelf life. It is important to take into consideration that minor temperature fluctuations during storage can induce thawing of liquids within the frozen food material. This freeze-thaw process may generate large ice crystals that can deteriorate the quality of the product and cause injury/death to live microbial culture subsiding within the food matrix (Labuza & Schmidl, 1985).

Inulin: A term applied to a wide range of naturally occurring polysaccharides from the class of fructans. Inulin is stored by plants typically in the roots as a form of energy. Inulin is not digested in the upper digestive tract of humans and hence may be considered as dietary fiber. Inulin has a low caloric value, which makes it a good fat and sugar replacement in processed

food. Inulin has been shown to enhance the bioavailability of calcium and magnesium and promote growth of intestinal bacteria (Niness, 1999).

Lactic Acid Bacteria: Bacteria that produce relatively large amounts of lactic acid by fermenting carbohydrates. The group is formed generally by the genera *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Lactobacillus* and *Streptococcus* (Ray, 1996).

Micro-encapsulation: Micro-encapsulation is a process of entrapping solid particles, droplets of liquids, and gases in thin polymer coats. The content is protected from the immediate environment conditions such as light air and moisture (Jackson & lee, 1991).

MRS Agar: Bacterial growth medium, invented by De Man, Rogosa and Sharpe**.** It contains 0.5% sodium acetate trihydrate, 0.1 % polysorbate 80, 0.02 % magnesium sulfate heptahydrate and 0.005 % manganese sulfate tetrahydrate which are considered growth promoting factors for *Lactobacilli*. The medium is also composed of : 1.0 % peptone, 0.8 % meat extract, 0.4 % Yeast extract, 2.0 % Glucose (energy source), 0.2 % Triammonium citrate, 1.0 % Agar and 0.2 % Dipotassium hydrogen phosphate which is a buffer agent that keeps a low pH to eliminate many competing bacteria. The medium when plated is of a light brown color (De Man, Rogosa, & Sharpe, 1960).

Probiotics: Live microorganisms, which when administered in adequate amounts; confer a health benefit on the host (FAO, 2001).

Limitations

Due to time constraints, the study of ice cream samples and enumeration of *Lactobacillus* bacteria was conducted over a period of 28-days. This duration is typically not representative of commercial ice cream shelf-life. Typically, the shelf life and storage period of ice cream varies from a few months to one year depending on the formulation, process and storage conditions

(Whelan, Regand, Vega, Kerry, & Goff, 2008). Therefore, subsequent studies with a longer time frame should be considered to deduce practical conclusions and recommendations that can be valid for commercial settings.

Chapter II: Literature Review

In this chapter, the survival rate of microorganisms, especially probiotics, and the effect of freeze-thaw conditions on the survival of LAB in ice cream are introduced. In this review, the role of inulin as a fat replacer and its effect on the probiotic culture survival in low-fat-probiotic ice cream are discussed.

Lactic Acid Bacteria (LAB)

In the food industry in general and in the dairy industry specifically, microorganisms are of great importance. One specific group of relevance is the lactic acid bacteria (LAB). LAB are characterized by their capacity to ferment lactose to lactic acid and they are naturally present in raw milk (Salminem, Wright, & Ouwehand, 2004). LAB are used as starter cultures in the production of fermented dairy products such as yogurt, cheese, frozen yogurt/ice cream, and buttermilk. They are also used in fermented non-dairy products such as fermented meat, bakery products such as sourdough bread, malo-lactic fermentations in wine, and fermented vegetable products such as dill pickles and sauerkraut (Marth & Steele, 1998). Therefore special attention is given to the selection and balancing of LAB to obtain food and dairy products with desirable texture, flavor and nutritional value characteristics. Furthermore, organoleptic quality of the final dairy product is directly related to the initial composition of the starter culture and milk flora (Ahmed & Kanwal, 2004).

LAB contains microorganisms from various genera that produce lactic acids as the primary or secondary end product of fermentation. They are classified mainly as the *Lactobacillales*, which is composed of important genera such as *Lactobacillus, Streptocococcus, Leuconostoc, Lactococcus*, and *Pediococcus* (Salminem, Wright, & Ouwehand, 2004). Common characteristics of these genera are being gram-positive, non-spore forming, nonpigmented and

unable to produce iron-containing porphyrin compounds (catalase and cytochrom); they grow anaerobicaly but are also aerotolerant; and they have obligated fermentation of sugar with lactic acid as a major end product (Marth & Steele, 1998).

The present research study focused on the genus *Lactobacillus* and *Bifidobacterium* because of their established probiotic effect in human health (Gilliland & Walker, 1989; Havenaar et al., 1992; Jardine, 2009). Microorganisms in the *Lactobacillus* genus are gram positive and include a large number of species characterized by their rod shapes and inability to produce spores. They are facultative anaerobes and produce lactic acid by growing on media that contain glucose (Ray, 1996). Few other species of this genus are heterolactic fermentors and can produce a mixture of lactic acid, ethanol, acetic acid and carbone dioxide by fermenting a wide range of sugars such as lactose, sucrose, fructose and galactose. Generally, the optimal growth temperature of *Lactobacillus* is between 25 and 40˚C. LAB are ubiquitous and are found in plants, vegetables, seeds and milk (Marth & Steele, 1998). Some of the most prominent species of this genus are *Lab. acidophilus, Lab. kefir*, *Lab. fermetatum*, *Lab. brevis* and *Lab. casei,* which are broadly considered a beneficial intestinal and digestive tract micro flora (Ray, 1996).

The genus *Bifidobacterium* is similar to the *Lactobacillus* since they are mostly of rod-shaped with different sizes, Gram-positive and characterized by their inability to produce spores. Unlike LAB, Bifidobacteria are mostly anaerobes with the exceptions of a few species that can tolerate O_2 in the presence of CO_2 (Ahmed & Kanwal, 2004). Optimal growth temperature for *Bifidobacteria* is between 37 to 41˚C, and they are highly sensitive to pH above 8.0 or below 4.5 (Salminem, Wright, & Ouwehand, 2004). Many species of *Bifidobacteria* are isolated from the feces of human and animals. They can appear in the excretions of human babies after two to

three days and appear in high numbers in breast-fed babies. They include *Bif. Bifidum*, *Bif. Longum*, *Bif. Infantis,* and *Bif. Adolescentis* (Ray, 1996).

Survival of lactic acid bacteria in various food and dairy products.

At the beginning of the twentieth century, the need for large-scale industrial production of cheese, ice cream and fermented milk products marked the national and international market to satisfy consumer's demands. These innovations came at time when the processing technology of dairy products witnessed a revolution. This was achieved by the introduction of mechanization and short processing time, leading to enormous milk quantity being processed on a daily basis with a variety of products with different flavors, texture, colors and nutritional values (Tamime, 2005). This in turn, led to a great demand for starter cultures and the study of their stability, activity and resistance. These studies metamorphosed into a science able to predict the behavior of each strain and specie in specific media or functional food (Salminen & Wright, 1998).

The survival of LAB in milk and milk products is a function of many variables, from the milk composition which could vary by region where the milk is produced to the feed given to cows (Ahmed & Kanwal, 2004). Furthermore, milk and dairy products are generally not an optimal growth medium for LAB. Different strains have different nutritional requirements, especially with regard to vitamins and nonprotein nitrogen (NPN). For instance, *Lactobacilli* thrive in media where there is calcium pantothenate, niacin and nitroflavine whereas *Lactococci* needs media rich in niacin, pantothenic acid, pyridoxine and biothin (Tamime, 2005). Additionally, milk contains inhibitory compounds that affect the growth of LAB. The two major inhibitory compounds are antibiotics residues and bacteriophages; antibiotics were introduced in the late 1940's to treat mastitis in dairy cows. To this day, mastitis remains one of the most

serious diseases affecting cows, often resulting in economic loss, intestinal disorder, and bacterial resistance (Tamime, 2005). Several approaches have been introduced to dairy production to reduce the level of antibiotic residues in milk. These are based on good agriculture and hygiene practices that allow withdrawal periods, impose strict penalties on dairy farmers and tighten monitoring and testing systems by the governing agencies. The second major inhibitory compounds are bacteriophage viruses that infect bacterial cells and use their cell enzymes for propagation (Durmaz & Klaenhammer, 1995; Marth &Steele, 1998). The bacteriophage viruses inhibit the growth of LAB by causing the leakage of the bacterial cell's content out of the membrane. Therefore, numerous research studies concentrated on the classification of bacteriophages and their mechanism of action based on the host strain, DNA/ DNA hybridization and morphology. Table 1 summarizes a classification example of *Lactococcal* bacteriophages (Durmaz & Klaenhammer, 1995).

Table 1

Classification of Lactococcal bacteriophages by DNA/DNA hybridization (Durmaz & Klaenhammer, 1995)

DNA homology group	Percent of phages in the group	Head morphology	Gene size (kb)
I	29	Prolate	19-22
II	21	Small isometric	30-40
III	48	Small isometric	30-35
IV	1	Large isometric	53
V	1	Large isometric	134

In general, LAB live culture in fermented milk starts to decrease immediately after the end of the growth cycle (Salminen & Wright, 1998). This follows an exponential kinetic death curve expressed by the formula: $X_t = X_0 \exp^{(-kt)}$

X_t = Cell surviving after time t

X_0= Initial bacterial load at t_0

k= Specific death rate (strain and environment dependent)

t = time

Storage temperatures play an important role in the survival of the bacteria. When stored at room temperature, *Lactobacillus casei* in fermented milk products can decrease by two log-cycles in a period of two weeks (Magariňos et al., 2007). *Lactobacillus acidophilus* and *Bifidobacterium bifidum* are more thermo-sensitive strains and can incur higher decrease in numbers at the same temperature. However, low storage temperatures can extend the viability of the LAB and preserve the microbial load (Salminen & Wright, 1998).

Survival of lactic acid bacteria in ice cream.

In the United States, probiotics could fit into any of the following categories: conventional foods, dietary supplements, food for special dietary use and medicinal foods (Tamime, 2005). However, only a small number of conventional foods in the US contain probiotics, and they mainly constitute dairy product such as yogurt, milk and cultured milk (Jardine, 2009). For yogurt, the standards set by the Food and Drug Administration (FDA) require the use of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* with no levels of microbial load being a factor in regulatory enforcement. However, the National Yogurt Association (NYA) has introduced a voluntary 'Live Active Culture' seal for products that contain live cultures. The seal calls for a minimum of live bacterial load to be delivered by

each serving of probiotic food. This requires refrigerated yogurt to contain 10^8 CFU/g and frozen yogurt 10^7 CFU/g at the time of manufacture (Tamime, 2005).

Monitoring and characterizing the survival of the probiotic bacteria in ice cream after freezing is of high importance. This will allow the acquisition of the probiotic food label and the sales and advertisement certification for the product. Furthermore, knowing that the freezing step in the process of making ice cream could cause a reduction in the concentration of the bacterial load indicates that quantification of this reduction is important to maintain the bacterial load within the food industry standards.

In a study conducted at Austral University of Chile (Magariños et al., 2007) on the viability of probiotics microorganism: *Lactobacillus acidophilus* and *Bifidobacterium lactis* in ice cream, it was demonstrated that when these probiotics were inoculated into ice cream and stored at -25˚C for 60 days, the rate of survival was 87% for *L. acidophilus* at a final concentration of $2x10^6$ CFU g^{-1}. The *B. lactis* survival rate was 90% with a concentration of $9x10^6$ CFU g^{-1}. When both microorganisms were inoculated together, the survival rate was 86%. Furthermore, a substantial loss occurred at the freezing step (Figure 1), quantified by more than two logs of bacterial death (Magariños et al., 2007). The ice cream mix formula consisted of 8.0% fat, 11.2% nonfat milk solids, 14.0% sugar and 0.4% stabilizer-emulsifier (Magariños et al., 2007).

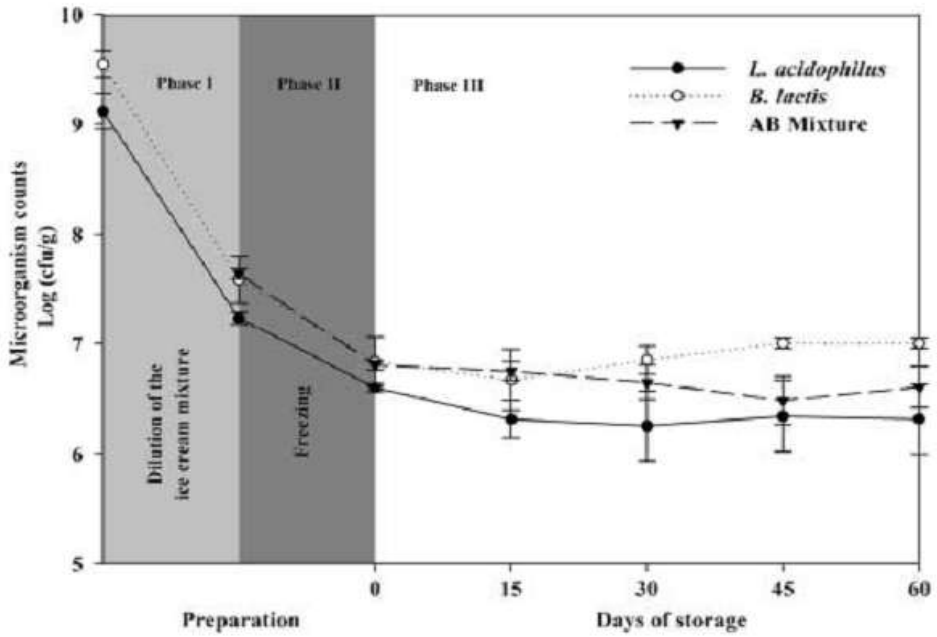

Figure 1. Effects of dilution, freezing, and storage of ice cream at -25°C on the levels of *Lactobacillus acidophilus*, *Bifidobacterium lactis*, and the combined mixture of the two probiotic microorganisms (Magariños et al., 2007).

Inulin

Inulin and oligofructose are beta (2-1) fructans found naturally in abundance within a wide variety of plants, bacteria and some fungi. They are the second most occurring non-structural natural polysaccharides after starch. Fundamentally, plants that contain inulin can be categorized in two groups; the Liliales and the Compositae. Table 2 summarizes a list of some plants that could be used in the daily diet of humans and contain inulin (Jardine, 2009).

Table 2

Inulin content in plants used for human consumption (Jardine, 2009)

Source	Edible part	Inulin content (% of fresh weight)
Onion	Bulb	2-6
Jerusalem artichoke	Tuber	16-20
Garlic	Bulb	9-16
Artichoke	Leaves-heart	3-10
Banana	Fruit	0.3-0.7
Rye	Cereal	0.5-1.0
Barley	Cereal	0.5-1.5
Camas	Bulb	12-22
Wheat	Cereal	1-4
Chicory	Root	15-20
Yacon	Root	3-19
Dandelion	Leaves	12-15

Chemistry.

Chemically, inulin is a polydisperse beta (2-1) fructan composed of a mixture of oligo- and polysaccharides (De Leenher & Hoebregs, 1994). They are mostly formed of linear chains of fructose (Figure 2) structured as GF_n, where G= glucosyl unit, F= fructosyl unit, and n= the number of fructosyl units linked to each other (Figure 2). For instance, in chicory inulin the number of fructose units that are linked together is of the order of 2 to 65, with a degree of

polymerization (DP) ranging from 10 to 12 (Jardine, 2009). Physical removal of the lower DP fraction allows the obtainment of long-chain inulin (high performance inulin) that is used for textural improvement and fat replacement. Oligofructose obtained from inulin contains GF_n chains ranging from 2 to 6 (De Leenher & Hoebregs, 1994).

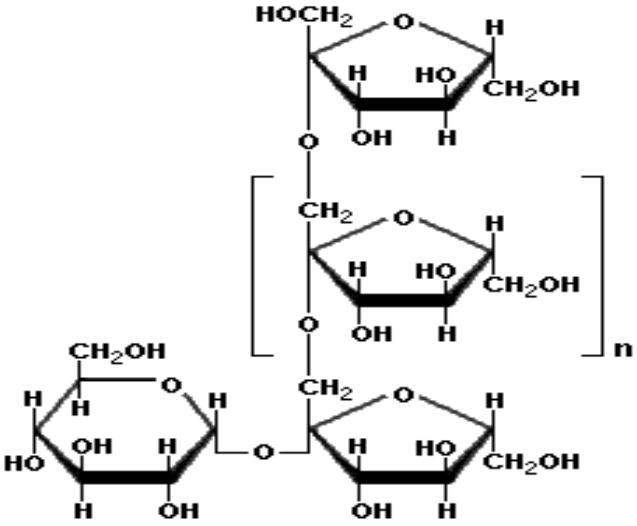

Figure 2. Chemical structure of inulin (n= number of fructosyl units) (Zamora, 2005)

The organoleptic characteristics of inulin manifest as a bland neutral taste and no off-flavor or aftertaste (De Leenher & Hoebregs, 1994). Standard inulin is 10% as sweet as sugar while the high performance inulin does not display any sweetness. High performance inulin is a low soluble substance in water (10% at room temperature) and presents low viscosity (< 2 mPa.s for a 5% solution in water) (Jardine, 2009).

Inulin as a fat replacer in food and frozen desserts.

Inulin is considered to have remarkable functional properties such as the ability to act as a fat or sugar replacer without adversely affecting flavor (Jardine, 2009). When mixed with water or other liquid solutions, inulin forms a pasty gel network with creamy structure and a

short, spreadable texture that allows it to be easily incorporated into food to replace up to 100% of fat (Franck, 1993). When inulin is used as a fat replacer, it allows the manufacturer of dairy desserts such as yogurt and ice cream to offer healthy food product choices. They can provide low fat content, organoleptic properties, and rheological characteristics similar to as regular yogurt or ice cream. This is due in part to the ability of inulin to stabilize the structure of the emulsion phase, which creates an improved 'creaminess' and mouth-feel (Franck, 1993).

In a research study conducted by Nagar, Clowes, Tudorica, Kuri and Brennan (2002), the researchers found that the addition of inulin to yog-ice cream was shown to increase the viscosity of the dairy dessert and improve the hardness of the resulting product. To run the rheological test, the authors used 5 different samples: high fat, low fat, 5% inulin, 7% inulin and 9% inulin. The research pointed out the close relationship between the polysaccharide (inulin) concentration used in the yog-ice cream and the improvement of its melting properties. The addition of inulin to the yog-ice cream mix resulted in better consistency index than the addition of fat only (Figure 3). The percentage of inulin incorporated to the yog-ice cream mix correlates to the overall consistency index. At higher levels of inulin in the product formulation, a higher consistency index was achieved.

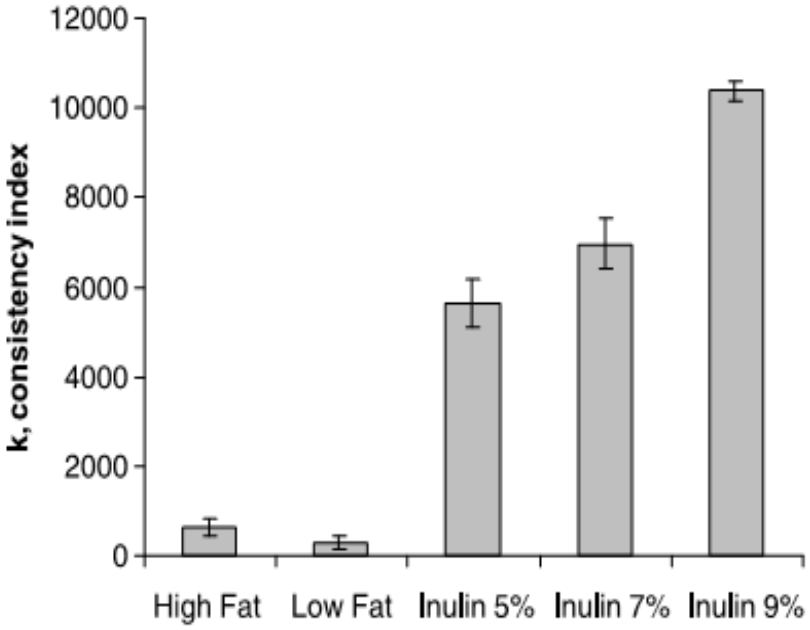

Figure 3. Consistency index of yog-ice cream mixes as determined using Brookfield Rheometer. Results derived from Power Law model (Pr = 0.91; means of triplicates ± SD) (Nagar et al., 2002).

The above results are significant when considering the production of fat free yog-ice cream (Figure 3). The substitution of fat by inulin can play the same role in forming the protein-fat matrix that gives the product its cohesiveness and yields a uniform inulin-liquid emulsion. This may be due to the hygroscopic properties of inulin and its ability to bind to water and form a gel-like network with high consistency index (Franck, 1993).

In Figure 4, the effect of inulin on the melting properties of ice cream is shown. Samples with high inulin content demonstrated a low percentage of meltdown in a 60-minute period. Thus, the increase in viscosity appears to be directly correlated to the melting properties of ice cream. This effect of increased inulin concentration on the melting rates is due to the liquid biding characteristic of inulin. This property of inulin confers the ability to stabilize and enhance the formation of cohesive network of gel-matrix that results in more stable ice cream (Nagar et al., 2002).

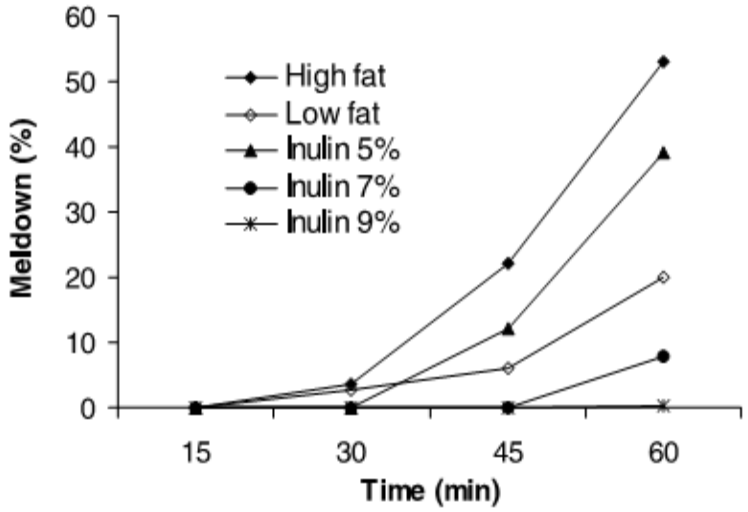

Figure 4. Meltdown characteristics of yog-ice cream expressed as percentage of yog-ice cream melted over 60 min (values represent means of duplicates) (Nagar et al., 2002).

Effect of Freeze-Thaw Conditions on the Survival of Lactic Acid Bacteria

The composition and characteristics of the ice cream can provide some challenges to the survival of LAB (Whelan et al., 2008). The environment in ice cream is similar to that of a frozen foam with a continuous phase constituted by viscous syrup and a suspended phase made from air bubbles, colloidal substances (casein and stabilizing gums) and ice crystal (Tamime, 2005). Lactic acid bacteria are suspended in the continuous phase and are affected by the pH, osmotic pressure, high salt concentrations and physical forces exercised by the ice crystals (Marth &Steele, 1998).

To study the effect of freezing on the survival of LAB, it is important to know that microorganisms are unable to grow at freezing temperatures and that freezing itself is considered a preservation method for certain foods (Marth &Steele, 1998). Furthermore, there is a sudden mortality immediately after freezing with a species and strain dependent severity (Magariňos et al., 2007). The surviving cells will enter a gradual death curve during storage that is also strain and species dependent. One important factor to consider is that temperatures just below the

freezing point (-5˚C) are far more lethal than lower temperatures (In the order of -20˚C). This is explained by the physical proprieties of the media at the phase below the glass transition curve (Salminen & Wright, 1998; Whelan et al., 2008). In this phase and due to low temperatures, there is no liquid movement, and large ice crystal formation is inhibited. Moreover, gram-positive bacteria rank above average on the survival scale when compared to vegetative cells of molds, yeast and gram-negative bacteria (Marth &Steele, 1998).

Effect of freeze-thaw on the survival of *Lactobacillus sp*.

Considering what occurs when cells freeze is important to understand the effect of freeze-thaw patterns on the lactic acid bacteria survival. When a temperature reaches critically low levels, free water freezes by forming ice crystals. The growth of ice crystals occurs by accretion (Jay et al., 2005). In slow freezing, ice crystals are large and extracellular while in fast freezing (Figure 5), ice crystals are small and intracellular (Whelan et al., 2008). The formation of ice crystals depletes the viable liquid from the cells and dehydrates them. This results in an increased viscosity, the loss of cytoplasmic gases such as O_2 and CO_2, pH change, change in electrolytes concentration and denaturation of cell protein (Marth &Steele, 1998). Cell freezing is a complex mechanism and the major factors contributing to cell death by freezing are the solute concentration and the ice crystal formation (Tamime, 2005; Whelan et al., 2008).

The thawing process is equally important, if not more detrimental, to the survival of *lactobacillus* bacteria. Repeated freezing and thawing will destroys the microorganism's membrane (Marth &Steele, 1998). The faster the thaw, the smaller is the damage incurred; this is due to the complicated process toward restoration of cell viability by reversing the steps above. The thawing is a slower process than freezing when conducted under comparable temperatures (Jay et al., 2005). The maximum temperature differential permissible under thawing is smaller

than that of freezing. Time-temperature patterns during thawing are different from patterns during freezing since, in thawing, temperatures rise rapidly to a near melting point and remain there for the rest of the process. This pattern permits chemical reactions, recrystallization and even microbial growth to occur when thawing is extremely slow (Marth &Steele, 1998; Jay et al., 2005).

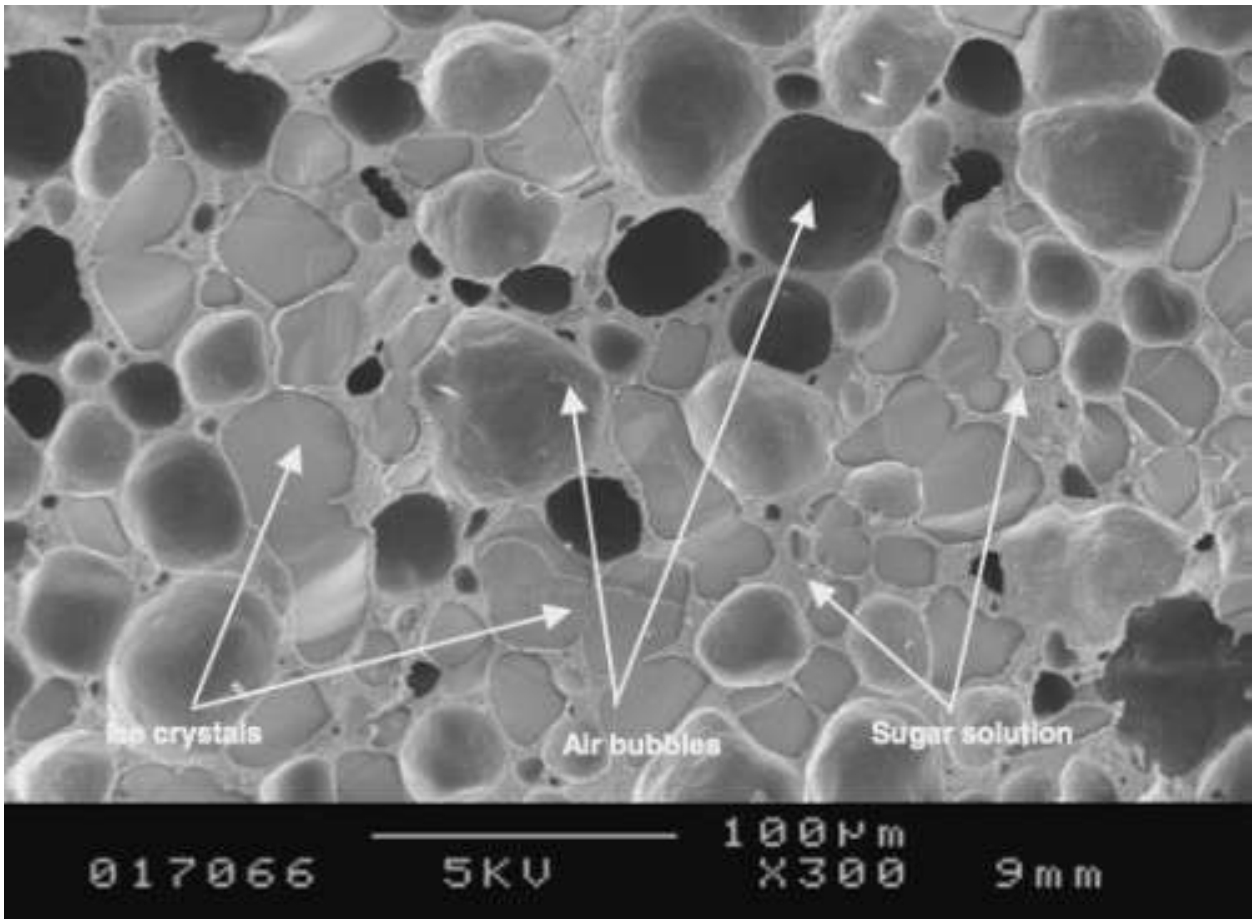

Figure 5. Photo showing the formation of ice crystals in ice cream (Clarke, 2003)

Use of inulin as a protective agent for the survival of lactic acid bacteria in food.

Ice cream and frozen desserts have the potential to be carriers of probiotic bacteria. Special consideration needs to be given to the freeze stress endured by the live bacteria during preparation phase and throughout storage periods (Tamime, 2005). Generally, two methods of

delivery are used: direct blending by mixing the probiotics cells and ice cream mix prior to the freezing phase and fermentation (Akalin & Erisir, 2008). Frozen yogurt is produced when the starter culture is allowed to ferment the ice cream mix before blending and freezing.

Probiotics used in ice cream are usually a combination of *Lactobacilli* and *Bifidobateria* due to their partial resistance to the freezing steps and storage period (Salminen & Wright, 1998). Kebary, Hamed, Salem and Yousef (2004) demonstrated the maintenance of 10^7 CFU/g of *Lactobacillus johnsonii* La1 for 10 weeks in ice cream. In another study, Haynes and Playne (2002) where able to keep a combination of *B. longum* and *B. infantis* for 52 weeks in stored ice cream at the same count levels (10^7 CFU/g). The ability of probiotics to survive in different freezing products is also strain dependent (Salminen & Wright, 1998).

Besides the known role of inulin as a fat replacer in different food product, few studies demonstrated the ability of inulin to increase the survival of probiotic culture in ice cream and yogurt (Akalin & Erisir, 2008; Franck, 1993; Magariňos et al., 2007). This is an important finding to extend the health benefits of the probiotics to ice cream, especially when these microorganisms need to be consumed in sufficient quantities to create the desired health benefits (Havenaar et al., 1992).

In a research study that was conducted by Akalin and Erisir (2008), supplementation of inulin or oligofructose showed a significant effect on the rheological characteristics and the survival of *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* Bb-12 in low-fat ice cream when stored at -18˚C for 90 days. The addition of inulin significantly increased the viscosity, overrun, and improved the melting properties and texture of ice cream. The major result of the study was that the addition of inulin to ice cream also improved the viability of *L. acidophilus* and *B. animalis* significantly (Table 3). Even if the total viable count of both

bacteria decreased throughout the storage period, a minimum of 10^6 CFU/g concentrations of *L. acidophilus* La-5 *and B. animalis* Bb12 was achieved by the addition of inulin (Akalin & Erisir, 2008).

Table 3

Viable counts of L. acidophilus La-5 and B. animalis Bb12 (mean ± SD, n = 3) in ice cream during storage (log CFU/g) (Akalin & Erisir, 2008).

Ice cream	1st day	30th day	60th day	90th day	Mean of storage
L. acidophilus La-5					
P	5.98 ± 0.25^{aB}	5.53 ± 0.18^{aAB}	5.02 ± 0.48^{aA}	5.13 ± 0.28^{aA}	5.41 ± 0.48^{a}
PO	6.21 ± 0.02^{aB}	5.77 ± 0.11^{bA}	5.79 ± 0.15^{bA}	5.70 ± 0.10^{bA}	5.87 ± 0.23^{b}
PI	6.00 ± 0.09^{aB}	5.47 ± 0.14^{aA}	5.24 ± 0.10^{aA}	5.12 ± 0.46^{aA}	5.46 ± 0.41^{a}
B. animalis Bb12					
P	6.27 ± 0.19^{bB}	5.97 ± 0.07^{bA}	5.93 ± 0.26^{bA}	5.94 ± 0.20^{abA}	6.03 ± 0.23^{b}
PO	6.60 ± 0.20^{cB}	6.40 ± 0.17^{cAB}	6.45 ± 0.28^{cAB}	6.25 ± 0.11^{bA}	6.43 ± 0.22^{c}
PI	5.96 ± 0.13^{aB}	5.36 ± 0.35^{aA}	5.51 ± 0.19^{aAB}	5.47 ± 0.55^{aAB}	5.57 ± 0.39^{a}

P = probiotic ice cream, PO = probiotic ice cream with oligofructose, PI = probiotic ice cream with inulin.
a,b,c Means with different letters in the same column are different ($P < .05$).
A–C Means in the same row with different superscripts are significantly different ($P < .05$).

Chapter III: Methodology

Culture Selection and Inoculation

The probiotic culture was obtained from "Dairy Connection Inc" (Madison, Wisconsin, USA). The freeze-dried culture was stored in airtight containers and kept at -20˚C throughout the experimentation period. The cultures contained a mixture of: *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium lactis*. The culture was used in the study because of its prominent application in the commercial production of yogurt and frozen yogurt.

Lactobacillus acidophilus and *Bifidobacterium lactis* were the probiotic bacteria present in the mixed culture used in the study (Jardine, 2009; Tamime, 2005). A viable count of 10^9 CFU/g of total bacteria was present in the culture mix and was verified by enumeration on MRS agar (Difco, Franklin Lakes, USA). Two grams (2g) of the viable culture was added to 40ml of raw milk and set at room temperature (25˚C) for sixty minutes. Two milliliters of this inoculum was added to 1litre of the ice cream mix.

Instrumentation and Ice Cream Preparation

For ice cream preparation, a Lolla Musso Pola scraped-surface freezer (described in Table 4), with a capacity of two quarts and a draw temperature of -10°C, was used. The ice cream mix was prepared with a Stephan mixer (UMC5 EL, 1989, Germany) linked to a hot water circulator bath (F12-ED, Julabo, USA) with a temperature set at 85˚C and a rotation speed of 7000rpm to blend the ingredients and to pasteurize the mixture.

Table 4

Lolla Musso Pola 5030 ice cream maker characteristics

Instrument characteristic	Specification
Model No	5030
Type of machine	Electric
Capacity	2-qt
Dimensions	20 x 14 x 12.25-in
Weight	72.5 lbs
Volts/Watts	300 watts/110 Volts
Origin	Italy
Material	Stainless Steel

Whole milk and cream were obtained from the local grocery store and stored in the refrigerator at 3.6˚C. Simple granulated table sugar was used as sucrose. Inulin and stabilizer were obtained from Cargill, Inc (Minneapolis, MN). The stabilizer was a hydrocolloid composed of alginates, carrageenans, guar gum, locust bean gum, pectins, xanthan gum, and scleroglucan. Table 5 shows the ingredients used to prepare the batches of ice cream mix for this experiment. After weighing the ingredients, duplicate batches were prepared by mixing in a Stephan mixer at 85˚C and 7000rpm rotation for 15 minutes. The ice cream mixes were left to cool at (25˚C) in 1litre glass containers for 45 minutes before they were placed in the refrigerator at 3.6˚C.

Table 5

Ingredients formulation used in preparing ice cream mix treatments

Ingredient	Control %	Control g/l	1.5 Inulin %	1.5 Inulin g/l	3 Inulin %	3 Inulin g/l
Cream	27.19	271.91	27.41	274.09	27.63	276.28
Whole milk	52.65	526.49	50.78	507.77	48.91	489.05
NFDM	4.86	48.60	5.01	50.14	5.17	51.67
Sucrose	15	150	15	150	15	150
Inulin	0	0	1.5	15	3	30
Stabilizer	0.3	3	0.3	3	0.3	3
Total	100%	1000g	100%	1000g	100%	1000g

In the freezing step, 2ml of the bacterial inoculum was added to 1 liter of duplicate ice cream mixes. Inoculated ice cream mixes were frozen to -10˚C using the Lolla Musso Pola 5030 ice cream maker (Figure 6). Samples were taken before and after freezing to -10 ˚C to analyze the effect of the initial freezing phase on the survival of the added microorganisms in ice cream. Figure 9 shows a flowchart for ice cream preparation.

To determine the effect of inulin on bacteria under steady-frozen conditions, ice-cream treatments containing inulin (0% inulin, 1.5% inulin, and 3.0% inulin w/w) were stored under steady-state conditions in a -20˚C freezer. The experimental storage period was 28-days with enumerations of the bacterial load conducted on 0, 3, 6, 10, 14, 21, and 28-days.

To determine the effect of inulin on bacterial survival during thaw-freeze conditions, one liter batches of treatment ice creams (0% inulin, 1.5% inulin, and 3.0% inulin w/w) were subjected to a thaw-freeze cycles (temperature increase from -20˚C to -5˚C over a 2 hour period in a refrigerator maintained at 3.6 ˚C followed by returning the samples back to -20˚C). The thaw-freeze cycles were repeated every three days for the 28-days storage period. Lactic acid bacterial enumeration was conducted on 0, 3, 6, 10, 14, 21, and 28-days. Methodology for bacterial enumeration is discussed in the next paragraph.

Figure 6. Lolla Musso Pola 5030 ice cream maker

Live Culture Enumeration of Lactic Acid Bacteria

Enumeration of bacteria in the ice cream treatments were done by aseptically sampling ice cream on 0, 3, 6, 10, 14, 21 and 28-days of frozen storage. One milliliter (1mL) of ice cream sample was mixed in 9 ml solution of buffered peptone water (BPW, Difco). The samples were serially diluted in BPW, plated on MRS (de Man, Rogosa and Sharpe, Difco) agar and the plates were incubated in an environmental chamber (Steridium, i170, USA) for 48 hours at 37˚C. Colonies on plates were counted using a Dark Field Quebec colony counter to determine the bacterial load in ice cream samples during storage (Figure 8).

Yield Stress Test and Viscosity Analysis of Ice Cream

Textural and rheological measurements were conducted on ice cream samples that had been tempered overnight at -16°C for the yield stress test and at -6°C for the viscosity analysis. Apparent viscosity, yield stress coefficients and flow behavior indices were evaluated after 28-days of storage at (-20˚C) using a Brookfield DV-III Ultra Rheometer (Brookfield Engineering Laboratories, Stoughton, Massachusetts, USA) (Figure 7). Samples were tested in duplicate at room temperature with spindle # 3 for stress strain test and spindle # 2 for viscosity. The shear stress and apparent viscosity of each sample was measured at rotation speeds of 200 rpm. Apparent viscosity of each sample was recorded at 100 rpm. Plots of shear stress versus shear rate and apparent viscosity were created from each flow curve as described by Akalin, Karagözlü, and Ünal (2008).

Figure 7. Brookfield DV-III Ultra Rheometer

Data Analysis

Statistical analysis to determine the effect of inulin addition on the dependent variables was done by two-way Analysis of Variance (ANOVA) conducted on log survival data of bacterial numbers, stress-strain data and viscosity values using the SPSS 17 statistical software (IBM, New York, USA).

Limitations

During the storage period, temperatures fluctuations may have occurred due to the usage of commercial refrigerators and freezer.

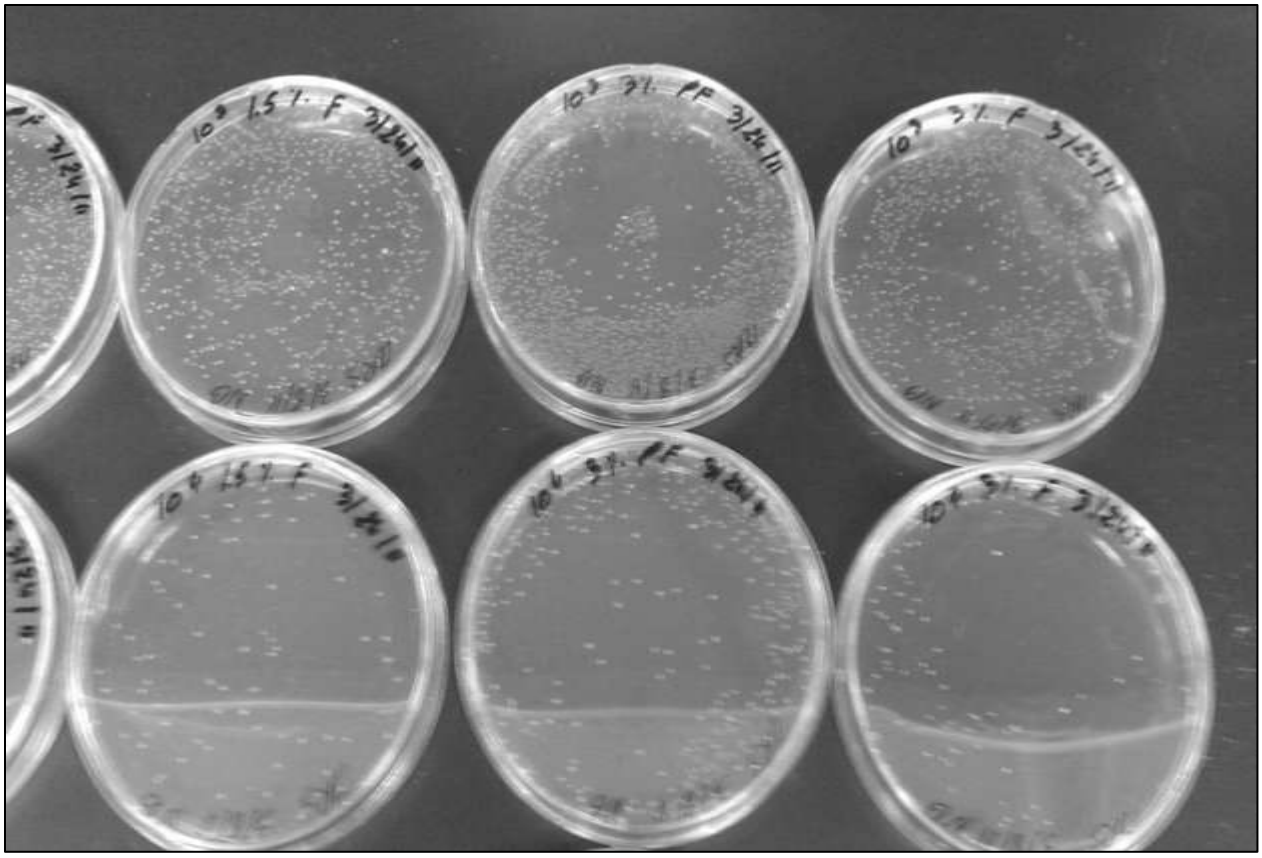

Figure 8. Serially diluted plates (left to right) of MRS Agar showing lactic acid bacteria in ice cream when stored at -20˚C for 28-days.

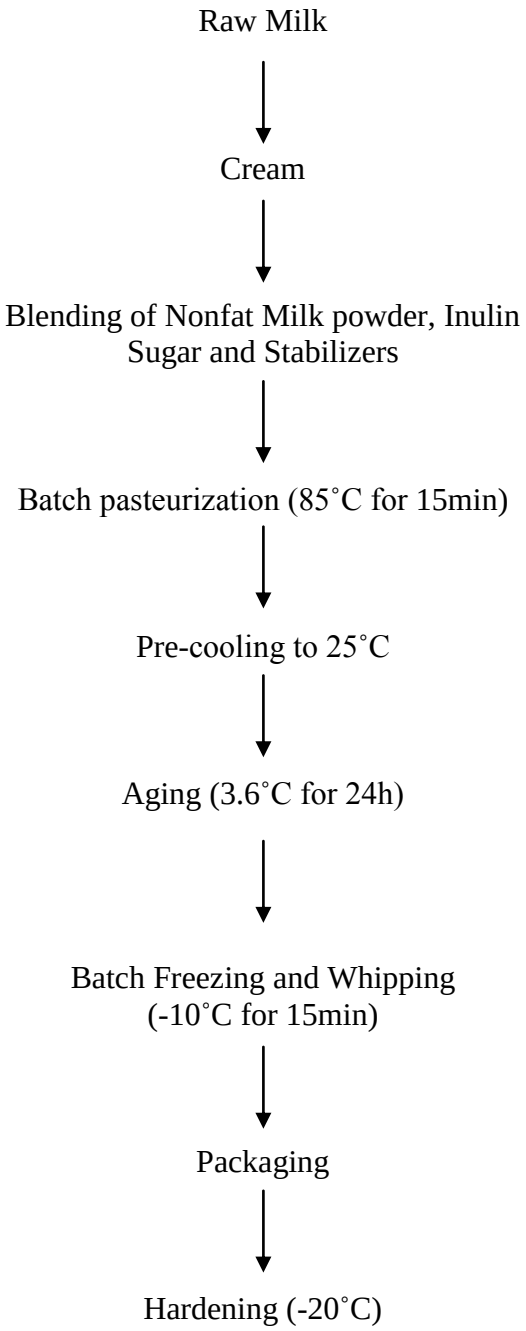

Figure 9. Flowchart for ice cream preparation

Chapter IV: Results and Discussion

Effect of Inulin on the Survival of Inoculated Bacteria in Ice Cream During the Initial Freezing Phase (from Liquid Ice Cream Mix at 3.6˚C to Frozen Ice Cream at -10˚C)

The effect of inulin concentration (0% control, 1.5% and 3.0% w/w) on bacterial load before and after the freezing phase is shown in Figure10. Results indicate that the mean population level of LAB and the probiotic bacteria in the ice cream samples after the freezing phase were in the ranges of 5.9 to 6.5 log CFU/g. This observation is consistent with the study conducted by Akalin and Erisir (2008) where the authors observed a mean population level of probiotic bacteria in ice cream samples after the freezing phase ranging from 5.9 to 6.6 log CFU/g. For the control treatment, (0% inulin), the bacterial numbers during pre-freeze and post-freeze was 6 log CFU/g and 6.1 log CFU/g, respectively. Similarly, for 1.5% of inulin in the ice cream mix, the bacterial count was 6.1 log CFU/g at pre-freeze and 6.0 at post-freeze. For the 3.0% inulin concentration, a drop from 6.5 log CFU/g at pre-freeze to 5.9 log CFU/g at post-freeze was recorded. The freezing phase had no significant effect ($F(1;5) = 1.24$, $p > .05$) on bacterial numbers in ice cream mix with varying inulin levels. This finding contradicts the findings of Magariños et al. (2007), where a two log reduction in bacterial numbers were observed in the initial freezing phase under experimental conditions similar to the current study.

This finding may be explained due to the differences in the bacterial genera and species among the two studies. In the current study, the bacterial culture contained a combination of microbial genera and species including *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium lactis*, whereas the study by Magariños et al. (2007) used a bacterial culture mix composed of only *Lactobacillus acidophilus* and *Bifidobacterium lactis*.

Further comparative studies are needed to measure the synergistic effect of combining multiple strains of LAB and probiotics in ice cream. Single strain samples in addition to the mixed culture need to be included in the experimental design of future studies to verify the microbial load and behavior of single strains during the freezing phase.

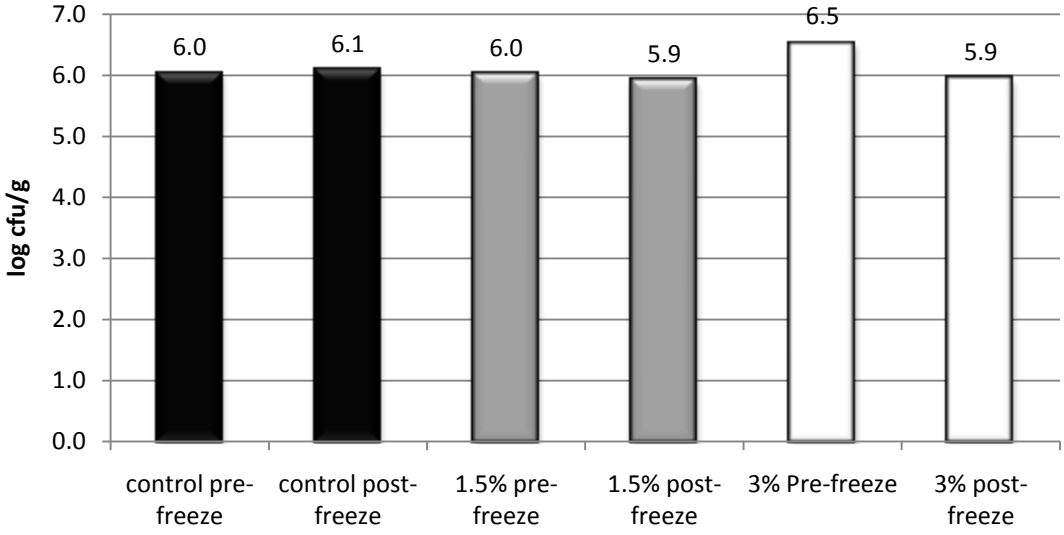

Figure 10. Surviving bacterial counts (CFU/g) in ice cream mix with varying levels of inulin (0% control, 1.5% and 3.0% w/w) during pre and post freezing phases (from liquid ice cream mix at 3.6˚C to frozen ice cream at -10˚C)

Effect of Inulin on the Survival of Inoculated Bacteria During Storage

Effect of inulin on the survival of inoculated bacteria during 28-days storage period at steady-state conditions (-20˚C).

Enumeration during the 28-day storage period at steady-state conditions (-20˚C) (Figure 11 and Table 6) showed no significant decrease in the bacterial load for all inulin concentrations (0% control, 1.5% and 3.0% w/w) ($F(2;17)$= 0.86, p >.05).

The bacterial number on day three was 5.9 log CFU/g for the control treatment, 6.1 log CFU/g for 1.5% inulin and 6.2 log CFU/g for the 3.0% inulin concentration. After 28-days of storage at steady-state conditions (-20˚C), the bacterial count was 5.9 log CFU/g for the control treatment,

5.9 log CFU/g for 1.5% inulin and 5.9 log CFU/g for the 3.0% inulin concentration. These results are in accord with other findings Magariños et al. (2007) and Marth and Steele (1998); where a non-significant decrease in microbial count during storage periods up to 90 days at -25˚C was reported. Marth and Steele (1998) suggested that freezing temperatures in the range of -20˚C to -25˚C has a protective effect on bacteria, and a live microbial load of 10^6 CFU/g was remained constant throughout the storage period.

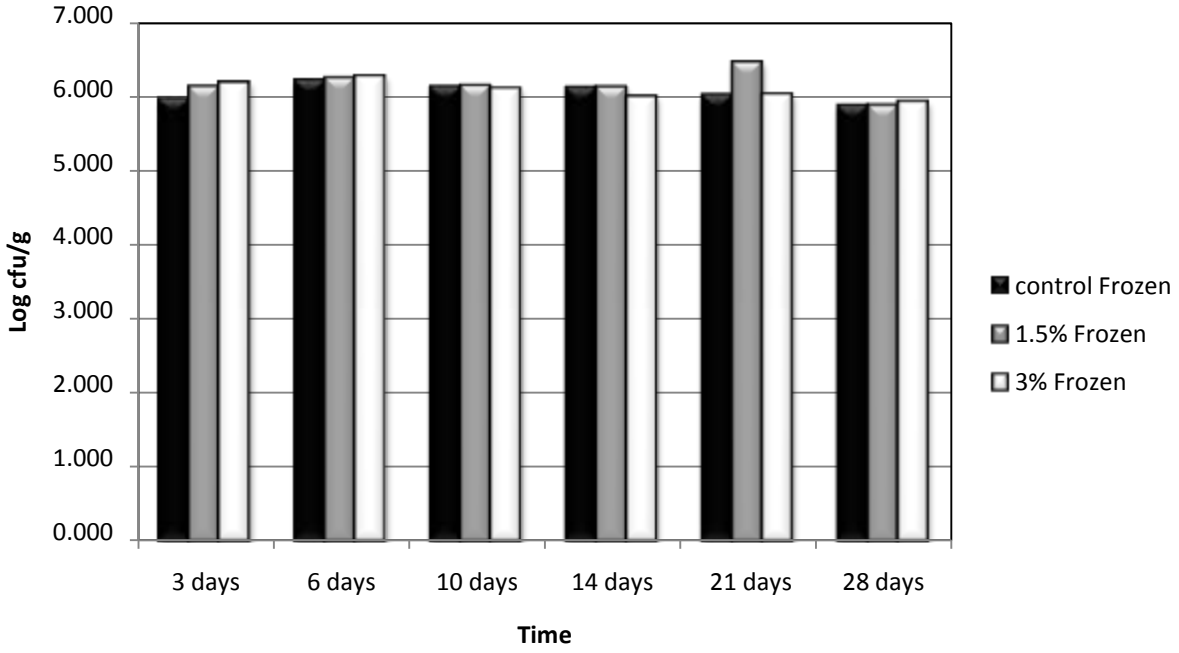

Figure 11. Surviving bacterial count (CFU/g) in ice cream with varying levels of inulin (0% control, 1.5% and 3.0% w/w) during 28-days storage period at steady-state conditions (-20˚C)

Effect of inulin on the survival of inoculated bacteria during 28-days storage period with repeated thaw-freeze cycles (-20˚C/-5˚C).

Enumeration of surviving bacterial numbers during a 28-day storage period with repeated thaw-freeze cycles (-20˚C/-5˚C) (Figure 12 and Table 6) showed no significant decrease in the bacterial load during this period in ice cream containing varying inulin concentrations (0% control, 1.5% and 3.0% w/w) ($F(2;15)$=0.17, p >.05).

The bacterial number at day three was 5.9 log CFU/g for the control treatment, 6.0 log CFU/g for 1.5% inulin and 6.2 log CFU/g for the 3.0% inulin concentration. After a 28-day storage period with repeated thaw-freeze cycles, the bacterial count was 5.8 log CFU/g for the control treatment, 5.8 log CFU/g for 1.5% inulin and 5.9 log CFU/g for 3.0% inulin concentration.

The results conflict with the results of Jay et al. (2005), Tamime (2005), and Whelan et al. (2008) that indicated minor fluctuations in storage temperatures could negatively affect the survival of probiotics in ice cream. The contradictory results obtained in the current research may be explained due to the short s storage period under thaw-freeze conditions (28-days). A significant microbial decrease may have occurred if the experiment was conducted for longer time duration.

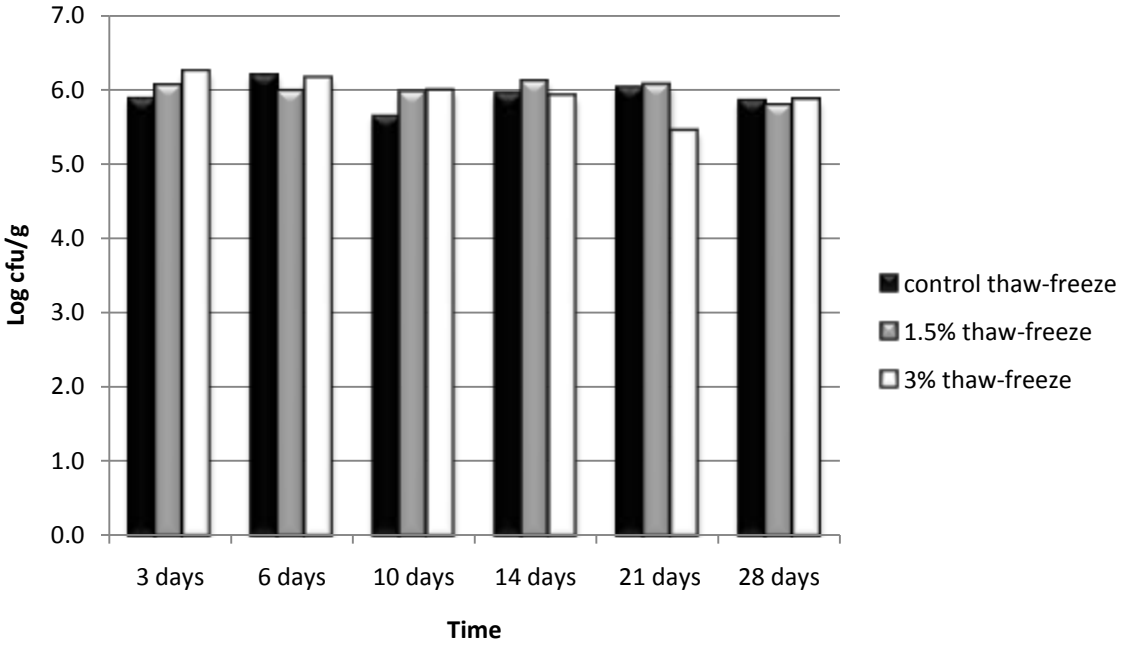

Figure 12. Surviving bacterial count (CFU/g) in ice cream with varying levels of inulin (0% control, 1.5% and 3.0% w/w) during 28-days storage period with repeated thaw-freeze cycles (-20˚C/-5˚C)

Table 6

Surviving bacterial count (Log CFU/g) for the varying levels of inulin concentrations during 28-days storage period at (-20˚C) and repeated thaw-freeze conditions

Treatments	**3 days**	**6 days**	**10 days**	**14 days**	**21 days**	**28 days**	**Average**
Control thaw-freeze	5.87	6.19	5.64	5.94	6.03	5.85	5.92
control (-20˚C)	5.95	6.20	6.12	6.10	6.00	5.86	6.04
1.5% thaw-freeze	6.04	5.96	5.95	6.09	6.05	5.77	5.98
1.5% (-20˚C)	6.13	6.25	6.15	6.12	6.46	5.88	6.17
3% thaw-freeze	6.23	6.14	5.97	5.90	5.43	5.85	5.92
3% (-20˚C)	6.20	6.28	6.11	6.00	6.03	5.93	6.09

During the 28-day storage period, the alternation of thaw-freeze cycles caused a small decrease in the mean population level of bacterial number throughout the three variations of inulin concentrations (0% control, 1.5% and 3.0% w/w) (Figure 13). For the control treatment, 6 log CFU/g of bacterial number was recorded during the freezing period at steady-state conditions (-20˚C) and 5.9 log CFU/g in repeated thaw-freeze cycles conditions. At 1.5% inulin concentration, the mean population level of bacterial number was 6.2 log CFU/g at (-20˚C) and 6.0 log CFU/g during repeated thaw-freeze cycles. At 3.0% inulin concentration, a decrease of bacterial number from 6.1 log CFU/g in (-20˚C) storage conditions to 5.9 log CFU/g during repeated thaw-freeze cycles was observed. This decrease in bacterial count was not statistically significant ($F(1;5) = 6.25$, $p > .05$). These results contradict the observation of Jay et al. (2005), Tamime (2005), and Whelan et al. (2008) and as discussed previously, longer storage times may

result in higher bacterial death.

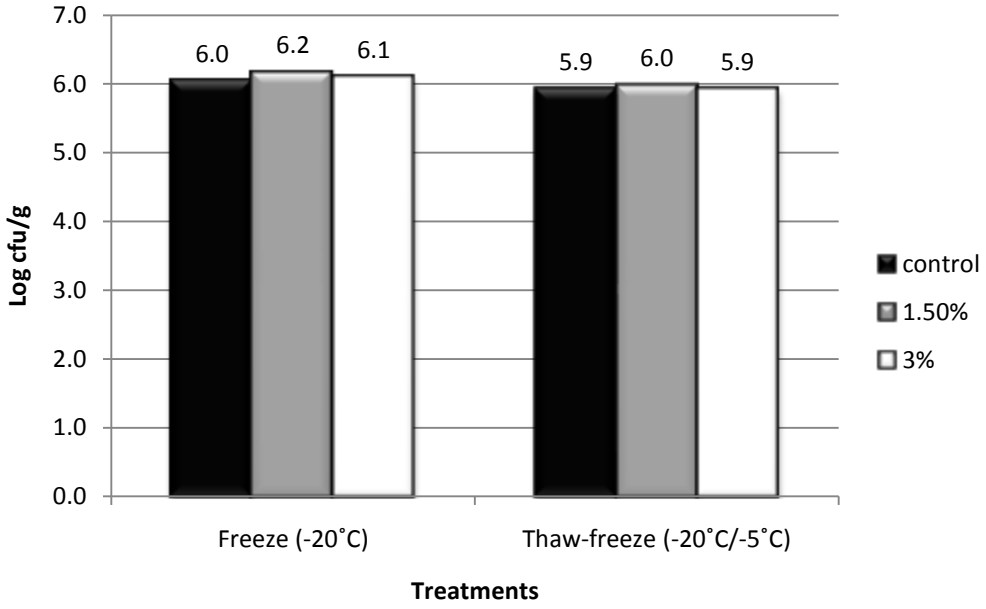

Figure 13. Comparison of mean population levels of bacterial number (CFU/g) during the 28-days storage period under repeated thaw-freeze cycles (-20˚C/-5˚C) versus steady-state freezing conditions (-20˚C)

Effect of Inulin on the Textural Structure in Ice Cream

Results of textural analysis studies done on ice cream samples with varying inulin concentrations (0% control, 1.5% and 3.0% w/w) are presented in Figures 14 and 15. The yield stress (minimum shear stress required to achieve flow) of ice cream made with three concentrations of inulin and stored at steady-state conditions (-20˚C) for 28-days is shown in Figure 14. Yield stress values are plotted as shear stress (pa) as a function of shear strain (Ra). The value of yield stress in the control treatment (0% inulin) ice cream (1153.4 Pa) was found to be very similar to the value of yield stress in 1.5% inulin ice cream (1141.2 Pa) and slightly higher than the value of yield stress in 3.0% inulin ice cream (1065.2 Pa). The difference in yield stress values between ice cream samples was not statistically significant ($F(2;281) = 0.53$, $p > .05$). This indicates that inulin when supplemented in small quantities into ice cream have no

significant effect on the textural structure and flow compared to the control. It should be noted that the fat content of the ice cream treatments had no variation. A previous study by Nagar et al. (2002) was conducted to determine the effect of higher inulin concentrations (5%, 7%, and 9% w/w) on ice cream texture. This study concluded that inulin had a significant effect on ice cream textural structure. However, fat free and regular ice cream samples were used to infer the effect of inulin.

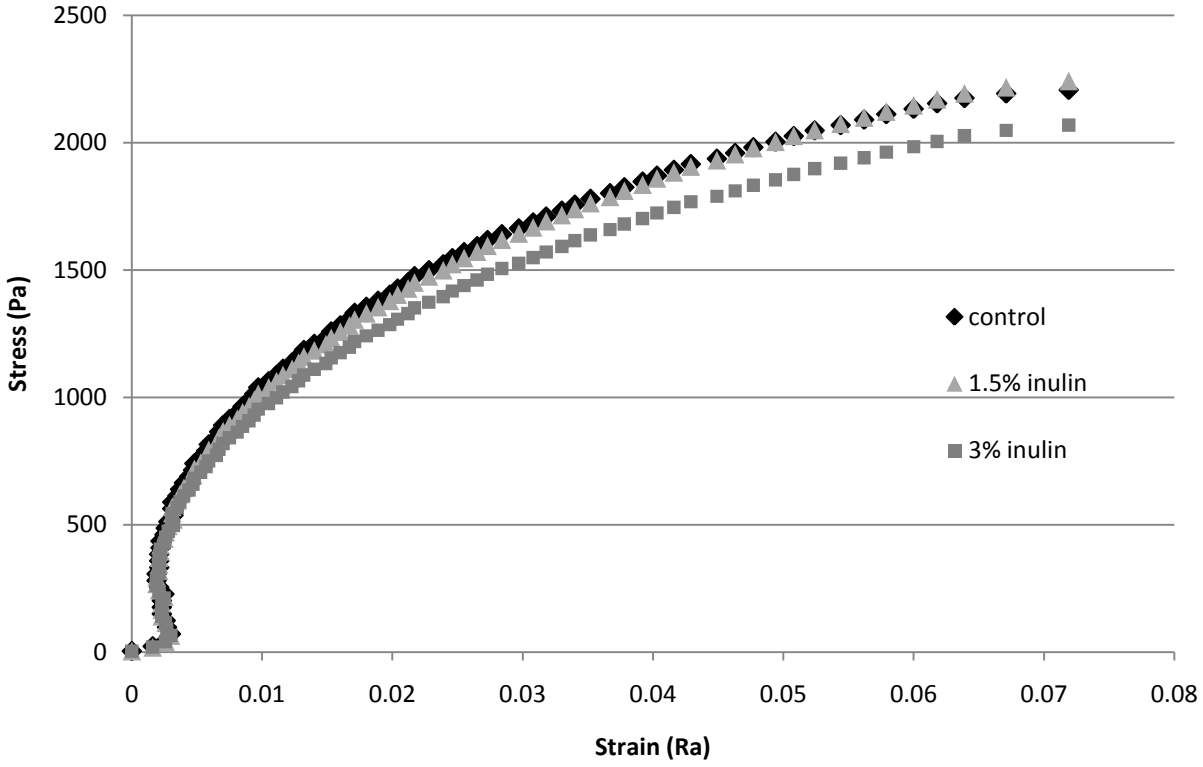

Figure 14. Stress-strain curves showing typical yield of ice cream formulated with three variations of inulin concentrations (0% control, 1.5% and 3.0% w/w) and stored for 28-days at steady-state conditions (-20˚C)

The apparent viscosity of ice cream samples made with three concentration of inulin (0% control, 1.5% and 3.0% w/w) and stored at steady-state conditions (-20˚C) for 28-days is shown in Figure 15. Apparent viscosity corresponds to the amount of force required to move one layer of fluid in relation to another in the ice cream mix. Viscosity values (cP) are plotted as a

function of time (s) in Figure 15 to show the flow behavior of ice cream samples with different inulin concentrations. The value of apparent viscosity in the control treatment (0% inulin) ice cream (856.1 cP) was found to be similar to the value of apparent viscosity in 1.5% inulin ice cream (853.4 cP). Apparent viscosity values of controls and 1.5% inulin treatment were higher than the 3.0% inulin treatment (731.1 Pa). The difference in apparent viscosity values between ice cream samples was statistically significant ($F(2;68) = 4.97$, $p < .05$). This indicates that inulin when supplemented at 3% concentration into ice cream had a significant effect on the flow behavior of the product. This result is consistent with the observation of Nagar et al., 2002 where high inulin concentrations produced high consistency index and low viscosity. This observation may be due to interaction between inulin molecules and liquid components in ice cream. Inulin's hygroscopic properties are responsible for binding water and forming a gel network that modifies the rheology and flow behavior of ice cream samples.

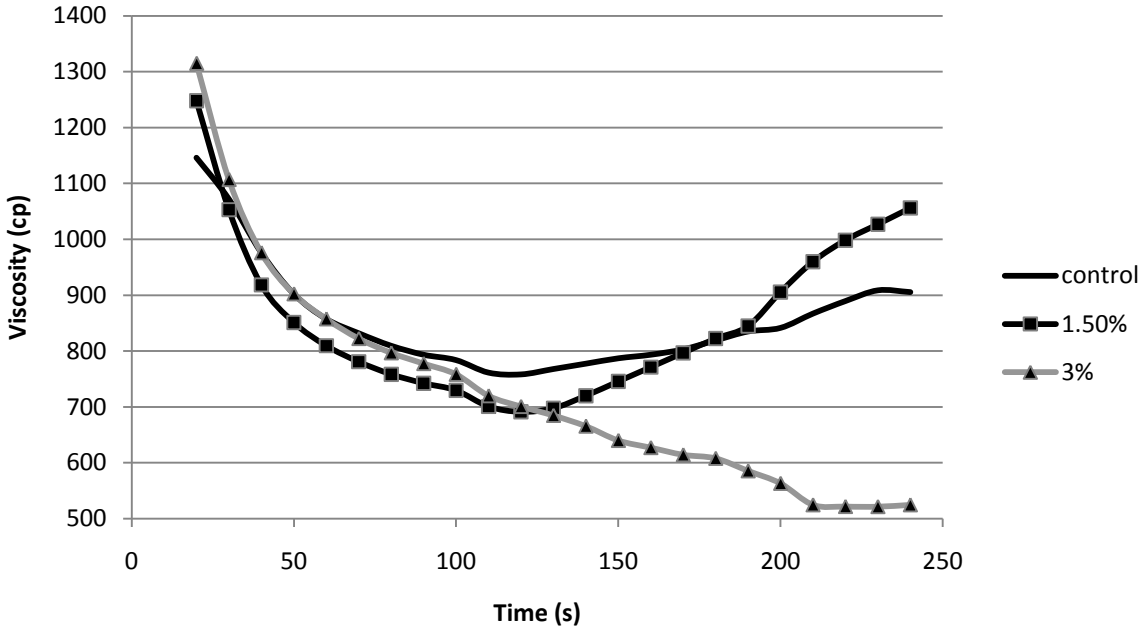

Figure 15. Viscosity (cp) as function of time (s) in ice cream formulated with three variations of inulin concentrations (0% control, 1.5% and 3.0% w/w) and stored for 28-days at steady-state condition (-20˚C)

Chapter V: Conclusion

The objective of the study was to explore the protective effect of three levels of inulin (0% control, 1.5% and 3.0% w/w) on the survival of LAB and probiotic bacteria in ice cream during the freezing phase. The effect was also studied throughout a 28-days storage period at constant temperature (-20˚C) and under repeated thaw-freeze cycles (-20˚C/-5˚C). Yield stress and viscosity analysis was conducted on the treatment samples to determine the effect of inulin addition on the texture of ice cream.

The effect of the freezing phase on the reduction of bacterial populations in the three variations of inulin concentration was not statistically significant ($F(1;5) = 1.24$, $p > .05$).

Bacterial enumeration during the 28-days storage period at steady-state conditions (-20˚C) showed no significant decrease in the bacterial load during this period for all three concentration of inulin (0% control, 1.5% and 3.0% w/w) ($F(2;17) = 0.86$, $p > .05$).

Bacterial counts during 28-days storage period under repeated thaw-freeze cycles showed no significant decrease in the bacterial load for all three concentration of inulin (0% control, 1.5% and 3.0% w/w) ($F(2;15) = 0.17$, $p > .05$)

The yield stress test of ice cream made with three concentration of inulin and stored at steady state conditions (-20˚C) for 28-days showed no significant difference in texture between the samples ($F(2;281) = 0.53$, $p > .05$).

The difference in apparent viscosity (cP) of ice cream samples made with three concentration of inulin (0% control, 1.5% and 3.0% w/w) and stored at steady-state conditions (-20˚C) for 28-days was statistically significant ($F(2;68) = 4.97$, $p < .05$). The addition of 3.0% inulin concentration to ice cream has a significant effect on the flow behavior of the product compared to the control (0% inulin) and 1.5% inulin.

Recommendations

The following suggestions are recommended for further research:

1. Evaluate the effect of inulin on the survival of LAB and probiotic bacteria using higher concentrations not determined in this present study (> 3.0% w/w).
2. Study the survival of LAB and probiotic bacteria in ice cream for longer storage periods not determined in this present study (> 28-days up to 1 year depending on commercial considerations).
3. Conduct studies to determine the survival of single and mixed bacterial cultures (to analyze synergistic interactions) in ice cream made with varying levels of inulin.
4. The current study has determined that ice cream made with varying inulin levels has no significant textural differences. However, this finding will have a commercial implication only after conducting sensory evaluation studies that may account for organoleptic characteristics (appearance, flavor, body, and texture).

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