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A NOVEL YOGURT PRODUCT WITH *LACTOBACILLUS ACIDOPHILUS*

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

In

The Interdepartmental Program in Animal, Dairy and Poultry Sciences

by
Senthil Ganesh
B.Sc., Bharathidasan University, 1993
M.Sc., NDRI, 1999
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DEDICATED TO GOD ALMIGHTY WHO MADE IT ALL POSSIBLE.

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ABSTRACT

Health benefits of *Lactobacillus acidophilus* include providing immune support for infections or cancer, providing a healthy replacement of good bacteria in the intestinal tract following antibiotic therapy, reducing occurrence of diarrhea in humans, aiding in lowering cholesterol and improving the symptoms of lactose intolerance. Consumer demand exists for new dairy products. There are several types of yogurt like stir curd, set curd and drinkable yogurt and they all need to be refrigerated. Moreover there are very few dairy products that can be stored at room temperature and not many dairy foods are finger foods. A novel yogurt product like a yogurt jerkey with *L.acidophilus* could be a dairy product that is a finger food, which can be stored at room temperature and have health benefits. The objectives of the research were to study the effects of 0, 1, 10 and 100g of *Lactobacillus acidophilus* /gal of novel yogurt product on *L. acidophilus*, yogurt bacteria, coliform, yeast and mold counts and TPA (Texture Profile Analysis) hardness, springiness, chewiness, cohesiveness, and adhesiveness over 0, 1, 2 and 3 months of storage of the novel yogurt product at room temperature. The interaction effect of treatment and time was significant for all attributes studied except adhesiveness. Yogurt bacterial counts were significantly higher in all treatments at month 3 compared to control. With the use of 10g and 100g/gal addition of *L.acidophilus* there was a significant decrease in *L.acidophilus* counts at month 2 and month 3 when compared to month 0. Hardness of product with *L.acidophilus* use at 100g/gal was significantly lower when compared to the control and treatments 1, 10g/ gal over months 1, 2 and 3. Springiness and chewiness of all treated samples at month 2 were significantly higher

than control. Cohesiveness was significantly higher with all levels of *L.acidophilus* compared to control.

Use of probiotics favorably affected some characteristic of the novel yogurt product. Use of probiotic *L.acidophilus* at 100g/gal can be recommended in the manufacture of a healthy novel yogurt product such as a yogurt jerkey or bite sized chewable yogurt capable of being stored at room temperature.

CHAPTER 1: INTRODUCTION

1.1 Importance of Milk and Milk Products in Diet

Fluid milk is not only nature's food for a new born infant, but also a source for a whole range of dairy products consumed by mankind. Fluid milk is about 87% water and 13 % solids. The fat portion of the milk contains fat-soluble vitamins. The solids other than fat include proteins, carbohydrate, water-soluble vitamins, and minerals. Milk products contain high quality proteins. The whey proteins constitute about 18% of the protein content of the milk. Casein, a protein found only in milk, contains all of the essential amino acids and accounts for 82 % of the total proteins in milk. Milk also contains calcium, phosphorus, magnesium, and potassium. The calcium found in milk is readily absorbed by the body; Vitamin D plays a role in calcium absorption and utilization. Milk is also a significant source of riboflavin (vitamin B_2), which helps promote healthy skin and eyes (Dairy Facts 2003). Dairy products such as yogurts, cheeses and ice creams contain nutrients such as proteins, vitamins and minerals. Consumption of dairy products been associated with decreased risk of osteoporosis, hypertension, colon cancer, obesity and insulin resistance syndrome (IRS). The main dietary source of calcium and vitamin D are dairy products (Weaver, 2003).

1.2 Fermented Milk Products

The introduction of fermented milk products such as cheeses and yogurts in to the diet of man is thought to date back to the dawn of the civilization (Mckinley, 2005) Consumption of fermented-milk products is associated with several types of human health benefits partly because of their content of lactic acid bacteria. Several experimental observations have indicated a potential effect of lactic acid bacteria (LAB)

against the development of colon tumors (Wollowski et al. 2001). Recently, the role of fermented milks containing lactic acid bacteria (LAB), such as *Lactobacillus, Bifidobacterium* and *Streptococcus thermophilus,* has been studied (Saikali et al. 2004). A wide range of other health benefits, including improved lactose digestion, diarrhea prevention, immune system modulation and serum cholesterol reduction, have been ascribed to fermented milk consumption.

1.3 Yogurt

Yogurt is a product of the lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. In some countries less traditional microorganisms, such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii* ssp. *lactis*, are sometimes mixed with the starter culture (McKinley, 2005). Although fermented milk products such as yogurts were originally developed simply as a means of preserving the nutrients in milk, it was soon discovered that, by fermenting with different microorganisms, an opportunity existed to develop a wide range of products with different flavors, textures, consistencies and more recently, health attributes. The market now offers a vast array of yogurts to suit all palates and meal occasions. Yogurts come in a variety of textures (e.g. liquid, set and stirred curd), fat contents (e.g. regular fat, low-fat and fat-free) and flavors (e.g. natural, fruit, cereal, chocolate), can be consumed as a snack or part of a meal, as a sweet or savory food. This versatility, together with their acceptance as a healthy and nutritious food, has led to their widespread popularity across all population subgroups (Mckinley, 2005).

Yogurt was introduced to the American diet during the 1940s. By the 1980s, it had become the product for dieters, and the lunch of choice for young women. The use of

yogurt as a calcium source has made it one of the most rapidly growing dairy products, but presently it is more than just a calcium source. Yogurt, Kefir, and similar fermented milk products are on the way to becoming major nutraceuticals aimed at treating a variety of disease conditions (Katz, 2001). Yogurt's nutritional profile has a similar composition to the milk from which it is made but will vary somewhat if fruit, cereal or other components are added. Yogurt is an excellent source of protein, calcium, phosphorus, riboflavin (vitamin B_2), thiamin (vitamin B_1) and Vitamin B_{12}, and a valuable source of folate, niacin, magnesium and zinc. The protein it provides is of high biological value, and the vitamins and minerals found in milk and dairy foods including yogurt are bio-available. Yogurt particularly the low-fat varieties, provide an array of important nutrients in significant amounts in relation to their energy and fat content, making them a nutrient-dense food. Eating dairy products, such as yogurt, helps to improve the overall quality of the diet and increases the chances of achieving nutritional recommendations. (Mckinley, 2005). Vitamins and minerals may be added and often are for products given to children. Yogurts may be spoonable or drinkable, and may be considered dietary supplements for infant consumption. So they cross the line between dietary supplements, medical foods, and conventional foods (Katz, F. 2001).

Yogurt gels are formed by the fermentation of milk with thermophilic starter bacteria; milk is normally heated at high temperatures (e.g., 85°C for 30 min), which causes the denaturation of whey proteins. Denatured whey proteins interact and cross-link with κ-casein on the surface of casein micelles. There is increased casein-casein attraction as the pH of milk decreases from ~6.6 to ~4.6 during yogurt fermentation, which results in gelation as casein approach their iso-electric point. Physical properties of

yogurt gels, including whey separation play an important role in quality and consumer acceptance. An understanding of gelation process during fermentation is critical in manipulating physical properties of yogurt (Lee and Lucey, 2004).

1.4 Various Ingredients Used in Dairy Products

In some dairy products, sucrose is commonly used but surprisingly little systematic work has been reported on gel formation at low water activity. Sucrose concentration is a very important parameter for controlling the kinetics of casein gel formation as well as the strength and stability properties of casein gels. The effect of sucrose varies depending on acidification or enzymatic gelation route used. It was reported that during acid gelation, the addition of up to 30% (wt/wt) sucrose allows casein gels to be formed more rapidly and at higher pH, at sucrose levels above 30% (wt/wt); a more grained and porous gel is formed. At higher sucrose levels the casein micelle structure is more swollen and the aggregates are softer. It is also reported that combined acidification and renneting leads to instantaneous gelation and a stronger gel. In the absence of sucrose, large water-containing pores are present between the casein aggregates. In the presence of sucrose the gel is more homogeneous with smaller aggregates linked together to form a fine meshed network. The network remains intact on storage suggesting that sucrose can prevent the local phase separation normally present in acid- or rennet-induced casein gels (Scorsch et al, 2002).

Starches are extensively used in a variety of food products such as ice cream, chocolate, milk based sweets, jellies, sauces, custards and desserts. In these products the method of preparation such as water content, temperature and the presence of other

organic/inorganic materials is an important factor that determines the rheological behavior of starch dispersions (Abu-Jdayil et al. 2004).

The hydrocolloids used in dairy desserts are typically starch and carrageenan, starch imparts body and mouth feel to the product while carrageenan provides the desired texture, depending on the type and the concentration used. Advantages of the use of starch/carrageenan blends in comparison to starch alone include the reduction of the starch content and thus of the caloric value of the dessert and a lower viscosity during processing. In dairy desserts carrageenan gelation is importantly affected by the presence of milk proteins and starch, an attractive interaction takes place between K-carrageenan molecules and milk proteins in sterilized dairy desserts. Due to this interaction, milk proteins are involved in the formation of the carrageenan gel network and contribute to the physicochemical properties of the desserts. Starch granules act as non-interacting fillers and cause a concentration of the other ingredients in the continuous phase as a result of the exclusion effect (D.Verbeken et al. 2006).

The effect of the heating temperature on the rheological behavior of heated starch-milk-sugar (SMS) systems was studied by Abu-Jdayil et al. (2004) They used corn and wheat starch and also three types of sugars namely; glucose, fructose and sucrose were used. The corn starch-milk-sugar (CMS) paste prepared at 60 and 75°C have nearly the same rheological behavior as the heating temperature increased to 95°C the apparent viscosity of the paste increased. The heating temperature changes the structure of CMS paste from a dispersion fluid type at 75°C to a medium gel structure at 85°C and to a strong gel product at 95°C. The addition of sugars to starch-milk system elevated the starch gelatinization temperature.

Gelatin is one of the hydrocolloids or water- soluble polymers that can be used as a gelling, thickening or stabilizing agent; it is a totally digestible protein, containing all the essential amino acids except tryptophan (Poppe, 1997). Gelatin is an ingredient compatible with the milk proteins and contributes good palatability to the end product, giving a fat-like sensory perception because of its unique property of melting at mouth temperature. It eliminates syneresis and considerably reinforces the mechanical resistance of the gels making it possible to obtain a wide range of texture (Fiszman and Salvador, 1999).

In the food industry gelatin is used not only for its functional properties, but also because of its importance as a source of protein in the daily diet. The primary properties of gelatin include gel formation, texturizing, water binding, and surface effects such as emulsion and foam formation. Increases in the concentration of gelatin in yogurt and variations in the pH of acid-heat-induced gels caused changes in the shape of the force/displacement curves. Potentially, the use of different concentrations of gelatin would make it possible to obtain a wide range of textures including creamy, slightly gelled and firm, "mouldable" gel of yogurt (Fizman and Salvador, 1999).

One of the most important properties of gelatin is its ability to form thermo-reversible gels. Gelatin swells in cold liquid absorbing 5-10 times its volume of water. When heated to temperatures of approximately 50 to 60°C it dissolves and forms a gel when cooled. This sol- gel conversion is reproducible and can be repeated several times. This versatile property is used in many food applications. The gelling power of gelatin is determined by its Bloom value, which is a standardized measurement of the firmness of a standard gel under precisely determined conditions. The Bloom values of gelatin range

from 80 to 300 g. In general, viscosity increases with increasing Bloom strength (Schott, 2001).

1.5 The Texture Profile Analysis

Casein gels are responsible for many rheological properties of cheese and other dairy products that gel, stretch and fracture. Rheological studies are performed as a quality control method and as a technique to study the structure of the product (Tunick, 2000). Instrumental texture measurements, which have been devised, to relate to human perception have been of both an imitative and empirical nature. Imitative tests generate several instrumental parameters while empirical tests usually yield only one instrumental parameter. The generation of multiple parameters is one of the main advantages of imitative tests over empirical tests. The instrumental texture profile analysis (TPA), first developed for the General Foods Texturometer, is an imitative test. It was later adapted to the Instron Universal Testing Machine (I.U.T.M) and refined by Bourne (1966, 1978) has become a standard (Meullenet, 1997).

1.6 Quality and Shelf Life of Yogurt

Yogurts shelf life is based on whether the products display any of the, physical chemical, microbiological or sensory characteristics that are unacceptable for consumption. Studies of changes in these quality characteristics during storage would be instrumental in predicting the shelf life of the product (Salvador and Fiszman, 2004). Salvador and Fiszman (2004) compared yogurts stored at 10, 20 and 30°C and found that the samples evolved differently at the 3 temperatures studied, their results of physical properties, sensory and microbiological analysis showed that negative characters such as syneresis, appearance defects, atypical texture / mouth feel increased with storage time,

the results also indicate that, to some extent the changes observed over the storage period could be considered the result of a combination of time and temperature.

Concentrated yogurt, known as labneh in the Middle east is widely consumed, chiefly as a sandwich spread, the shelf life of cloth-bag labneh was adequately determined by Weibul distribution using flavor deterioration or yeast counts as failure indices. Flavor defects develop at a higher rate than textural changes during storage and seem to be the earliest manifestation of product failure. The shelf life of cloth-bag labneh is expected to range from 8.5 to 10.5 days at a storage temperature of 5°C. The end of shelf life of cloth bag labneh is accompanied by an increase of 10 to 15% of free whey and 0.5 to 0.6% of lactic acid and drop of 0.3 to 0.4 pH units. The deterioration in sensory quality and changes in physico-chemical parameters of cloth-bag labneh are largely governed by the growth of yeasts and molds (Al-Kadamany, et al. 2002).

1.7 Functional Foods

It is now well established that there is a clear relation between diet and health, more recent discoveries support the hypothesis that, beyond nutrition, diet may modulate various functions in the body. Functional foods are described as foods claimed to have a positive effect on health (Stanton, et al, 2001). Such products are gaining more widespread popularity and acceptance throughout the developed world and are already well accepted in the Japan and the United States. The origin of the term functional food can be traced to Japan, where the concept of foods designed to be medically beneficial to the consumer evolved during the 1980s. The term refers to the practice of fortifying foods with added ingredients that can confer health effects on the consumer, current definitions

of what constitutes a functional food vary considerably as a result of the rapid growth of the area in recent years outside Japan (Stanton, et al, 2001).

Functional foods can include probiotics, prebiotics and synbiotics. Although definitions vary, probiotics can be defined as "live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance"(Champagne and Gardner, 2005). Prebiotics is defined as " a non digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" a synbiotic is a combination of prebiotics and prebiotics that " beneficially affects the host by improving the survival and the implantation of live microbial dietary supplements in the gastro intestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health promoting bacteria"(DiRienzo, 2000).

The concept of probiotics evolved from a theory first proposed by Nobel Prize winning Russian scientist, Elie Metchnikoff who suggested that the long life of Bulgarian peasants resulted from their consumption of fermented milk products. Probiotic bacteria can be found worldwide in a variety of products, including conventional food products, dietary supplements and medical foods. In the United States, the main outlets for probiotic bacteria are dairy foods and dietary supplements and medical foods. Dairy foods containing probiotic bacteria include most major brands of yogurt, culture-containing fluid milks, such as "Sweet Acidophilus Milk" and a few brands of cottage cheese. Dairy foods seem to fit naturally with probiotics because of the traditional association of beneficial fermentation bacteria and fermented dairy product. Consumers

naturally associate fermented dairy products with live cultures and perceive a benefit in the presence of these cultures (Sanders, 2000).

1.8 Health Benefits of *Lactobacillus acidophilus*

Lactobacillus acidophilus offers a range of health benefits which include: providing immune support for infections and cancer are a healthy replacement of good bacteria in the intestinal tract following antibiotic therapy, reducing occurrence of diarrhea in humans (children and adults), aiding in lowering cholesterol, improving the symptoms of lactose intolerance. Anti-tumor effect of *L.acidophilus* was reported by Goldin and Gorbach (1984). Oral dietary supplements containing viable cells of *L.acidophilus* decreased ß- glucuronidases, azoreductase, and nitroreductase, bacterial enzymes, which catalyze conversion of procarcinogens to carcinogens. Anticarcinogenic effect of *L.Acidophilus* may be due to direct removal of procarcinogens and activation of body's immune system. Animal studies have shown that dietary supplementation with *L.acidophilus* decrease the number of colon cancer cells in a does dependant manner (Rao et al., 1999).

1.9 The New Trend

Consumer demand exists for new dairy products. There are several types of yogurt like stir curd, set curd and drinkable yogurt. All these yogurts need to be refrigerated. More over there are very few dairy products that can be stored at room temperature and not many dairy foods are finger foods. A novel yogurt product like a yogurt jerkey with *L.acidophilus* could be a dairy product that is a finger food, which can be stored at room temperature and have health benefits.

1.10 Objectives

The objectives of this research were to study the effect of various levels of *Lactobacillus acidophilus* namely 0, 1, 10 and 100g/gal of yogurt product on the following characteristics over 0, 1, 2 and 3 months of storage at room temperature.

a. Counts of *Lactobacillus acidophilus*, yogurt bacteria, coliform, yeast and mold.
b. Textural characteristics namely hardness, springiness, chewiness, cohesiveness, and adhesiveness.

CHAPTER 2:MATERIALS AND METHODS

2.1 Experimental Design

Novel yogurt product was manufactured with 0g/gal, 1g/gal, 10g/gal and 100g/gal of *Lactobacillus acidophilus* culture and the samples were analyzed at 0,1,2 and 3 months for Texture (Hardness, Chewiness, Springiness, adhesiveness and Cohesiveness), *Lactobacillus acidophilus* count, lactobacilli count, coliform count, yeast and mold count. Randomized complete block design was used as the experimental design. Three replications were made for each treatment and the data was analyzed as repeated measures in time. Replications were blocks.

2.2 High Total Solid Yogurt Manufacture and Storage

Plain yogurt with high total solid was manufactured with skim milk and nonfat dry milk (1024g of Non Fat Dry Milk /gallon of Skim Milk). 1024g of Non Fat Dry Milk 1 gallon of Skim Milk were taken and mixed well. The mixes were preheated to 60ºC, homogenized at 1500 psi first stage and 500 psi second stage in a Gaulin homogenizer (Manton-Gaulin Manufacturing Company, Inc., Everett, MA), batch pasteurized at 85ºC for 30 min, and cooled to 40ºC. The yogurt culture CH-3 (Chr. Hansen, Inc., Milwaukee, WI) (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) (10 mL/gal) was added to the mix at 40ºC. The Yogurt mixes were poured into 355 mL Reynolds RDC212 – Del-Pak Combo-Pak containers (Alcoa, Inc., Pittsburgh, PA) and incubated at 40°C to pH 4.5 before cooling to 4°C. Samples were stored at 4ºC until further processing.

2.3 Concentrating Yogurt Solids

High total solids yogurt was taken in wide mouth screw cap round bottles (Nalgene, cat. #2105 0008) and centrifuged at 10000 rpm for 15 minutes using Beckman, Model-J2-21 centrifuge, with the rotor Type JA-14 at 4°C. After centrifugation the supernatant whey was decanted and the solids stored at 4°C for processing.

2.4 Novel Yogurt Product Manufacturing Process and Formulation

The high total solids yogurt was taken and vigorously agitated using a sterile spatula to make a homogenous paste. It was mixed with gelling agent and sugar base at the rate of 7: 3 (70% concentrated high total solids yogurt and 30% base).

The base was manufactured as follows:

The respective amounts of various ingredients were used according to the formulation in Table 1.

Table 1. Gelling agent and sugar base formulation

Ingredients	Quantity % (w/w)
Water	Specific quantity
A specific gelling agent	Specific quantity
Sugars from three different sources	
Sugar 1	Specific quantity
Sugar 2	Specific quantity
Sugar 3	Specific quantity

The base ingredients were cooked as follows:

The sugars were weighed out in a glass beaker and the gelling agent weighed out separately in another glass beaker. A specific part of the water taken for the process was added to the sugars and the rest was heated to a specific temperature and added to the weighed out gelling agent and mixed gently with out aeration and made in to a paste, this paste was kept covered over a steam bath to maintain the temperature at a specific range. The sugars were blended and cooked with the gelling agent in a specific sequence and process.

2.5 Mixing of Yogurt Solid and Base

The 70% concentrated high total solids yogurt and 30% base mixture was blended with sterile spatula and made in to a homogenous paste. Frozen culture concentrate of *Lactobacillus acidophilus* K pellets (Chr. Hansen, Inc., Milwaukee, WI) was freshly thawed at 4°C and added to the above yogurt paste at the rates of 0, 1g, 10g and 100g/gallon yogurt paste. Yogurt product was poured in to sterile trays for microbial counts and syringes for texture profile analysis.

2.6 Microbiological Analysis of Samples

2.6.1 Sample Preparation for Microbiological Analysis

The yogurt product base was poured in a clean tray wiped and sterilized with 70 % ethyl alcohol which was air dried and spread with clean aluminum foil again sterilized with 70 % ethyl alcohol and spread in the shape of long strips (like jerkey) that were approximately 0.5cm thick and 20cm long and 4cm wide. The tray was air dried in a clean ethanol sterilized incubator set at 22°C (Precision scientific, Low temperature incubator, model#815, Chicago, IL.) for 24 hrs; the strips were turned over after 12 hrs.

with sterilized forceps. After drying samples are divided separately for each month and vacuum packed in ultra violet sterilized Rival. Seal-a-Meal vacuum storage bags model# VSB4-A, Multivac model A300/52, (Kansas City, MO), vacuum packing machine was used. Care was taken to prevent contamination. The vacuum packed samples were stored at room temperature (22-25°C) till further analysis.

Eleven grams of the appropriate samples were weighed aseptically in to 99 mL of sterilized Butterfield buffer in pre-filled dilution bottles (Weber Scientific, Hamilton, NJ) and they were kept in the refrigerator for 24 hours for softening. After softening the entire content of the dilution bottle was transferred aseptically in to an ultra violet light sterilized stomacher bag (Seward medical stomacher 400) and homogenized for 10 minutes using a Stomacher 400 Lab blender (Seward medical, Model # BA 6021, London, UK). The resultant dispersion was the first dilution of that sample.

2.6.2 Determination of *Lactobacillus acidophilus* Counts

Lactobacillus acidophilus counts were determined by a modification of Tharmaraj and Shah (2003). The MRS base medium without dextrose was prepared by weighing the appropriate proportion of 10.0 g of proteose peptone #3 (United States Biological, Swampscott, MA), 10.0 g of beef extract (Becton, Dickinson and Co., Sparks, MD), 5.0 g of yeast extract (Becton, Dickinson and Co., Sparks, MD), 1.0 g of polysorbate 80 (Tween 80) (Sigma-Aldrich Inc., St. Louis, MO), 2.0 g of ammonium citrate (Fisher Scientific, Fair Lawn, NJ), 5.0 g of sodium acetate, anhydrous (EMD Chemicals Inc., Gibbstown, NJ), 0.1 g of magnesium sulfate, anhydrous (EMD Chemicals Inc., Gibbstown, NJ), 0.05 g of manganese sulfate, monohydrate (Sigma-Aldrich Inc., St. Louis, MO), 2.0 g of dipotassium phosphate (Fisher Scientific, Fair Lawn, NJ), and 15.0

g of agar (EMD Chemicals Inc., Gibbstown, NJ) and diluting these ingredients to the appropriate proportion of 1 L with distilled water. This mixture was autoclaved at 121ºC for 15 min. A 10% (w/v) sorbitol (EMD Chemicals Inc., Gibbstown, NJ) solution was prepared and filter sterilized with Nalgene Membrane Filter Units (Nalgene Co., Rochester, NY), and the appropriate amount of this solution was aseptically added to the MRS base medium to form a 10% sorbitol solution (final concentration of 1% sorbitol) and 90% MRS base medium mixture immediately before pouring the plates. The appropriate dilutions of yogurt product were made with 99 mL of sterilized Butterfield buffer in pre-filled dilution bottles (Weber Scientific, Hamilton, NJ). The pour plate method with this MRS-sorbitol agar was performed. Petri dishes were placed in BBL GasPaks (BBL, Becton, Dickinson and Co., Cockeysville, MD) and incubated anaerobically at 40ºC for 48 hr. A Quebec Darkfield Colony Counter (Leica Inc., Buffalo, NY) was used to assist in enumerating the colonies. The *L. acidophilus* counts were enumerated at 0, 1, 2, and, 3 months of storage.

2.6.3 Determination of Yogurt Bacterial Counts

Yogurt bacteria counts for yogurt samples serially diluted to the appropriate dilution with 99 mL of sterilized Butterfield buffer in pre-filled dilution bottles (Weber Scientific, Hamilton, NJ) were performed by the pour plate method using Difco Lactobacilli MRS agar (Becton, Dickinson and Co., Sparks, MD). Petri dishes were placed in BBL GasPaks (BBL, Becton, Dickinson and Co., Cockeysville, MD) and incubated anaerobically at 40ºC for 48 hrs. A Quebec Darkfield Colony Counter (Leica Inc., Buffalo, NY) was used to assist in enumerating the colonies. The Lactobacillus counts were enumerated at 0, 1, 2, and, 3 months of product storage at room temperature.

2.6.4 Coliform Counts

Coliform counts (cfu/ml) were determined according to (Richardson, 1985). Coliform count Petrifilms (3M, St. Paul, MN) were used to enumerate coliforms at 0, 1, 2, and, 3 months of storage.

2.6.5 Yeast and Mold Counts

Yeast and Mold counts (cfu/ml) were determined according to (Richardson, 1985). Yeast and Mold Petrifilms (3M, St. Paul, MN) were used to enumerate Yeast and Mold counts at 0, 1, 2, and, 3 months of storage.

2.7 Texture Profile Analysis (TPA) of Formulations

2.7.1 Sample Preparation

The concentrated high total solids yogurt (70%) and base (30%) mixes were poured in to syringes specially cut at the end to facilitate pouring of sample and allowing extracting at uniform diameter and shape for Texture Profile Analysis (TPA). BD brand syringe-20ml (disposable), Becton Dickinson, (Franklin Lakes, NJ), was used. The tip portion was cut neatly using a tube cutter. The concentrated high total solids yogurt and base mixture was poured in properly marked syringes and allowed to stand vertical in a test tube stand so that the sample formed in the shape of a cylinder. The internal diameter of the syringe was 19mm. The samples were allowed to stay overnight to facilitate proper setting. The cylinders were extracted and air-dried at 22 ºC for 24 hrs (Precision scientific, Low temperature incubator, model#815, Chicago, IL.). The samples in the form of cylinders were cut in to 20mm long pieces after carefully trimming the edges. Care was taken to keep the size uniform 20 mm in length and 19mm in diameter. The pre cut samples were divided separately for each month and vacuum packed in Seal-a-Meal

vacuum storage bags model# VSB4-A, Seal –a-Meal, (Milford, MA). Multivac model A300/52, (Kansas City, MO), vacuum packing machine. The vacuum packed samples were stored at room temperature (20ºC to 25ºC) till further analysis.

2.7.2 The TPA Method

TPA method was adapted from Bourne (1966,1978). Instron Software Catalog #2410-212 (Instron Corporation, Norwood, MA) was used with some modification. The TPA two bite method was used with sample height 20mm, anvil height at 40mm, crosshead speed 100mm/min, 75% compression, the samples were at room temperature. Compression anvil Model#2830-009 was used. The experiments were conducted by compression test that generated plot of force (N) vs. Time (Figure 1), from which texture values were obtained using the following formulae that was programmed in Instron software.

Hardness (N) = Maximum force of the first compression

$$\text{Cohessiveness} = \frac{\text{Area under } 1^{st} \text{ compression (A1)}}{\text{Area under } 2^{nd} \text{ compression (A2)}}$$

Adhesiveness (N) = Negative area in graph (A3)

$$\text{Springiness (mm)} = \frac{\text{Length1}}{\text{Length2}}$$

Chewiness (N) = Hardness x cohesiveness x springiness

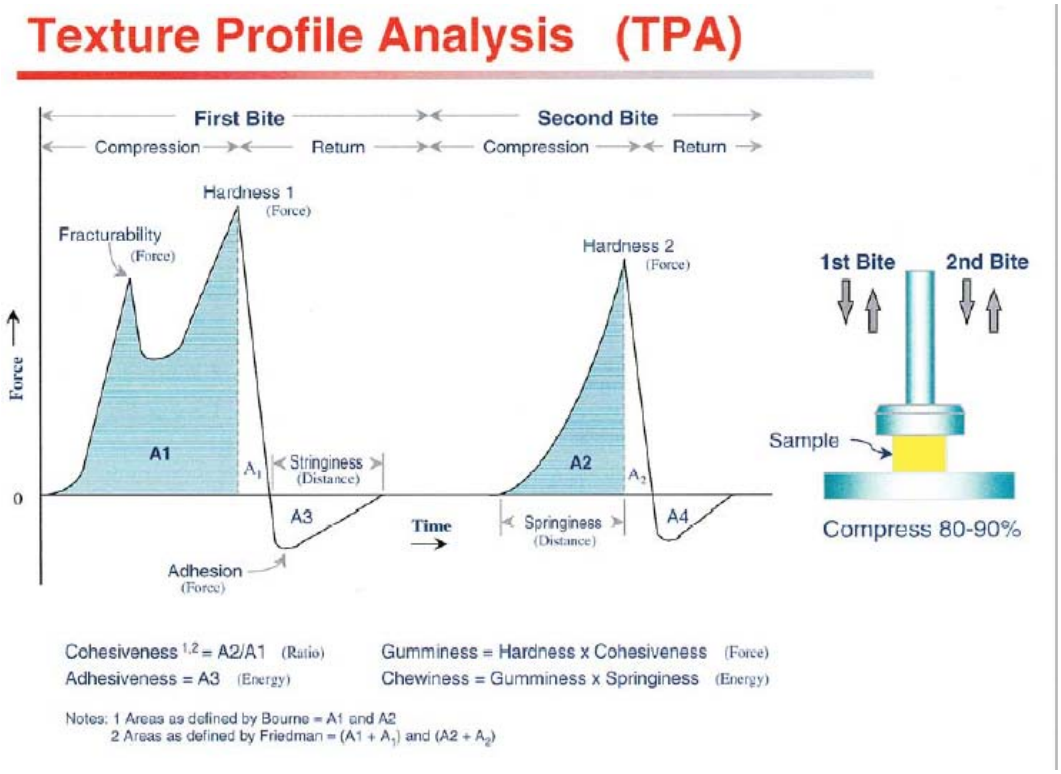

Figure 1. Typical Texture Profile Analysis curve generated by Instron

Source: http://www.instron.us/wa/library/streamfile.aspx?doc=477&Download=true

2.8 Statistical Analysis

The data obtained from TPA and microbial analysis of four treatments (0g/gal, 1g/gal, 10g/gal, 100g/gal) of Novel yogurt product were analyzed applying repeated measures in time design using PROC. MIXED procedure of SAS Version 9.1.3, (Cary, NC). First order Auto Regressive Covariance (AR(1)) structure was selected based on Akaike Information Criteria (AIC) and applied to all variables (Akaike, 1974). For each variable treatment, time, treatment x time, interactions were studied. Treatments 0g/gal, 1g/gal, 10g/gal and 100g/gal were compared. Significant differences ($p<0.05$) among treatments (1g/gal, 10g/gal and 100g/gal) with control (0g/gal) were analyzed using Dunnet's adjustment.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 *Lactobacillus acidophilus* Counts

The *Lactobacillus acidophilus* counts are reported in Figure 2. The interaction of treatment x time was significant ($p<.0001$)(table 2). There was no significant effect ($p>.05$) of 1g/gal starter addition on *L.acidophilus* count over time. With the use of 10g/gal and 100g/gal addition of *L.acidophilus* there was a significant decrease ($p<.05$) in counts at month 2 and month 3 when compared to month 0.

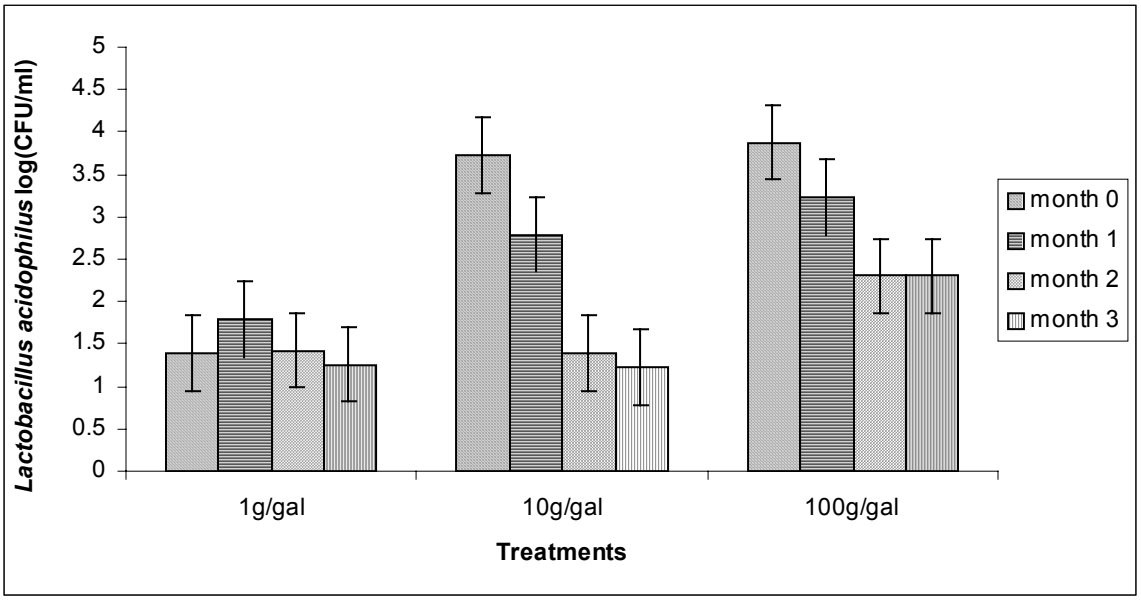

Figure 2. *Lactobacillus acidophilus* counts of Novel Yogurt product with 0, 1, 10, 100g/gal *Lactobacillus acidophilus* for 0, 1, 2, 3 months Mean (± SE)

Yogurts are made from the symbiotic growth of the two bacteria: *S.thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. These yogurt bacteria do not survive the gastric passage or colonize the gut; hence the recent trend is to add *L.acidophilus* and Bifidobacterium spp. to yogurt (Shah, 2000). According to National Yogurt Association, McLean, VA, for the refrigerated cup of yogurt the total population of organisms in live and active culture yogurt must be 10^8 cfu/g at the time of manufacture and in frozen yogurt it must be at least 10^7 cfu/g at the time of manufacture (Chandan, 1999). For probiotic organisms in order to obtain a desired therapeutic effects, it has been suggested that these organisms should be present in a food to a minimum level of 10^6 cfu/g, such high numbers might have been suggested to compensate for the possible reduction in the numbers of the probiotic organisms during passage through the stomach and the intestine (Shah, 2000)

The low *L.acidophilus* counts and the reduction over time could be attributed to several factors. Hull et al. (1984), observed *L.acidophilus* added to the yogurt before manufacture at the stage of starter addition multiplied during manufacture and reached 10^7 viable organisms per ml in the finished product and survived well at 5˚C for several weeks. Where as *L.acidophilus* culture added to set yogurt after its manufacture rapidly lost viability. Recoverable viable organisms dropped by 90-99% within 3-5 days; this dramatic loss termed acidophilus death was attributed to hydrogen peroxide produced by the starter lactobacilli. The better survival of *L.acidophilus* when added along with the starter was presumably due to the transient tolerance of the culture to hydrogen peroxide (Speck et al. 1977). Other factors that have been claimed to affect the growth of probiotic bacteria are the strains used, interaction between species present, culture conditions, final

acidity of the product, and the concentration of lactic and acetic acids. The viability also depends on the availability of nutrients, growth promoters and inhibitors, concentration of sugars, dissolved oxygen and oxygen permeation through packaging materials, incubation temperature, fermentation time, and storage temperature (Shah, 2000).

Dave et al. (1997) reported that pH was found to be the most crucial for *L.acidophilus* culture, yogurt pH <4.4 at the time of fermentation results in 3-4 log cycle reduction in *L.acidophilus* numbers within 20-25 days. The counts declined gradually during storage until 15days and faster thereafter and found the viability dependent on associative yogurt organisms. Same authors in their previous study (Dave et al.1996) reported that the viable counts of *L.acidophilus* were found to be affected by the oxygen content to a greater extent than that of temperature of storage. The dissolved oxygen content directly affected the increase in numbers and survival of *L.acidophilus* during storage. They also reported that oxygen content increased in plastic cups in storage compared to glass bottles. According to Champagne et al. (2005), oxygen affects the probiotic cultures in two ways. The first is a direct toxicity to cells. Certain probiotic cultures are very sensitive to oxygen and die in its presence, presumably due to the intracellular production of hydrogen peroxide. The second way the oxygen effects the probiotic cultures is indirect, when oxygen is in the medium certain cultures, particularly *Lb.delbrueckii*, excrete peroxide in the medium and a synergistic inhibition of bifidobacteria by acid and peroxide has been demonstrated, this suggests that probiotic strains can be affected by the H_2O_2 produced by the other cultures in the environment.

De Angelis et al. (2004) reported that lactobacilli are often exposed changes in the solute concentration of their natural habitats. Nevertheless, their cytoplasmic solute

concentration needs to be relatively constant. A sudden increase in the osmolarity of the environment (hyperosmotic stress) results in the movement of water from the cell to the outside, which causes a detrimental loss of cell turgor pressure, changes the intracellular solute concentration and changes the cell volume.

The increase in hardness of the sample over time, which indicates the drying of the product, shows that there is an increase in the solid content, which along with the sugar content of the product contributes to the osmotic stress. In order to increase viability of probiotic bacteria Shah (2000) proposed microencapsulation of probiotic bacteria where the cells are retained within the encapsulating membrane to reduce the cell injury or loss. He also suggested two-stage fermentation where initial fermentation was carried out with probiotic bacteria and followed by yogurt bacteria after 2 hrs.

3.2 Yogurt Bacteria Counts

The yogurt bacteria counts in log values are reported in Figure 3. The interaction of treatment x Time was significant (p=0.0446)(table 2). There was a significant difference (p=0.0001) between 0 month counts and 3rd month count of control sample. The yogurt bacterial counts decrease over the months. There was no significant difference among various levels of culture addition over the months.

The reduction in lactobacilli counts over time could be attributed to the possible inhibitory effect of *L.acidophilus* on the yogurt bacteria, the moisture loss due to drying over time. The increase in osmotic pressure could contribute to the reduction of viable organisms. Antagonism among the bacteria used in starter cultures caused by antimicrobial substances such as bacteriocins may decrease the numbers of any sensitive organisms that may be present in a product or starter culture (Shah, 2000). Joseph et al.

(1998) isolated 28 strains of yogurt and probiotic bacteria from commercial starter cultures, commercial yogurts and probiotic capsule. Inhibition of 5 *S.thermophilus* strains and 2 bifidobacterial strains observed was due to organic acids but not due to a bacteriocin or bacteriocin like substances. The other 2 strains of *S.thermophilus*, and 6 bifidobacterial strains were not inhibited by organic acids or low pH. *L.delbrueckii ssp.bulgaricus* were not inhibited by most *S.thermophilus* or bifidobacterial strains isolated from market preparations, but they showed strong inhibition due to bacteriocin like substances (BLIS) produced by 7 out of 8 strains. Vinderola et al. (2002) reported an important inhibitory activity of culture filtrates of *L.acidophilus* obtained from milk on the growth of lactic acid starter but not on propionic bacteria. They also mentioned if *L.acidophilus* can grow in the cold storage of fermented milk, its metabolic waste could jeopardize lactic acid starter bacteria viability.

Dave et al. (1996) observed that organisms multiplied in yogurt many more times with the lower level of inoculum than the higher level. However the final counts of organisms remained slightly higher at higher level of inoculation. They suggested that this could be due to *S.thermophilus*, which might be biologically more active in samples prepared with lower inoculum. This was supported by increase in their numbers for up to 10 days in the yogurt prepared with low level of inoculum as compared with 5 days in the yogurt prepared with the higher rate of inoculum. After initial increase up to 5-10 days, the number of these organisms decreased. The results indicate the need to focus on selecting suitable strains of starter culture and probiotic organisms and a proper fermentation combination.

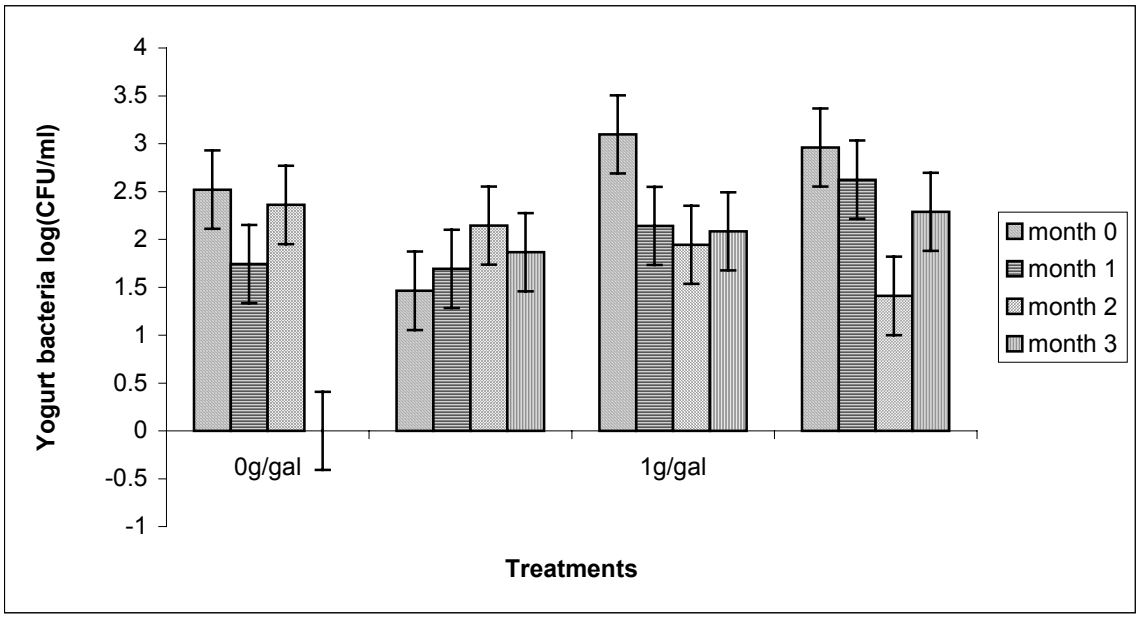

Figure 3. Yogurt bacteria count of novel yogurt product with 0, 1, 10, 100g/gal *Lactobacillus acidophilus* for 0, 1, 2, 3 months. Mean (± SE)

3.3 Coliforms

The coliform counts were <10 cfu/g for all the samples. Contamination with the coliform organism is a common problem in the industry and they are completely undesirable in any products. These organisms are killed during pasteurization and if they are present in the product they are the result of post pasteurization contamination (Kroger, 1976). The absence of coliform organisms indicates that proper care was taken during processing to avoid post-processing contamination and the product is of good quality.

Table 2. Mean Squares and Pr > F of treatments, storage time and their interaction *L.acidophilus* count, Lactobacilli count, coliform count, yeast and mold count

Mean Squares and Pr > F of treatments, storage time and their interaction *L.acidophilus* count, yogurt bacteria count, yeast and mold count.						
Source	*L. acidophilus* count		Yogurt bacteria		Yeast and mold count	
	MS	Pr > F	MS	Pr > F	MS	Pr > F
Tr	3.33255000	0.0032	2.34762500	0.0080	0.42261389	0.0943
Month	7.98454444	<.0001	1.93215833	0.0183	0.30629722	0.1911
Month x Tr	1.73667593	0.0116	1.12476759	0.0446	0.16283611	0.5425
Error	0.59062500		0.50059792		0.1823983	

3.4 Yeast and Mold Count

The yeast and mold counts are reported in Figure 4. There was no treatment x time interaction effect; also there was no treatment effect and no time effect ($p>0.05$)(Table 2). Yeast and mold count indicates contamination with these organisms; particularly with fruit yogurt yeast and mold problems are likely to arise (Kroger, 1976). Yeast and mold can also come from the environment where proper air control system is not in place. The results indicate that proper care was taken to avoid contamination through out the process and there was no post processing contamination.

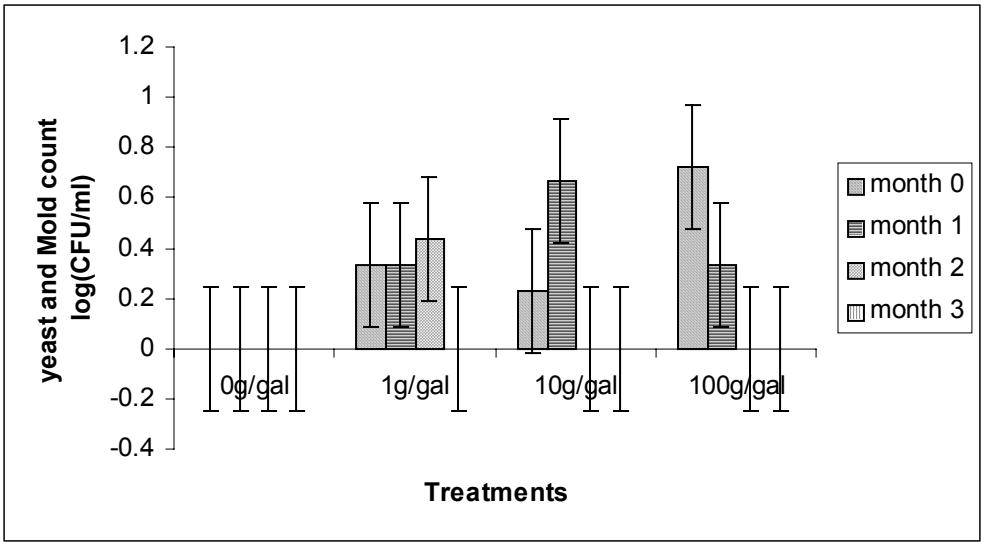

Figure 4. Yeast & mold counts of Novel Yogurt product with 0, 1, 10, 100g/gal *Lactobacillus acidophilus* for 0, 1, 2, 3 months. Mean (± SE)

3.5 Hardness

The hardness is defined as force necessary to attain a given deformation (Uprit and Mishra, 2004). The hardness (N) values are reported in Figure 5. The interaction of treatment x time was significant ($p<.0001$)(table 3). The hardness value for control at month 0 was significantly lower ($p=<.0001$) than the treatments at month 0. This could be attributed to the changes that were caused by the yogurt bacteria; there is evidence that starter organisms cause structural changes in the protein matrix. As cheese age, the protein matrix undergoes proteolysis. The components that make up the cheese undergo rearrangement of bonds, associations, and interactions that result in the infrastructure of the cheese being altered and the most of the changes take place in the first 8 weeks (Van Hekken et al., 2004).

Comparing month 0 to month 3 there was an increase in hardness in the control, and the treatments 1g/gal and 100g/gal. This could be attributed to drying of the product. La Torre et al. (2003) reported that probiotic fermented milk products made with starter cultures producing exopolysaccharides were firmer. Ropiness and the protein matrix are more responsible for hardness (Tunick 2000). Salvador and Fiszman (2004) reported that firmness in yogurt significantly increased with storage and the firmness values for whole yogurt were lower than for nonfat yogurt. They attributed this to lower fat and higher protein content of non-fat yogurt. 100g/gal of *L.acidophilus* culture added product had shown significantly lower hardness when compared to the control and treatments 1g/gal and 10gal/g over months 1,2 and 3. This decrease in hardness values for 100g/gal could be attributed to the increased rate of probiotic addition, resulting in increased moisture

content added along with the probiotic culture, since the culture was added as a freshly thawed culture concentrate. Uprit and Mishra (2004) studied the textural properties of

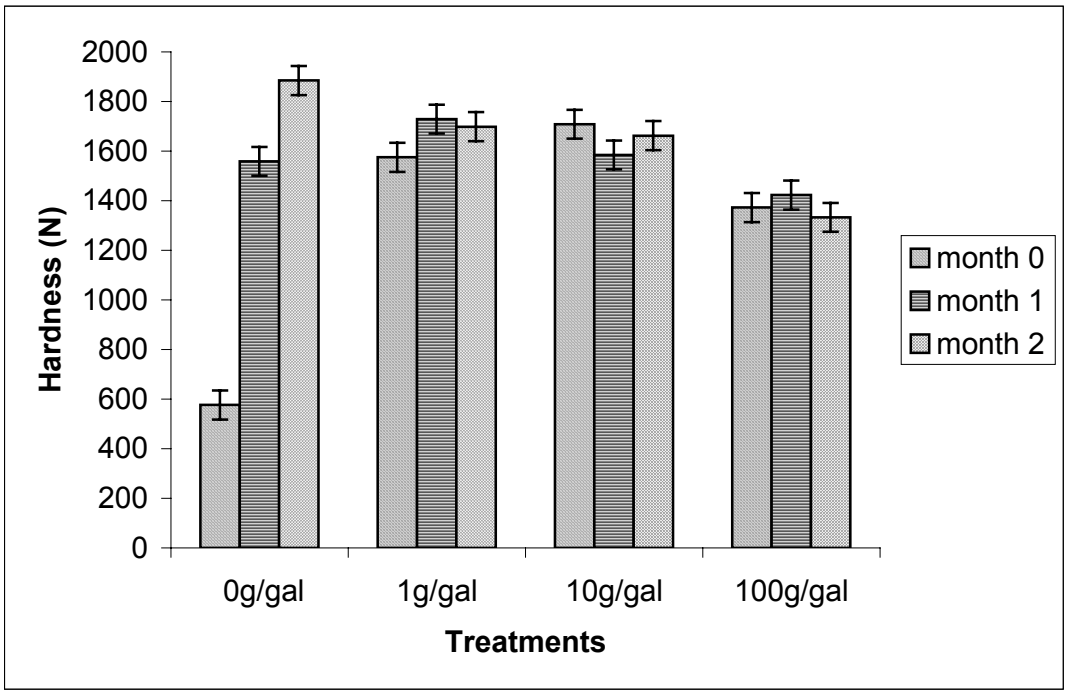

Figure 5. Hardness values of Novel Yogurt product with 0, 1, 10, 100g/gal *Lactobacillus acidophilus* for 0, 1, 2 months. Mean (± SE)

press chilled acid coagulated curd (Paneer) and reported that decrease in hardness can be explained by an increase in moisture content, they correlated the increase in hardness of the curd to the decrease in fat content and attributed it to the compact protein matrix with less open spaces, which are otherwise occupied by milk fat globules. With reduction in fat content, the compact appearance of the protein network increased and the number of milk fat globules dispersed within the network decreased. Tunick et al. (1995) reported that proteolytic breakdown of protein matrix led to decrease in hardness values. There

was no significant change in hardness for control after 2 months. Results are reported only until month 2 because at month 3 the hardness value of control reached beyond the maximum hardness value that could be recorded by the Instron machine, with a maximum 2000N load cell. This had an effect on other attributes (springiness, cohesiveness, chewiness, adhesiveness) of the control product at month 3.

The increase in hardness values in control and all treatments could be attributed to the drying of the sample. The high hardness values can be due to the individual and combined effects of high solid content, the presence of sugar and gelatin. Fizman and Salvador (1999) compared milk gel systems with equal concentrations of gelatin, and observed the forces were to be somewhat greater in the presence of solids, microstructure analysis of milk gels containing 10, 20 and 30% of solids showed 30% solids to appear considerably denser and hence firmer than the 10% solid gel. With a loss of moisture over time, the total solids tend to increase which results in increased hardness.

3.6 Springiness

TPA springiness is the rate and extent to which a deformed material goes back to its un-deformed condition after the deforming force is removed (Kahyaoglu et al., 2005). The springiness (mm) values are reported in Figure 6. The interaction of treatment x time was significant ($p<.0001$)(Table 3). Control sample showed significant decrease in springiness from month 0 to month 2. Bacteria have enzyme systems (Kosikowski 1982). Dairy lactic acid bacterium such as *L.acidophilus* has proteases that hydrolyze dairy proteins. Springiness of Gaziantep cheeses increased with a decrease in the fat content. This may be due to the fact that the absence of fat results in a more flexible protein network and more protein content in reduced fat cheeses supplies more matrix per unit of

volume to recover the cheese to its initial shape after compression. (Kahyaoglu et al., 2005).

Imm et al., (2003) studied the textural properties of bovine and caprine mozzarella cheese and observed that proteolysis could be responsible for textural differences including springiness.

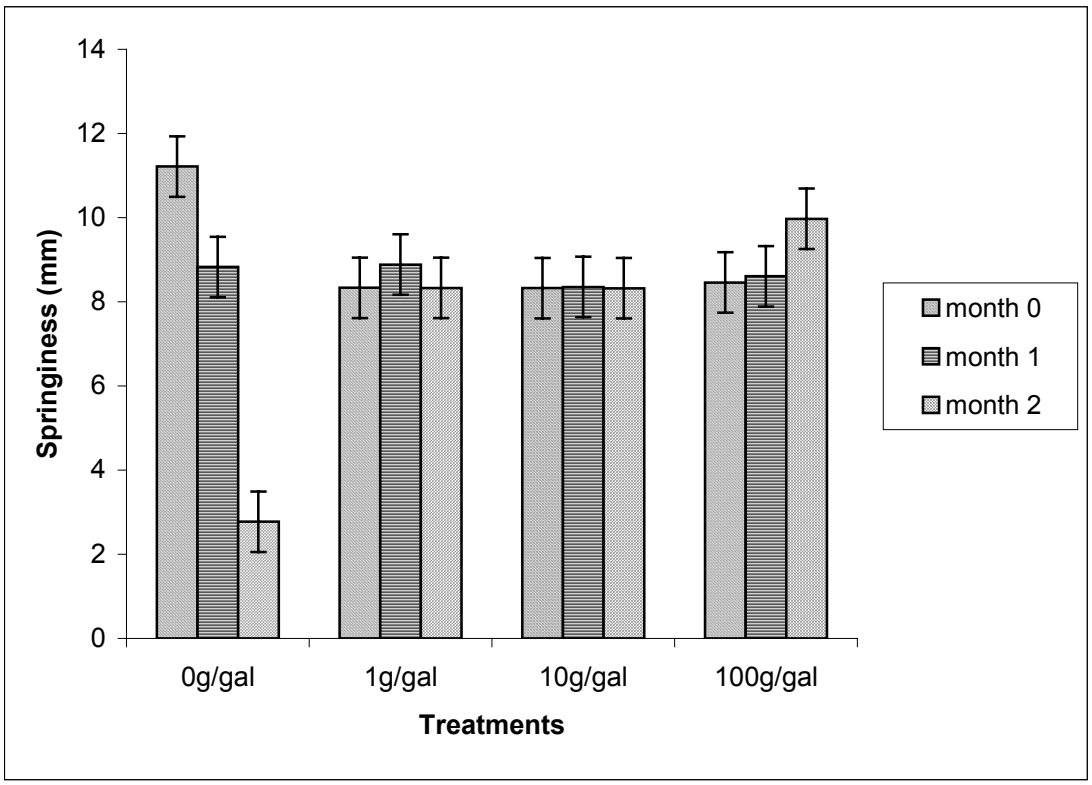

Figure 6. Springiness values of Novel Yogurt product with 0, 1, 10, 100g/gal *Lactobacillus acidophilus* for 0, 1, 2 months. Mean (± SE)

3.7 Chewiness

Chewiness is the energy required to masticate a solid food product to make it ready for swallowing (Uprit and Mishra, 2004). The chewiness values are reported in Figure 7. The interaction of treatment x time was significant (p=0.0002)(Table 3). Control sample showed significant (p<.0001) increase from month 0 to month 1 and a significant decrease from month 1 to month 2. There were no significant difference in chewiness between month 0 and month 2 of the control. The control month 0 and month 2 were significantly lower in chewiness compared to chewiness of all treatments at month 0 and month 2 respectively.

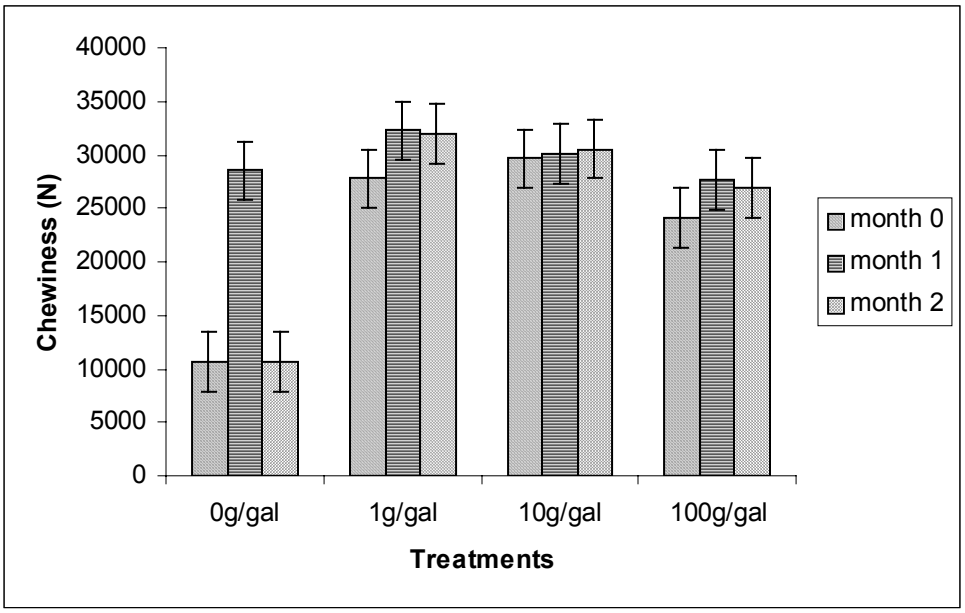

Figure 7. Chewiness values of Novel Yogurt product with 0, 1, 10, 100g/gal *Lactobacillus acidophilus* for 0, 1, 2 months. Mean (± SE)

Table 3. Mean Squares and Pr > F of treatments, storage time and their interaction for hardness, springiness and chewiness

Mean Squares and Pr > F of treatments, storage time and their interaction for hardness, springiness and chewiness						
Source	Hardness		Springiness		Chewiness	
	MS	Pr > F	MS	Pr > F	MS	Pr > F
Tr	207767.370	<.0001	32.2975910	<.0001	946950930	<.0001
Month	403887.989	<.0001	8.9732076	0.0028	99289140	0.0118
Month x Tr	274049.784	<.0001	26.0132891	<.0001	121741542	0.0002
Error	10236.443		1.5490854		23143482	

This increase in chewiness at month 0 and month 2 of the treatments compared to control may be because of action of enzymes of *L.acidophilus* on the yogurt product matrix. There is evidence that starter organisms cause structural changes in the protein matrix, as cheese age, the protein matrix undergoes proteolysis. The components that make up the cheese undergo rearrangement of bonds, associations, and interactions that result in the infrastructure of the cheese being altered (Van Hekken et al., 2004).

3.8 Cohesiveness

Cohesiveness is defined as the extent to which a material can be deformed before its rupture Cohesiveness depends upon the strength of the internal bonds. (Uprit and Mishra, 2004). The cohesiveness values are reported in Figure 8. The interaction of treatment x time was significant ($p<.0001$)(table 4). Control sample showed significant decline in cohesiveness comparing month 0 to month 2. There was a steady increase in

cohesiveness in all treatments from month 0 to month 2. This may have been because of action of enzymes of *L.acidophilus* in the product.

The protein matrix in yogurt is more responsible for cohesiveness (Tunick, 2000). As cheese age, the protein matrix undergoes proteolysis. The components that make up the cheese undergo rearrangement of bonds, associations, and interactions that result in the infrastructure of the cheese being altered (Van Hekken et al., 2004).

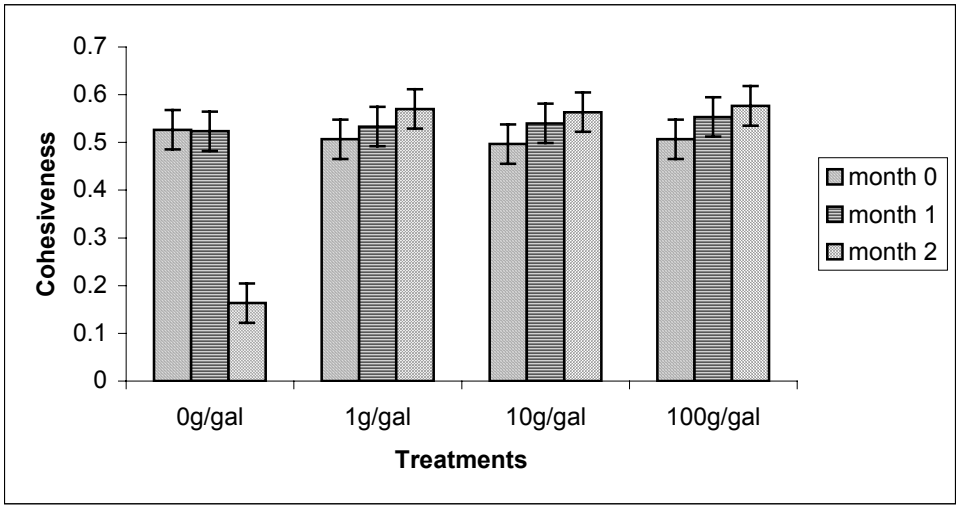

Figure 8. Cohesiveness values of Novel Yogurt product with 0, 1, 10, 100g/gal *Lactobacillus acidophilus* for 0, 1, 2 months. Mean (± SE)

3.9 Adhesiveness

Adhesiveness is the force necessary to remove the material that adheres to the mouth during eating (Uprit and Mishra, 2004). The adhesiveness (J) values are reported in Figure 9. There was no significant treatment x time effect. Neither was the treatment effect significant nor was the time effect significant (Table 4). The increased hardness and drying over time may have caused the loss of adhesiveness.

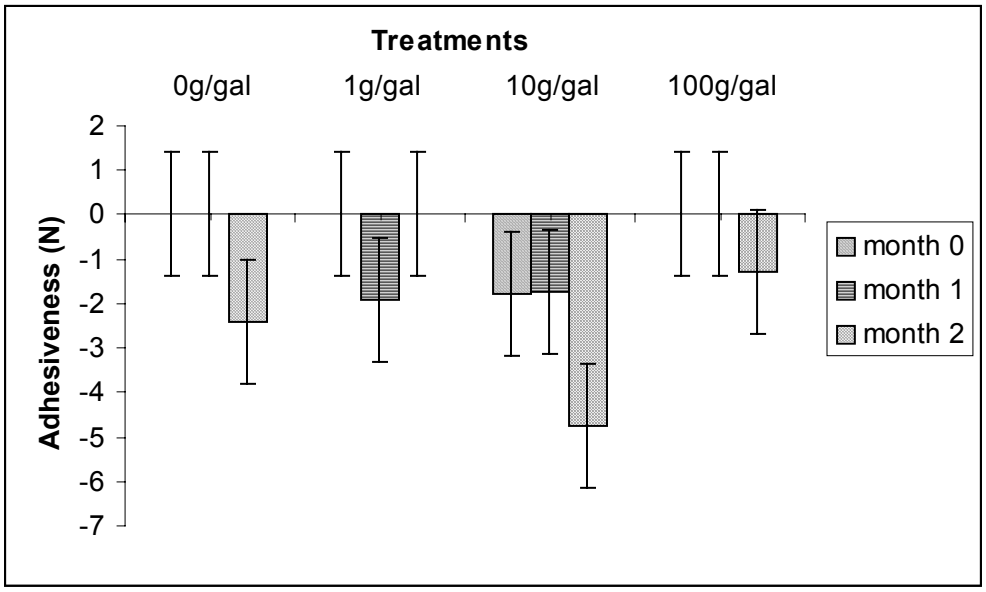

Figure 9. Adhesiveness values of Novel Yogurt product with 0, 1, 10, 100g/gal *Lactobacillus acidophilus* for 0, 1, 2 months. Mean (± SE)

Table 4. Mean Squares and Pr > F of treatments, storage time and their interaction for cohesiveness and adhesiveness

Mean Squares and Pr > F of treatments, storage time and their interaction for cohesiveness and adhesiveness.				
Source	Cohesiveness		Adhesiveness	
	MS	Pr > F	MS	Pr > F
Tr	0.17095208	<.0001	11.28274603	0.1450
Month	0.03442431	0.0012	5.95747027	0.3997
Month x Tr	0.06094653	<.0001	3.55956794	0.7808
Error	0.00511250		5.8541458	

CHAPTER 4:CONCLUSIONS

The results indicate that incorporation of *Lactobacillus acidophilus* at levels as high as 100g/gal in to the novel yogurt product can be achieved with out affecting its textural characteristics. *L.acidophilus* counts significantly decreased over months. The initial counts were not as high as regular yogurt. The stage of culture addition appears to play a significant role in the growth. There seems to be inhibitory effect on acidophilus as well as yogurt bacteria due to increased total solids content and added sugar content possibly of production of inhibitory substances by *L.acidophilus* and/or yogurt bacteria. Yogurt bacterial counts also significantly reduced over time. The drying of the product over time had a significant effect on viable counts of organisms as well as textural properties like hardness, springiness and chewiness. Hardness of the product increased over month, which affected chewiness and springiness. The low coliform and yeast and mold counts indicate that there was no post processing contamination and this novel product could be manufactured with out having contamination problems and can be stored at room temperature for extended period of time.

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