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Influence of "added" lactose on probiotic properties of yogurt culture bacteria and yogurt characteristics

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INFLUENCE OF “ADDED” LACTOSE ON PROBIOTIC PROPERTIES OF YOGURT CULTURE BACTERIA AND YOGURT CHARACTERISTICS

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 The School of Animal Sciences

by
Behannis J. Mena Chalas
B.S., Escuela Agrícola Panamericana Zamorano, 2010
August 2013

For my beloved brother: Cesar Antonio Mena

You are my inspiration and for that you will always live in my heart.

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ABSTRACT

Lactose sugar is a source of energy for Lactic Acid Bacteria (LAB) in dairy fermented products. Enrichment of yogurt with lactose addition may increase growth and viability of the yogurt starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) and enhance yogurt physico-chemical and sensory attributes. The objectives of this study were: to determine the influence of added lactose on (1) acid and bile tolerance of yogurt starter culture *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12, (2) the final lactose content of yogurt during its shelf life, (3) the physico-chemical characteristics of yogurt during shelf life, (4) the growth of the yogurt starter culture during yogurt's shelf life and (5) the sensory attributes of yogurt. Acid tolerance test was conducted on pure culture at 30 minutes intervals for 2 hours of incubation period and bile tolerance at 1 hour intervals during 12 hours. Fat free plain set-type yogurt was manufactured using 0, 1, 3 and 5% w/w added lactose to accomplish objectives 2, 3 and 4. For objective 5, a blueberry yogurt was manufactured using the same lactose levels. Analyses for plain set-type yogurt were done at 7 days intervals during 35 days of storage period. Sensory evaluation was conducted on yogurt 3 days after its manufacture. Data were analyzed using Proc Mixed model of SAS® 9.3 program and by analysis of variance (ANOVA) using Proc GLM. Significant differences between means were analyzed at $\alpha = 0.05$ using Tukey's adjustment. Lactose had a positive effect on acid tolerance and bile tolerance of both yogurt starter cultures, growth of *S. thermophilus* and sensory attributes of flavored yogurt. Mean overall liking scores were higher for samples containing added lactose compared to control as tested by 100 panelists. Lactose supplementation in yogurt might be a good approach to increase its health benefits, acceptability and purchase intent among consumers.

CHAPTER 1: INTRODUCTION

1.1 PROBIOTICS

Dairy products with incorporated probiotic bacteria are gaining popularity (Agrawal, 2005). Probiotics comprise approximately 65% of the world functional food market (Figueroa-Gonzalez *et. al.*, 2011). In 2010-2012 period, the global market retail value for probiotics increased from \$22 to \$27 million and is expected to reach about \$29 billion in sales by 2015 (Figueroa-Gonzalez *et. al.*, 2011) thus the importance on studying the behavior of these bacteria in dairy foods. Shah (2007) affirmed that the most extensively used organisms, for human gut health, in probiotic preparations are lactic acid bacteria, particularly the species of *Streptococcus* ssp. and *Lactobacillus* ssp. Parvez *et. al.* (2006) stated that among the health effects the consumption of lactic acid bacteria provides, there are: (1) improvement of intestinal tract health; (2) enhancement of the immune system, synthesis and enhancement of the bioavailability of nutrients; (3) reduction of lactose intolerance symptoms, reduction of allergy prevalence in vulnerable individuals; and (4) reduction of risk to suffer certain cancers.

There are several requirements microorganisms should meet to be consider as probiotics. The bacteria in the product should be metabolically stable and active, stay alive in large numbers during the passage through the upper digestive tract and have beneficial effects once in the intestine of the host (Gilliland, 1989). The requirement for any food sold with health claims from the addition of probiotics is that it must contain at least 10^6 - 10^7 CFU per gram of viable probiotic bacteria (FAO/WHO, 2001).

1.2 LACTIC ACID BACTERIA (LAB)

Many bacteria are present in milk, particularly *lactobacilli* species (Minard, 1990). Kumar-Anal and Singh (2007) defined LAB as “Gram-positive, rod-shaped, non-spore-forming, catalase-negative organisms that are devoid of cytochromes and are of non-aerobic habit but are aero-tolerant, acid-tolerant and strictly fermentative.” According to Axelsson (1993) “lactic acid is the major end-product of sugar fermentation from these bacteria.”

Lactic acid bacteria must survive the adverse conditions found in the gastrointestinal tract and intestine. These bacteria must be tolerant to acid and bile salts which enable a selected strain to survive, grow, and perform its therapeutic benefits in the gastrointestinal tract to be used as a probiotic culture or as food adjunct (Gilliland and Walker, 1989; Salminen and von Wright, 1993; Usman and Hosono, 1996). Probiotic bacteria should be resistant to the enzymes in the oral cavity (e.g., lysozyme) since they are delivered in a food system their journey begin via the mouth through the gastrointestinal tract (Fuller, 1992) and should also have the ability to resist the digestion process in the stomach.

Lactic acid bacteria genera are typically formed by low proteolytic activity-fermentative bacteria, which mean that they will ingest sugars to metabolize them and produce essentially lactic and acetic acids as their catabolic products. It is generally assumed that *lactobacilli* are the major inhabitants of the small bowel (Morelli, 2001). Klein *et. al.* (1998) affirmed that “physiological characteristics of LAB of interest for taxonomic considerations are carbohydrate fermentation patterns, resistance to different NaCl concentrations, growth on different nutrient media and temperatures, resistance against antibiotics and production of Short Chain fatty Acid (SCFA).”

1.2.1 Acid Tolerance of Lactic Acid Bacteria

Berrada *et. al.* (1991) reported that the time from entrance of the bacteria in the stomach to release from the stomach is 90 minutes. Cellular stress begins in the stomach, which has pH as low as 1.5 (Lankaputhra and Shah, 1995). Generally, the pH of the stomach ranges from 2.5 to 3.5 (Holzapfel *et. al.*, 1998). After traveling through this tough environment, the organism colonizes the epithelium of the lower intestinal tract (Conway *et. al.*, 1987). Therefore, strains selected to be used as probiotic bacteria should be able to tolerate acid for at least 90 minutes, tolerate bile salts concentration, attach to the epithelium, and grow in the lower intestinal tract before they can start providing any health benefits (Chou and Weimer, 1999).

The growth of lactic acid bacteria differs depending if the medium has acid conditions only or if it has a combination of acid and bile salts which is the expected scenario to be found in the gut. Therefore, these bacteria are required to tolerate the combined environment to be considered as probiotic. The pH values ranging from 2.0 to 8.0 can be found in the gastrointestinal tract (Hood and Zottola, 1988). According to Beal *et. al.* (1989) optimum growth conditions for *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are at pH 6.5 and 40° C and 5.8 and 44° C respectively.

1.2.2 Bile Tolerance of Lactic Acid Bacteria

According to Chou and Weimer (1999) after the bacteria pass through the stomach, they enter the upper intestinal tract where bile is secreted into the gut. The bile salt concentration in the gut is not static; it changes over time and in the different parts of the small intestine (Marteau *et. al.*, 1997); therefore, it is difficult to predict at any given moment (Lankaputhra and Shah, 1995).

Gastrointestinal conditions are determinant on the survival rate of lactic acid bacteria. Based on the results obtained in static in vitro models, some researchers (Floch *et. al.*, 1972; Simon and Gorbach, 1987) have reported that the bactericidal effects of conjugated bile acids are weaker than those of free bile acids. Bile salts that form micelles with phospholipids (as they are found in whole bile) have lower antibacterial activity than artificial solutions of pure bile salts (Stewart *et. al.*, 1987). Bacteria in yogurt after being consumed are exposed to bile in the intestines which alters the permeability of the bacterial cells so that lactose can enter the cells and be hydrolyzed.

The mechanism of lactose digestion seems to be linked to the release of β -galactosidase from bacterial cells during the transit through the small intestine; therefore the ability of the yogurt culture to hydrolyze lactose allows these strains to easily function as a source of enzyme in the intestinal tract (Gilliland and Kim, 1983).

Lactic acid bacteria including *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* are able to produce extracellular polysaccharides (EPS) that either encapsulate the bacteria or are excreted into the extracellular environment (Boke *et. al.*, 2010). The EPS functions as a natural encapsulation for the bacteria which offers a protective shield to adverse environmental conditions. There is a positive correlation ($r = 0.998$ for *streptococci* strains, $r = 0.992$ for *lactobacilli* strains) between the EPS production quantity of the strains and resistance to bile salts; this correlation is significant at 0.01 level (Boke *et. al.*, 2010). Goldin and Gorbach (1992) recommend concentrations between 0.15-0.3% of bile as a suitable concentration for selecting probiotic bacteria for human use.

Probiotic cultures must be capable to survive in the environment with gastric and bile acids (Pato, 2003). Standards for acid tolerance and bile tolerance of probiotic culture has been established as resisting at pH 3 for 2 hours and growing in the medium containing 0.1% of bile salts (Itoh, 1992; Gohran, 1994).

1.2.3 Influence of Sugar and Total Solids on growth of Lactic Acid Bacteria

Christensen (1970) states that the amount of sugar added to yogurt milk should not exceed 9% because it may inhibit culture growth. Tramer (1973) added increasing concentrations (5-11%) of dry sugar to skim milk, inoculated with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* and incubated at 42°C for 5 hours. The results showed a marked inhibition of *Lactobacillus bulgaricus* growth with increasing concentrations of sugar. On the contrary, *Streptococcus thermophilus* did not seem to be affected by the concentrations used (Tramer, 1973). Tramer (1973) added skim milk concentrate into yogurt manufacture to increase total solids content and analyzed the inhibitory effect of high total solids content. They concluded that it is not only the sugar but also the total solids which cause the inhibition of *Lactobacillus bulgaricus* due to reduction of water activity (a_w) and interference with metabolic activities. Cultures vary slightly in their resistance to total solids. The overall critical total solids concentration is around 22% above which severe inhibition will occur (Tramer, 1973).

1.3 LACTOSE

Lactose also known as “milk sugar” is a disaccharide carbohydrate, composed of two monosaccharide components: glucose and galactose (Adams, 2012). As a carbohydrate, lactose provides a ready source of energy to living organisms (Janine, 2011).

Lactose percent in milk varies among species and food product; in cow's milk is around 4.3-4.8% (Food Standards Agency, 2002). According to Minard (1990) "lactose is the only carbohydrate that mammals synthesize. It is synthesized in the mammary glands. Hydrolyzed, it yields one molecule of D-glucose and one of D-galactose." As the Code of Federal Regulation states, lactose is normally obtained from whey (CFR, 2012). According to Lee and Lucey (2010), lactose can be used as sweetener in low calorie products and β -galactosidase is added to hydrolyze it as the products are glucose and galactose, which are much sweeter than lactose itself.

1.3.1 Metabolism of Lactose

Lactic acid bacteria do not metabolize lactose directly. Lactose is transferred into the bacteria's cell where it is hydrolyzed to glucose and galactose by using lactose-permease enzymes (Neves *et. al.*, 2005), allowing its digestion. The mechanism by which lactose is transported determines largely the pathway for the hydrolysis of the internalized disaccharide and the fate of the glucose and galactose moieties.

There are two enzymes responsible of lactose hydrolysis. β -galactoside permease is a membrane-bounded enzyme which allows lactose to get inside the bacteria's cell where β -galactosidase enzyme breaks lactose. Several processes can occur inside the cell once lactose is hydrolyzed: Glucose is glycolysed to pyruvate through the Embden-Mayerhof-Parnas pathway by lactic dehydrogenase and converted to lactic acid (Venkatesh *et. al.*, 1993).

On the other hand, galactose is phosphorylated to galactose 1-phosphate by galactokinase and converted to glucose 1-phosphate and galactose 6-phosphate. For most species lactic acid and galactose will come out of the cell, but for some strains galactose will be metabolized to lactic acid as well. Biochemical and genetic studies have indicated that lactose can be transported via “phosphotransferase systems, transport systems dependent on ATP binding cassette proteins, or secondary transport systems including proton symport and lactose-galactose antiport systems” (De Vos and Vaughan, 1994).

Lactococcus species have a faster metabolism of lactose compared to other lactic acid bacteria (Marshall, 1987). According to Samarzija *et. al.*, (2001) the difference is in the simultaneous catabolism of glucose and galactose from *Lactococcus* spp. Lactose is phosphorylated by phosphoenolpyruvate (PEP) during translocation by PEP-dependant phosphotransferase system (PEP: PTS). The intracellular lactose phosphate is subsequently hydrolyzed to glucose and galactose by β -D-phosphogalactosidase enzyme (Samarzija *et. al.*, 2001). Marshall and Tamime (1997) reported that the galactose is then catabolized via the Tagatose pathway at the same time as the glucose is catabolized via Embden-Mayerhof-Parnas pathway for some bacteria strains.

1.3.2 Lactose and Yogurt Characteristics

1.3.2.1 Lactic acid production

Lactic acid is one of the flavor compounds in yogurt. De Vos and Vaughan (1994) affirmed that lactose utilization is the primary function of lactic acid bacteria used in industrial dairy fermentations. According to Tramer (1973) the rate of acid production varies with the temperature of incubation.

There is a noticeable difference in development of acidity within the first two hours of incubation depending on the temperature, but after three hours, it becomes less apparent especially in the 40-45°C range (Tramer, 1973). Lactic acid bacteria convert lactose into lactic acid which reacts with proteins in the milk, causing them to precipitate at pH 4.6, and make the milk creamier. The lactic acid has a sour taste, which causes a change in flavor of the fermented product, e.g. yogurt and cheese (Hendrickson, 2011). These lactic acid bacteria hydrolyze lactose and produce lactic acid mainly from the glucose portion of lactose (Minard, 1990); however some strains can utilize the galactose portion as well.

The capacity to ferment a type of sugar varies depending on the culture strain. *Streptococcus thermophilus* is capable to ferment lactose, sucrose, glucose and fructose, whereas, *Lactobacillus bulgaricus* ferments lactose, glucose, fructose and galactose, it does not ferment sucrose (Estevez *et. al.*, 2010). *Streptococcus thermophilus* ferments lactose in milk to L(+) lactic acid from 0.7 to 0.8% which is more readily metabolized by humans compared to D(-) lactic acid produced by *Lactobacillus delbrueckii* ssp. *bulgaricus* up to 1.7% in milk (Trachoo, 2002). According to Gilliland and Kim (1983) yogurt starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) do not utilize or hydrolyze lactose except as needed for growth. This indicates that the culture must be growing if it is to hydrolyze any lactose in milk.

1.3.2.2 pH

The pH is an important quality attribute of yogurt. As lactose is converted to lactic acid by the fermentation process conducted by lactic acid bacteria, the pH drops from 4.7 to 4.5 approximately.

Streptococcus thermophilus grows first and reduces the pH to 5.0 while *Lactobacillus bulgaricus* can bring the pH down up to 3.8 (Tramer, 1973). When the pH end point of 4.6 is achieved, the yogurt mixture is cooled to slow the reaction. Incorrect pH levels can lead to excessive free whey production and excess or insufficient tartness (Bakar, 2012). Gavin (1966) stored yogurt at 4°C and found that within six days of storage the pH dropped from 4.15 to 3.98 and from 4.62 to 4.15.

1.3.2.3 Titratable acidity (TA)

According to Trachoo (2002) yogurt has a titratable acidity of not less than 0.9%, expressed as lactic acid. Adding non-fat dry milk increases caloric value of yogurt and acid production since about 50% of non-fat dry milk is lactose (Kalab *et. al.*, 1983). Tramer (1973) affirmed that the increase in acidity it is what produces the characteristic coagulum of yogurt and thus a reasonable development of acidity is required to achieve the desired texture of the product. Goff (2009) affirmed that enzymes in the yogurt starter bacteria convert the disaccharide lactose into lactic acid. As the acid accumulates in milk and the acidity increases, proteins denature and the milk thickens and takes an acidic taste. Acidity variation can change the texture of yogurt. At lower acidity the yogurt will be sweeter and thinner, at high acidity yogurt will be thicker and sourer at higher acidity (Goff, 2009).

1.3.2.4 Viscosity

Viscosity of yogurt is greatly influenced by the total solids content of yogurt milk, especially the protein content (Lee and Lucey, 2010). Measuring viscosity of yogurt is challenging because it is a non-Newtonian fluid, i.e. viscosity changes as shear stress changes (Charm, 1971).

Therefore, in order to report apparent viscosity of yogurt, the measurement conditions used has to be precisely specified (Trachoo, 2002). According to Lee and Lucey (2010) rotational viscometers, such as the Brookfield viscometer, are often used to describe the flow behavior of yogurts. The fact that yogurt exhibit non-Newtonian behavior, as previously mentioned is a drawback of this method since viscosity is dependent on shear rate. The Brookfield viscometer only measures apparent viscosity at one spindle speed. Thus, only limited information on the major flow properties of yogurt can be obtained (Lee and Lucey, 2010). According to Trachoo (2002) viscosity of yogurt is affected by composition, type of starter cultures, heat treatment and stabilizer usage. As the total solids increase, viscosity and firmness increase (Becker and Puhon, 1989; Guirguis *et. al.*, 1984). Ropy strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* have been studied and used to produce smooth and viscous yogurt (Hess *et. al.*, 1997; Vedomuthu, 1991). These bacteria are called slime producing bacteria and increase viscosity by producing extracellular polysaccharides (Trachoo, 2002).

1.3.2.5 Syneresis

Syneresis or spontaneous whey separation on the surface of set yogurt is regarded as a defect (Amatayakul *et. al.*, 2006). This problem can be reduced by increasing the milk solids content to approximately 15% (Tamime and Deeth 1980; Shah 2003). Harwalkar and Kalab (1986) found that yogurt with higher total solids content was less susceptible to syneresis and that sodium caseinate was the most effective supplement to increase gel strength and reduce syneresis of yogurt. Soy protein isolates have also been investigated to replace non-fat dry milk in yogurt manufacturing to improve viscosity and reduce syneresis (Kolar *et. al.*, 1979).

According to Trachoo (2002), homogenization breaks down fat into smaller globules which prevents the formation of a cream line. This improves the consistency and viscosity of yogurt, thus a greater stability to syneresis can be obtained (Rasic and Kurmann, 1978; Tamime and Deeth, 1980; Tamime and Robinson, 1985). However, Schmidt and Bledsoe (1995) reported that homogenization has an adverse impact on yogurt with a lower fat content; it increases syneresis reducing water holding capacity due to empty spaces between casein matrices, and lack of native milk-fat globule membrane (FGM). In higher fat yogurts clusters of fat globules can fill up these spaces, thus syneresis can be minimized (Trachoo, 2002). Harwalkar and Kalab (1986) found that an increase in total solids increased the density of yogurt matrices which resulted in decreased syneresis.

Other studies showed that the increase in the concentration of available nutrients affected the EPS and lactic acid production by lactic acid bacteria (Amrane and Prigent 1998; Hassan *et. al.*, 2001; Zisu and Shah 2003). Nonfat yogurt is normally low in total solids (10 to 12%) and consequently suffers from whey separation or syneresis (Schellhaass and Morris, 1985).

The method used to determine syneresis in yogurt might influence the results. The most common techniques used to determine whey syneresis are drainage method and centrifugation method (Harwalkar and Kalab 1986; Guzman-Gonzalez *et. al.*, 1999, 2000; Bhullar *et. al.*, 2002; Jaros *et. al.*, 2002). Both of these methods give high-precision results; however they do not measure the actual value of spontaneous whey separation in set type yogurt since the breakage of the yogurt gel as well as the presence of EPS may influence the result (Amatayakul *et. al.*, 2006).

Lucey *et. al.* (1998) developed a method for the measurement of spontaneous whey separation in set type yogurt called the siphon method. This method determines the level of spontaneous whey separated on the surface of gels. By comparing between the three methods (drainage, centrifugation and siphon), the siphon method would be more appropriate in the determination of spontaneous whey separation level on the surface of set yogurt (Amatayakul *et. al.*, 2006).

1.4 YOGURT STARTER CULTURE

The Code of Federal Regulation defines yogurt as a culture food that contains the lactic acid-producing bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (CFR, 2012). Yogurt starter cultures use lactose as a source of energy fermenting it to lactic acid. These strains have a synergistic effect of one over the other. Initially *Streptococcus thermophilus* grows faster than *Lactobacillus bulgaricus* and releases lactic acid creating an acidic environment that favors the growth of *Lactobacillus bulgaricus*. *Streptococcus thermophilus* also produces formic acid and carbon dioxide (CO₂) which stimulates the growth of *Lactobacillus bulgaricus*. *Lactobacillus bulgaricus* has a high proteinase activity to produce peptides that are utilized by *Streptococcus thermophilus* which has a high peptidase activity to act on the peptides and release free amino acids that are utilized by both microorganisms (Trachoo, 2002).

Studies have been performed during the past trying to increase the number of viable cells of LAB that pass through the gastrointestinal tract. However, studies related to the effect of added lactose, as energy source, for LAB are scarce, so the relationship that exists between added lactose and the bacteria's behavior it is still unclear.

1.5 JUSTIFICATION

Lactose is important for the metabolic activities of lactic acid bacteria and has a beneficial role in the manufacture of fermented dairy products. Lactose is an energy source for lactic cultures, it is fermented to lactic acid which lowers pH and results in coagulation of caseins which forms a range of fermented products, e.g., yogurt which is a popular dairy product with sales in the US continuously increasing over the last 12 years. The relationship that exists between “added” lactose and probiotic properties of yogurt culture bacteria and yogurt characteristics is unknown. Would “added” lactose result in more benefits, particularly enhancing favorable characteristics of yogurt culture bacteria and yogurt attributes? If yes, at what level would the “added” lactose be beneficial? The hypothesis was that “added” lactose can stimulate bacterial growth, enhance their probiotic characteristics and improve the physico-chemical characteristics of yogurt. The objectives of this study were:

1. To determine the influence of “added” lactose at various concentrations on the acid tolerance and bile tolerance of the starter culture *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12.
2. To study the influence of “added” lactose on final lactose content of yogurt over its shelf life.
3. To elucidate the influence of “added” lactose on the physico-chemical characteristics of plain set yogurt over its shelf life.
4. To study the influence of “added” lactose at various concentrations in plain set yogurt on the growth of *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12 during yogurt’s shelf life.
5. To determine the influence of “added” lactose on the sensory attributes of a blueberry yogurt.

CHAPTER 2: MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

Treatments consisted of three concentrations of added lactose (1, 3 and 5% w/w) separately incorporated into a plain set-type yogurt. The control did not have any added lactose. For the first objective, acid tolerance was determined every 30 minutes for 2 hours while bile tolerance was determined every hour for 12 hours. Both analyses were conducted on pure culture and three replications were conducted. For the second, third and fourth objectives lactose content, physico-chemical and bacteriological attributes of plain set-type yogurts were analyzed at days 1, 7, 14, 21, 28 and 35. Three replications were conducted; replications were the blocks. For the fifth objective, a sensory study for consumer acceptance of blueberry yogurt with added lactose was performed with 100 panelists; panelists were the blocks. This study was conducted and analyzed as a Randomized Block Design (RBD).

2.2 YOGURT MANUFACTURE

This study was on lactose addition to yogurt. Two types of yogurt were manufacture. The first was lactose added plain set yogurt to avoid interference of flavorings to be used for physico-chemical and microbiological analyses. The second was lactose added blueberry yogurt for sensory evaluation. Plain set-type yogurt was manufacture according to standard procedure at the Louisiana State University Dairy Processing Plant. The yogurt mixture containing skim milk and added lactose at 1, 3 and 5% w/w was poured into previously cleaned and sanitized pails. Non-fat dry milk was added to keep total solids constant.

The mixture was preheated to 60°C then homogenized in a two stage homogenizer (Type: 300 DJP4 2PS, Gaulin, Manton-Gaulin MFG Co Inc., Everett, MA, USA) at 13.8 MPa for the first stage and 3.45 MPa for the second stage and later pasteurized at 85°C for 30 minutes. Yogurt mix formulations are reported in Table 1.

Yogurt mix was cooled to 40°C and inoculated. Freshly thawed frozen yogurt starter culture concentrate of *Streptococcus thermophilus* (ST-M5) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB-12) (CH-3, yogurt culture, Chr. Hansen's Laboratory, Milwaukee, WI, USA) was added at 0.75 mL per 3.78 L of milk for each bacteria strain, 7.56 L of skim milk per treatment were used. After mixing, the yogurt mix was poured into previously labeled 340 g plastic cups. The inoculated mixture was incubated at 40 °C until pH reached 4.65 ± 0.1 to obtain a set-type yogurt, and transferred to the cooler at 4°C for refrigeration until further analyses. Yogurt manufacture was replicated 3 times.

Table 1. Fat free plain set-type yogurt formulations.

INGREDIENTS	LACTOSE PERCENTAGES			
	0%	1%	3%	5%
Skim Milk	7.56 L	7.56 L	7.56 L	7.56 L
Non-fat Dry Milk	500 g	427.43 g	282.28 g	131.13 g
Lactose	0 g	72.57 g	217.72 g	362.87 g
Starter Culture	3 mL	3 mL	3 mL	3 mL

A separate batch of yogurt was manufactured for sensory evaluation. This yogurt was blueberry flavored with the same lactose treatments (0, 1, 3 and 5% w/w added lactose). The same manufacture process was used with the exception that 15% w/w blueberry puree was added after plain yogurt manufacture and refrigerated at 4°C.

2.3 PREPARATION OF MEDIA

2.3.1 Peptone Water

Peptone water (0.1%) was prepared by dissolving 1g of peptone powder (Bacto™ Peptone, Difco, Dickinson and company, Sparks, MD) in 1L of distilled water, then autoclaved in 99mL bottles at 121°C for 15 minutes.

2.3.2 *Lactobacilli* MRS Broth

The MRS broth for *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 growth was prepared according to the instructions given by the manufacturer (Difco™, Dickinson and company, Sparks, MD): 55g of MRS broth powder were weighed and suspended into 1L of distilled water, then autoclaved at 121°C for 15 minutes.

2.3.3 M17 Broth

M17 broth for *Streptococcus thermophilus* ST-M5 growth was prepared according to the instructions given by the manufacturer (Oxoid, Basingstoke, UK): 37.25g of M17 broth powder were weighed and suspended into 950 mL of distilled water, then autoclaved at 121°C for 15 minutes.

2.3.4 M17 Agar

M17 agar was prepared according to manufacturer's directions (Oxoid, Basingstoke, UK), and used as a selective media for *Streptococcus thermophilus* (Saccaro *et. al.*, 2011): 37.25g of M17 broth powder and 11g of agar powder were suspended in 950 mL of distilled water. The mixture was autoclaved at 121°C for 15 minutes then cooled to 50°C, and 50 mL of a sterile lactose solution (10% w/w) were aseptically added. To prepare the 10% (w/w) lactose solution, 1 g of lactose were dissolved in 100 mL of distilled water and sterilized by membrane (0.2 µm) filtration.

2.3.5 *Lactobacilli* MRS Agar

Difco *Lactobacilli* MRS agar for *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 growth was prepared according to manufacturer's directions (Difco™, Dickinson and company, Sparks, MD): 55g of MRS broth powder and 15g of agar powder were weighed and suspended into 1L of distilled water. A few drops of 1 N HCL were added to reduce pH to 5.2 ± 0.1 then autoclaved at 121°C for 15 minutes (Dave and Shah 1996).

2.3.6 *Streptococcus thermophilus* Agar

Streptococcus thermophilus agar was prepared according to Dave and Shah (1997): 10g of tryptone, 10g of sucrose, 5g of yeast extract and 2g of dipotassium phosphate (K_2HPO_4) were dissolved in 1L of distilled water. The pH of mixture was adjusted to 6.8 ± 0.1 by adding a few drops of 1 N HCL and 6 mL of 0.5% bromocresol purple solution was added as an indicator, and 12g of agar was added to the mixture. The medium was autoclaved at 121°C for 15 minutes.

2.4 MICROBIOLOGICAL ANALYSES

2.4.1 Acid Tolerance

Acid tolerance was determined according to the method proposed by Pereira and Gibson (2002), with slight modifications for the two cultures. The control and added lactose samples were inoculated separately with 1% (v/v) of pure culture in a previously acidified broth adjusted to pH 2 using 1 N HCL. The MRS broth (Difco™, Dickinson and company, Sparks, MD) was used for *Lactobacillus bulgaricus* LB-12 and M17 broth (Oxoid, Basingstoke, UK) for *Streptococcus thermophilus* ST-M5. The inoculated acidified broths were incubated at 43°C for *Lactobacillus bulgaricus* LB-12 and at 37°C for *Streptococcus thermophilus* ST-M5 during 2 hours of incubation period. Acid tolerance for *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12 was determined separately by plating the control and added lactose samples every 30 minutes up to 2 hours. An aliquot of the inoculated broths were serially diluted in peptone water (0.1% w/w) and pour plated in duplicate. *Lactobacillus bulgaricus* LB-12 was enumerated using *Lactobacilli* MRS agar (Dave and Shah, 1997) and *Streptococcus thermophilus* ST-M5 was enumerated using M17 agar (Jordano *et. al.*, 1992). Petridishes were kept anaerobically at 43°C for 72 hours for *Lactobacillus bulgaricus* LB-12 and aerobically at 37°C for 24 hours for *Streptococcus thermophilus* ST-M5. After the incubation period a colony counter (Darkfield Quebec Colony Counter, American Optical, Buffalo, NY) was used to assist the enumeration of colonies.

2.4.2 Bile Tolerance

Bile tolerance was determined according to the method proposed by Pereira and Gibson (2002) and Dave and Shah (1996), with slight modifications.

The bile tolerance of the two cultures was analyzed in THIO broth [MRS broth (Difco™, Becton, Dickinson and company, Sparks, MD) for *Lactobacillus bulgaricus* and M17 broth (Oxoid, Basingstoke, UK) for *Streptococcus thermophilus*]. Both, THIO broths and M17 broths, were individually supplemented with 0.3% (w/w) oxgall (bovine bile) (US Biological, Swampscott, MA). Oxgall was added to test bile tolerance of both bacteria and 0.2 % (w/w) sodium thioglycolate (Acros Organics, Fair Lawn, NJ) was added as oxygen scavenger to the THIO broth for *Lactobacilli* only. Control and added lactose samples were individually inoculated with 10% (v/v) of pure culture in THIO broth and M17 broth and incubated for 12 hours at 43°C for *Lactobacillus bulgaricus* LB-12 and at 37°C for *Streptococcus thermophilus* ST-M5. Each hour for 12 hours of incubation period, an aliquot of the inoculated broths was serially diluted in peptone water (0.1% w/w) and pour plated in duplicate. *Lactobacillus bulgaricus* LB-12 was enumerated using *Lactobacilli* MRS agar (Dave and Shah, 1997) and *Streptococcus thermophilus* ST-M5 was enumerated using M17 agar (Jordano *et. al.*, 1992). *Lactobacillus bulgaricus* LB-12 petridishes were kept anaerobically at 43°C for 72 hours and aerobically at 37°C for 24 hours for *Streptococcus thermophilus* ST-M5. The colonies were counted after the incubation period.

2.4.3 Growth

Growth of *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB-12) and *Streptococcus thermophilus* (ST-M5) was determined using the pour plate technique with serial dilutions of yogurt samples. Yogurts were sampled at days 1, 7, 14, 21, 28 and 35 of storage period. The yogurt in the cup was agitated and 1g of yogurt was pipetted from the center of the cup into a sterile bottle containing 99mL of sterile 0.1% peptone water (Difco, Detroit, MI, USA).

Contents in bottle were agitated to prepare serial dilutions and plated on 5.2 modified pH *Lactobacilli* MRS agar for *Lactobacillus bulgaricus* LB-12 and *Streptococcus thermophilus* agar for *Streptococcus thermophilus* ST-M5. Pour plates were incubated anaerobically at 43°C for 72 hours for *Lactobacillus bulgaricus* LB-12 (Tharmaraj and Shah, 2003) and aerobically at 37°C for 24 hours for *Streptococcus thermophilus* ST-M5 (Dave and Shah, 1996). The colonies were counted after the incubation period.

2.4.4 Coliform Counts

The blueberry yogurt was tested for coliforms before conducting the sensory evaluation using petrifilms (3M®, St. Paul, MN) which contain violet red bile agar (VRBA). The procedure was performed by weighting 11g of yogurt samples and pouring into a sterile bottle containing 99mL of sterile 0.1% peptone water (Difco, Detroit, MI, USA). Contents in bottle were agitated to prepare serial dilutions. Aliquots of 1mL were taken from dilutions 10^{-1} and 10^{-2} and plated in duplicate for control and added lactose samples. Previously labeled petrifilms were kept aerobically at 32°C for 24 hours.

2.5 ANALYTICAL PROCEDURES

2.5.1 Lactose Content

2.5.1.1 Sample preparation

Lactose concentration on yogurts was determined using the Lactose/D-Galactose determination kit from R-Biopharm AG® (Washington, MO, USA). Lactose concentration was measured every 7 days for 35 days of storage time for each replication. This UV method was performed using spectrophotometry analysis.

For control and added lactose samples, 1g of sample was accurately weighed into a 100mL volumetric flask. To dilute the sample 60mL of distilled water was added and flasks were incubated for 15 minutes at 70°C; shaking from time to time. For clarification, 5mL of Carrez-I-solution (3.60g potassium hexacyanoferrate(II), $K_4[Fe(CN)_6] \times 3 H_2O/100mL$), 5mL of Carrez-II-solution (7.20g of zinc sulfate, $ZnSO_4 \times 7 H_2O/100mL$) and 10mL of NaOH (0.1 M) were added and mixed after each addition; the sample solution was adjusted to 20-25°C and filled up to 100mL with distilled water, then filtered using a 12.5 cm Whatman® filter paper. The clear solution was used for the assay.

2.5.1.2 Assay procedure

From solution 1 (citrate buffer, NAD, magnesium sulfate) 0.20mL were pipetted into a plastic cuvette for lactose blank, lactose sample, D-galactose blank and D-galactose sample. From suspension 2 (β -galactosidase) 0.05mL were pipetted into the cuvette for lactose blank and lactose sample only. The 0.10mL of sample solution from each treatment was pipetted into the cuvette for lactose and D-galactose samples but not blanks. Cuvettes were mixed using a Fisher vortex (Scientific Industries, Inc., New York, USA) and incubated for 20 minutes at 20-25°C. After this period, 1mL of solution 3 (potassium diphosphate buffer) was added into the cuvette for lactose blank, lactose sample, D-galactose blank and D-galactose sample. The 2mL of distilled water was added to lactose blank, 1.90mL to lactose sample, 2.05mL to D-galactose blank and 1.95mL to D-galactose sample. Cuvettes were vortexed and after 2 minutes absorbance was read (A_{11}). Wavelength was previously set to 340 nm and the spectrophotometer was zeroed. Cuvettes were wiped down before reading.

After reading absorbance (A1) 0.05mL of suspension 4 (galactose dehydrogenase) was added to all cuvettes to start the enzymatic reaction. Cuvettes were vortexed and absorbance (A2) was read after 30 minutes.

2.5.1.3 Calculations

$$c = \frac{V \times MW}{\varepsilon \times d \times v \times 1000} \times \Delta A \text{ [g/l]}$$

$$c = \frac{3.300 \times 342.3}{\varepsilon \times 1.00 \times 0.100 \times 1000} \times \Delta A_{\text{lactose}} \text{ [g/l]} = \frac{11.30}{\varepsilon} \times \Delta A_{\text{lactose}} \text{ [g lactose/l sample solution]}$$

Where,

V = final volume [mL]

v = sample volume [mL]

MW = molecular weight of the substance to be assayed [g/mol]

d = light path [cm]

ε = extinction coefficient of NADH at 340 nm = 6.3 [l x mmol⁻¹ x cm⁻¹]

To calculate the lactose concentration in the sample, the absorbance differences (A2-A1) for blanks and samples were determined. The absorbance difference of the lactose sample was subtracted from the absorbance difference of the blank sample: $\Delta A = (A2-A1)_{\text{sample}} - (A2-A1)_{\text{blank}}$. Next, the $\Delta A_{\text{lactose} + \text{D-galactose}}$ (from lactose sample) was subtracted from $\Delta A_{\text{D-galactose}}$ (from D-galactose sample) to obtain the true lactose amount.

The result corresponds to ΔA lactose value to be substitute in the equation. The equation (see above) was applied to obtain lactose concentration. The result was obtained in grams of lactose per liter of sample solution. This result must be converted to the dilution used (100 mL, see assay procedure). For that, 100 mL was divided by the amount of sample weighted and this result was multiplied by the value obtained with the equation, then divided by 10 to express the final result in percentage of lactose.

2.5.2 Syneresis

Syneresis was determined with the method described by Amatayakul *et. al.* (2006) with slight modifications. The 300 mL of yogurt mix was poured into plastic cups. The cup of set yogurt was kept at an angle of 45° and spontaneous whey was collected at the side of the cup with a pipette. Amount of whey in mL was measured at 22°C. The yogurt gel was allowed to stand for 1 minute and any further surface whey was pipetted and total whey release (mL) was measured.

2.5.3 Titratable Acidity (TA)

The titratable acidity was determined by weighing 9 g of yogurt. The 6 drops of phenolphthalein indicator solution was added and samples were titrated with 0.1 N NaOH as until color changed to rose pink and persists for 30 seconds.

2.5.4 pH

The pH of the yogurts was determined using the Oysters Series pH meter (Extech Instruments, Waltham, MA).

The instrument was calibrated using commercial pH 4.00 and 7.00 buffers (Fisher Scientific, Fair Lawn, NJ) and instrument's temperature was adjusted to the sample's temperature of $8^{\circ}\text{C} \pm 2$ before reading. Two measurements were taken.

2.5.5 Apparent Viscosity

Apparent viscosities were measured using a Brookfield DV II + viscometer (Brookfield Engineering Lab Inc., Stoughton, MA, USA) with a helipath stand at $10^{\circ}\text{C} \pm 2$. A T bar B spindle was set to 10 rpm to obtain a torque force between 10-90%.

The T bar B spindle was inserted in the sample at a constant depth of 2 cm from the top level of the sample container. The helipath was set in downward motion to cut new circular layers at increasing depths of the sample. Sample's container geometry was 4.55" top diameter, 3.25" bottom diameter and 2.45" height with 340 g capacity. The data was gathered using the Wingather® software (Brookfield Engineering Lab Inc., Stoughton, MA, USA). The viscosity measurements were continuous over 33 seconds required to collect one hundred data points averaged per sample per replication.

2.6 SENSORY STUDY

The sensory study was approved by the LSU Institutional Review Board with the IRB exempt number of HE13-6 (Appendix A). Blueberry yogurt containing the four treatments (0, 1, 3 and 5% w/w added lactose) was poured into 2.5 oz. previously labeled shuffle cups. A 3-digits random number code was used to label the cups. Consumer acceptance study was performed with 100 panelists.

One cup per treatment, that is four cups in total, were given to each panelist along with the evaluation questionnaire (Appendix B) which consisted of a 9-points rating scale (1= Dislike extremely, 9 = Like extremely), and acceptability and purchase intent questions (yes/no questions). Panelists were asked to evaluate each yogurt sample for the following attributes: Appearance, Color, Aroma, Taste, Sourness, Sweetness, Thickness, Graininess and Overall liking.

2.7 STATISTICAL ANALYSIS

The *Lactobacillus bulgaricus* LB-12 and *Streptococcus thermophilus* ST-M5 counts were converted to \log_{10} scale before analyzing the data by SAS. Data were analyzed using Proc Mixed of the SAS® 9.3 program. Differences of Least Square Means were used to determine significant differences at $P < 0.05$ for main effects (lactose concentration and time) and interaction effects (lactose concentration* time). Sensory data are presented as mean \pm standard deviation of the means. Significant differences between means were analyzed at $\alpha = 0.05$ using Tukey's adjustment to determine the best treatment.

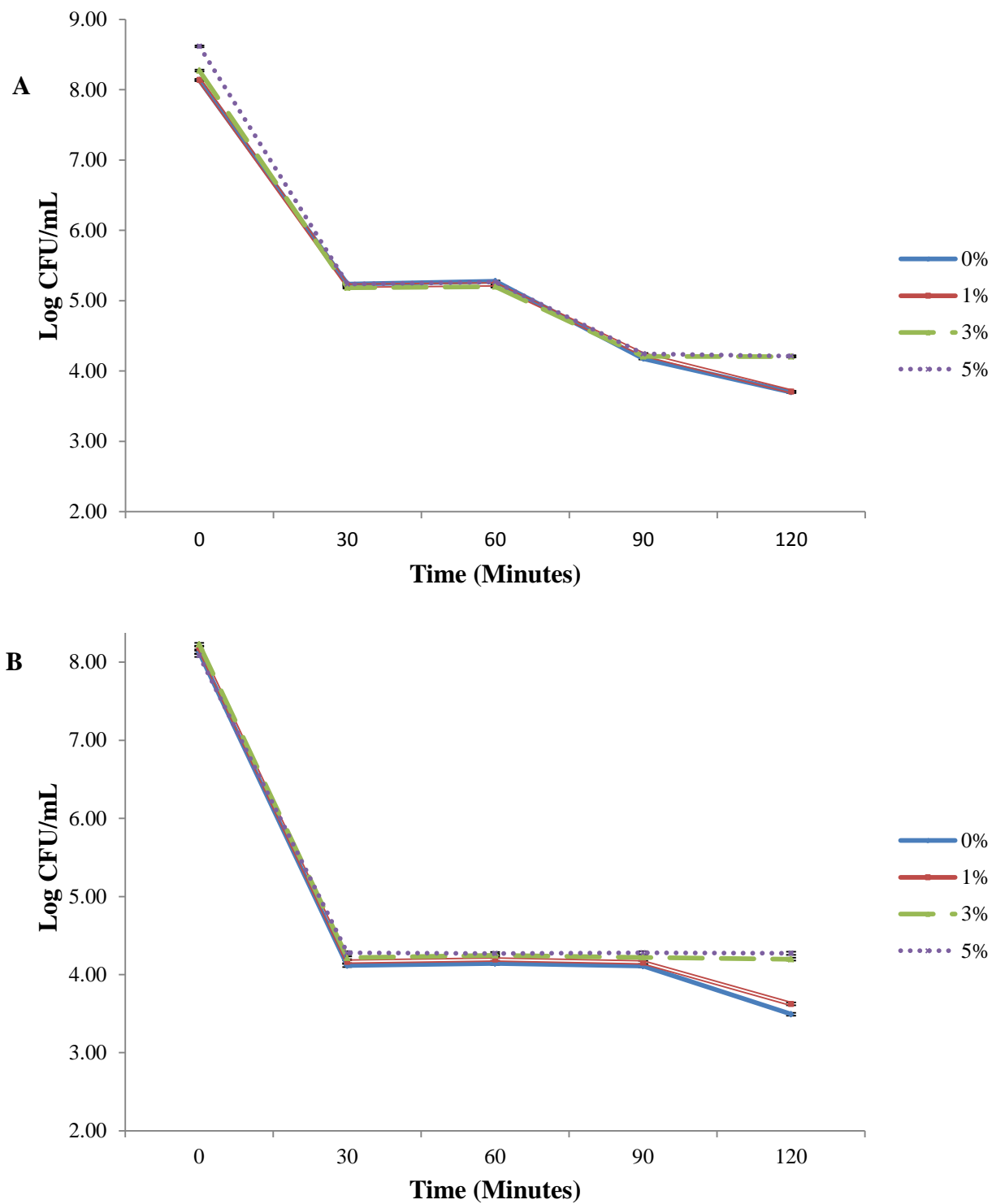


Figure 1. Acid tolerance of *Streptococcus thermophilus* ST-M5 (A) and *Lactobacillus bulgaricus* LB-12 (B) as influenced by added lactose concentration over the incubation period of 120 minutes.

CHAPTER 3: RESULTS AND DISCUSSION

SECTION 1: Yogurt Culture Bacteria

3.1 ACID TOLERANCE

3.1.1 *Streptococcus thermophilus* ST-M5

The acid tolerance of *Streptococcus thermophilus* ST-M5 as influenced by lactose addition over incubation of 120 minutes is shown in Figure 1A. Treatment*minutes interaction effect was significant ($P<0.05$). The treatment effect and minutes effect were also significant ($P<0.05$) (Table 2). At 0 and 120 minutes 3 and 5% w/w added lactose showed significantly higher viable cell counts compared to control and 1% w/w added lactose (Tables 3). Mean log difference in the counts of *Streptococcus thermophilus* ST-M5 was obtained by subtracting log CFU/mL of 120 minutes from 0 minutes of incubation. A low number indicates lower bacterial death. The bacterial death was the lowest for 3% w/w added lactose compared to the rest (Table 4). According to Van de Guchte *et. al.* (2002) the LAB, including *Streptococcus thermophilus*, are neutrophils (i.e., optimal pH for growth is between 5 and 9) except for some species of the genera *Lactobacillus*, *Leuconostoc* and *Oenococcus*. *Streptococci* ssp. are susceptible to low pH. Papadimitriou *et. al.* (2007) reported that exposure of *Streptococcus macedonicus* at pH 3.5 for 45 minutes resulted in almost 100% death.

3.1.2 *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12

Acid tolerance of *Lactobacillus bulgaricus* LB-12 as influenced by lactose addition over incubation of 120 minutes is shown in Figure 1B. Treatment*minutes interaction effect was significant ($P<0.05$) and also treatment effect and minutes effect (Table 2).

Table 2. Probability > F Value (Pr > F) for fixed effects of *Streptococcus thermophilus* ST-M5 and *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 counts in the presence of 0, 1, 3 and 5% w/w of added lactose under the influence of acid.

Effect	<i>Streptococcus thermophilus</i> ST-M5	<i>Lactobacillus bulgaricus</i> LB-12
Treatment	<0.0001	<0.0001
Minutes	<0.0001	<0.0001
Treatment*Minutes	<0.0001	<0.0001

From 90 minutes of incubation 3 and 5% w/w added lactose had a significantly higher counts ($P<0.05$) compared to control (Table 3). At 30, 90 and 120 minutes of incubation, 5% w/w added lactose showed significantly the highest viable cell counts compared to control (Table 3). Mean log difference in the viable counts of *Lactobacillus bulgaricus* LB-12 was obtained by subtracting log CFU/mL of 120 minutes from 0 minutes of incubation period. A low number indicates a low bacterial death. The bacterial death was the lowest for 5% w/w added lactose compared to the rest (Table 4). Both bacteria exhibited a similar acid tolerance behavior. *Lactobacillus bulgaricus* is known as a more acid tolerant strain compared to *Streptococcus thermophilus*. Shah and Jelen (1990) reported that at pH 1.5 *Lactobacillus delbrueckii* ssp. *bulgaricus* proved to be more acid tolerant than *Streptococcus thermophilus*. Liong and Shah (2005) reported that the most acid tolerant strains are *Lactobacillus acidophilus* and *Lactobacillus casei*.

Table 3. Least Square Means (Log CFU/mL) for acid tolerance of *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12 as influenced by added lactose over the incubation period of 120 minutes.

Added Lactose Concentration (%)	<i>Streptococcus thermophilus</i> ST-M5					<i>Lactobacillus bulgaricus</i> LB-12				
	Time (Minutes)									
	0	30	60	90	120	0	30	60	90	120
Control One Three Five	8.14 ^C	5.24 ^{DEF}	5.28 ^D	4.17 ^H	3.70 ^I	8.13 ^{AB}	4.12 ^{FG}	4.14 ^{EFG}	4.11 ^G	3.49 ^I
	8.14 ^C	5.21 ^{DEF}	5.23 ^{DEF}	4.23 ^{GH}	3.71 ^I	8.19 ^{AB}	4.17 ^{DEFG}	4.20 ^{CDEFG}	4.16 ^{DEFG}	3.62 ^H
	8.27 ^B	5.18 ^F	5.20 ^{EF}	4.21 ^{GH}	4.21 ^{GH}	8.23 ^A	4.21 ^{CDEF}	4.25 ^{CD}	4.22 ^{CDE}	4.19 ^{CDEFG}
	8.62 ^A	5.23 ^{DEF}	5.26 ^{DE}	4.25 ^G	4.21 ^{GH}	8.09 ^B	4.28 ^C	4.27 ^C	4.28 ^C	4.27 ^C

^{ABC} LSMeans with different letter within the table are significantly different.

Table 4. Mean log difference in the viable counts of *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12 as influenced by added lactose concentration in the presence of acid.

Added Lactose Concentration (%)	<i>Streptococcus thermophilus</i> ST-M5	<i>Lactobacillus bulgaricus</i> LB-12
	Log CFU/mL	
Control	4.44	4.64
One	4.43	4.56
Three	4.06	4.04
Five	4.41	3.81

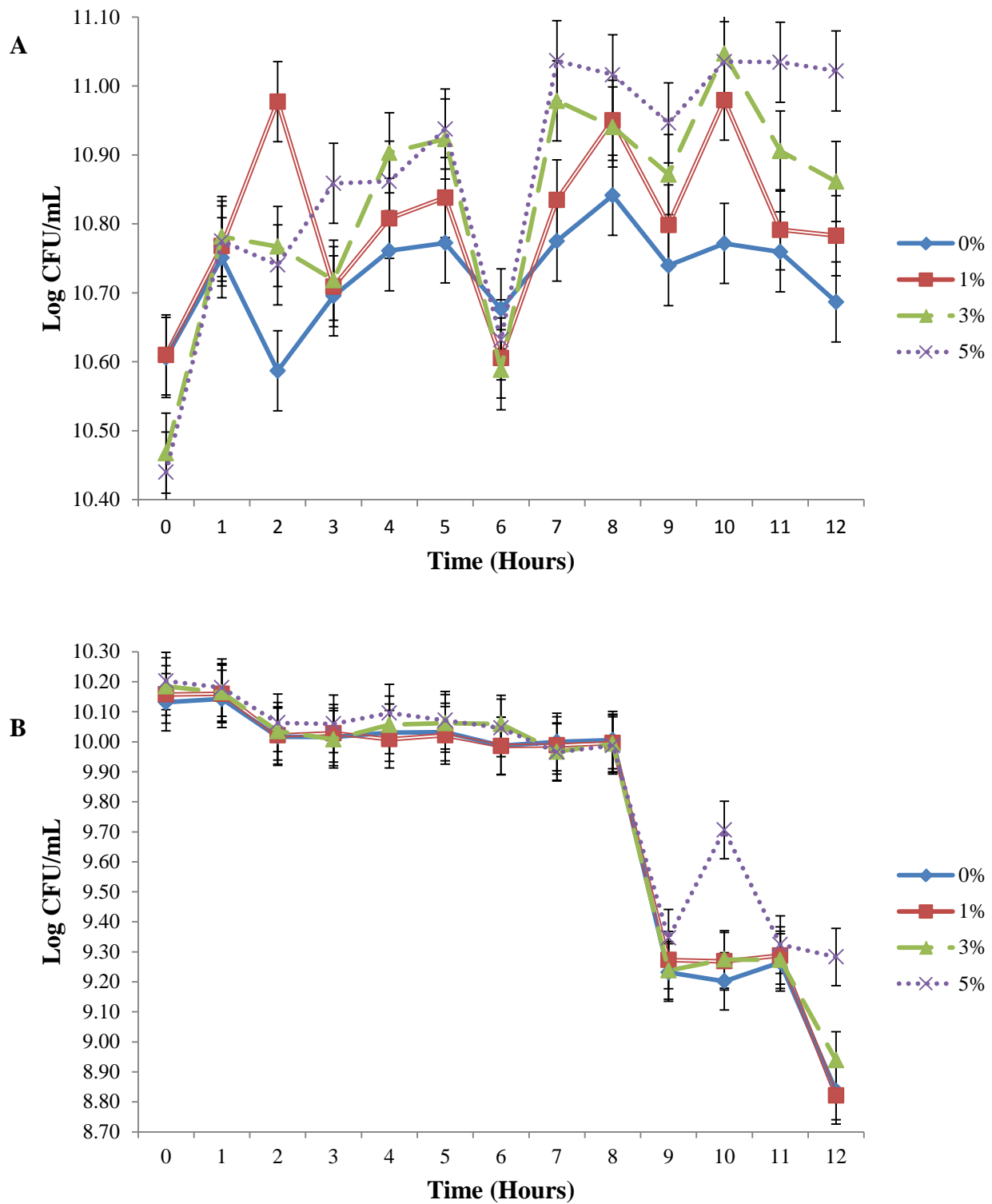


Figure 2. Bile tolerance of *Streptococcus thermophilus* ST-M5 (A) and *Lactobacillus bulgaricus* LB-12 (B) as influenced by added lactose concentration over the incubation period of 12 hours.

3.2 BILE TOLERANCE

3.2.1 *Streptococcus thermophilus* ST-M5

The bile tolerance of *Streptococcus thermophilus* ST-M5 as influenced by addition of lactose over incubation of 12 hours is shown in Figure 2A. Treatment*hour interaction effect was significant ($P<0.05$). The treatment effect and hour effect were also significant ($P<0.05$) (Table 5). At hour 2 of incubation added lactose at 1% w/w showed significantly higher counts ($P<0.05$) compared to control (Table 6). Gilliland and Kim (1983) reported that the addition of 0.5 and 1% oxgall increased the lactose hydrolyzing activity of yogurt starter culture to 19.8 and 16.7 units respectively ($P < 0.01$). Martini *et. al.* (1987) reported that 0.5 or 1% oxgall increased lactase activity by 3-fold in yogurt containing starter culture. The researchers suggested that the permeability of the bacterial cell is changed when exposed to bile in the intestine (Martini *et. al.*, 1987). Mean log difference in the viable counts of *Streptococcus thermophilus* ST-M5 was obtained by subtracting log CFU/mL of 0 hours from 12 hours of incubation. A high number indicates higher bacterial survival. The bacteria survival was the highest for 5% w/w added lactose compared to the rest (Table 9). Pereira and Gibson (2002) reported no significant differences on growth of *Streptococcus thermophilus* in MRS broth containing 0.2 and 0.4% (w/v) oxgall for 12 hours of incubation at 37°C and monitored hourly for growth.

3.2.2 *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12

Bile tolerance of *Lactobacillus bulgaricus* LB-12 as influenced by lactose addition over incubation of 12 hours is shown in Figure 2B. Treatment*hour interaction effect was not significant ($P>0.05$) while treatment effect and hour effect were significant ($P<0.05$) (Table 5).

Table 5. Probability > F Value (Pr > F) for fixed effects of *Streptococcus thermophilus* ST-M5 and *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 counts in the presence of 0, 1, 3 and 5% w/w of added lactose with the influence of bile (oxgall).

Effect	<i>Streptococcus thermophilus</i> ST-M5	<i>Lactobacillus bulgaricus</i> LB-12
Treatment	<0.0001	0.0162
Hour	<0.0001	<0.0001
Treatment*Hour	0.0028	0.8967

Table 6. Least Square Means (Log CFU/mL) for bile tolerance of *Streptococcus thermophilus* ST-M5 as influenced by added lactose over the incubation period of 12 hours.

Treatment	<i>Streptococcus thermophilus</i> ST-M5												
	Time (Hours)												
	0	1	2	3	4	5	6	7	8	9	10	11	12
Control	10.61 ^{FG} HI	10.75 ^{ABCD} EFGHI	10.59 ^{GHI}	10.70 ^{BCDE} FGHI	10.76 ^{ABCD} EFGHI	10.77 ^{AB} CDEFGHI	10.68 ^{DEF} GHI	10.78 ^{AB} CDEFGHI	10.84 ^{AB} CDEFG	10.74 ^{AB} CDEFGHI	10.77 ^{AB} CDEFGHI	10.7 ^{ABC} DEFGHI	10.69 ^{CDEF} GHI
One	10.61 ^{FG} HI	10.77 ^{ABCD} EFGHI	10.98 ^{AB} CD	10.71 ^{ABCD} EFGHI	10.81 ^{ABCD} EFG	10.84 ^{AB} CDEFG	10.61 ^{FG} HI	10.84 ^{AB} CDEFG	10.95 ^{AB} CDE	10.80 ^{AB} CDEFGH	10.98 ^{AB} CD	10.79 ^{AB} CDEFGH	10.79 ^{ABC} DEFGH
Three	10.47 ^{HI}	10.78 ^{ABCD} EFGH	10.77 ^{AB} CDEFGHI	10.72 ^{ABCD} EFGHI	10.90 ^{ABCD} EFG	10.92 ^{AB} CDEFG	10.59 ^{GHI}	10.98 ^{AB} CD	10.94 ^{AB} CDEF	10.87 ^{AB} CDEFG	11.05 ^A	10.91 ^{AB} CDEFG	10.86 ^{ABC} DEFG
Five	10.44 ^I	10.77 ^{ABCD} EFGHI	10.74 ^{AB} CDEFGHI	10.86 ^{ABCD} EFG	10.86 ^{ABCD} EFG	10.94 ^{AB} CDEF	10.63 ^{EFG} HI	11.04 ^A	11.02 ^{AB} CD	10.95 ^{AB} CDEF	11.03 ^{AB}	11.04 ^{AB}	11.02 ^{ABC}

^{ABC} LSMeans with different letter within the table are significantly different.

Table 7. Least Square Means (Log CFU/mL) for bile tolerance of *Lactobacillus bulgaricus* LB-12 as influenced by added lactose concentrations.

Added Lactose Concentration (%)	<i>Lactobacillus bulgaricus</i> LB-12
	LS Means
Control	9.76 ^b
One	9.77 ^b
Three	9.79 ^{ab}
Five	9.87 ^a

^{ab} LSMeans with different letter within the column are significantly different.

Table 8. Least Square Means (Log CFU/mL) for bile tolerance of *Lactobacillus bulgaricus* LB-12 as influenced by the incubation period of 12 hours.

Incubation Period (Hours)	<i>Lactobacillus bulgaricus</i> LB-12
	LS Means
0	10.17 ^a
1	10.16 ^a
2	10.03 ^a
3	10.03 ^a
4	10.05 ^a
5	10.05 ^a
6	10.02 ^a
7	9.98 ^a
8	9.99 ^a
9	9.27 ^b
10	9.36 ^b
11	9.29 ^b
12	8.97 ^c

^{abc} LSMeans with different letter within the column are significantly different.

The highest counts for *Lactobacillus bulgaricus* LB-12 were obtained from treatment containing 5% w/w added lactose (Table 7).

Table 9. Mean log difference in the viable counts of *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12 as influenced by added lactose concentration in the presence of bile (oxgall).

Added Lactose Concentration (%)	<i>Streptococcus thermophilus</i> ST-M5	<i>Lactobacillus bulgaricus</i> LB-12
	Log CFU/mL	
Control	0.08	1.29
One	0.18	1.33
Three	0.40	1.25
Five	0.59	0.92

The highest counts of *Lactobacillus bulgaricus* LB-12 were obtained at the first 8 hours of incubation (Table 8). Mean log difference in the viable counts of *Lactobacillus bulgaricus* LB-12 was obtained by subtracting log CFU/mL of 12 hours from 0 hours of incubation. A low number indicates lower bacterial death. The bacterial death was the lowest for 5% w/w added lactose compared to the rest (Table 9). The mechanisms of bile tolerance vary between LAB strains (Van de Guchte *et. al.*, 2002).

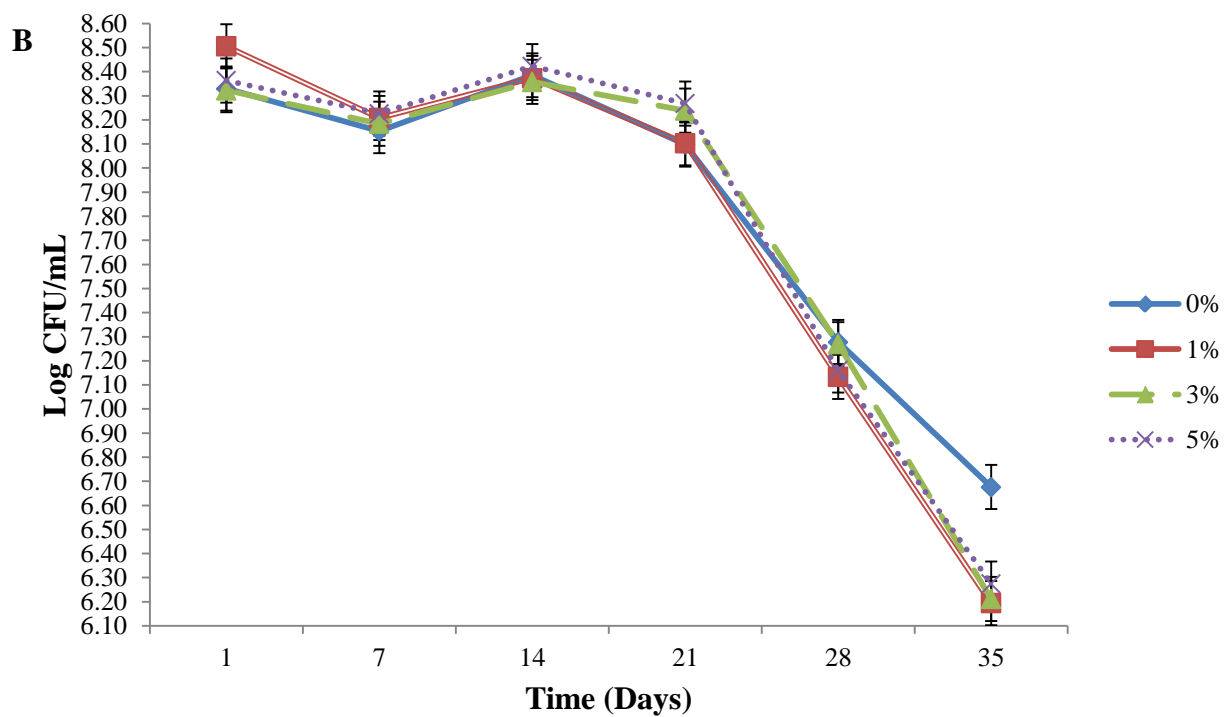
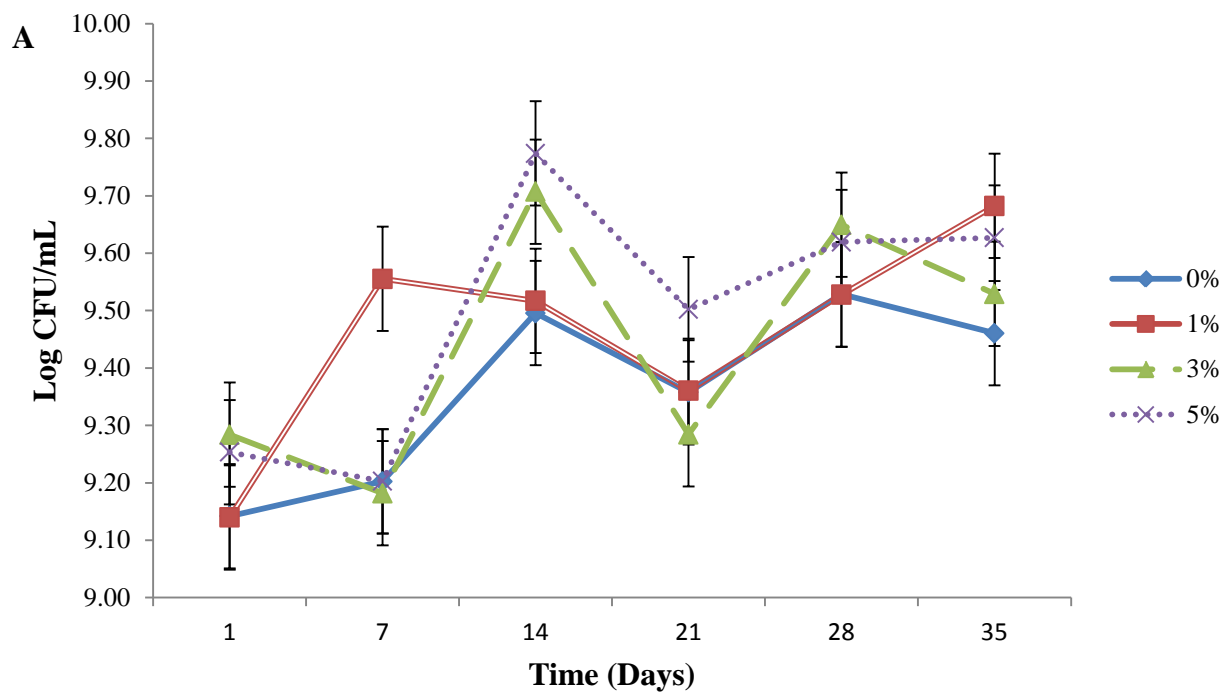


Figure 3. Growth of *Streptococcus thermophilus* ST-M5 (A) and *Lactobacillus bulgaricus* LB-12 (B) as influenced by added lactose concentration over storage period of 35 days.

SECTION 2: Yogurt Analysis

3.3 GROWTH

3.3.1 *Streptococcus thermophilus* ST-M5

The growth of *Streptococcus thermophilus* ST-M5 as influenced by added lactose concentration over storage of 35 days is shown in Figure 3A. Treatment*day interaction effect was not significant ($P>0.05$) while the treatment effect and day effect were significant ($P<0.05$) (Table 10). In general, upon addition of lactose there was an increase in viable counts at day 35 compared to day 1 (Figure 3A).

Table 10. Probability > F Value (Pr > F) for fixed effects of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 counts, *Streptococcus thermophilus* ST-M5 counts, lactose content, viscosity, pH, titratable acidity and syneresis in the yogurts containing 0, 1, 3 and 5% w/w of added lactose over storage period of 35 days.

Effect	<i>S. thermophilus</i> ST-M5	<i>L. bulgaricus</i> LB-12	LC	Apparent Viscosity	pH	TA	Syneresis
Treatment	0.0225	0.5903	<0.0001	<0.0001	0.0003	<0.0001	<0.0001
Day	<0.0001	<0.0001	<0.0001	0.6735	<0.0001	<0.0001	<0.0001
Treatment*Day	0.0859	0.1000	0.0970	0.8809	0.9988	<0.0001	<0.0001

LC = Lactose Content, TA = Titratable Acidity

Use of 5% w/w added lactose resulted in significant higher counts compared to control (Table 11). Ding and Shah (2010) studied the effect of 2% lactose on probiotic bacteria in soymilk. Counts of soymilk containing lactose were significantly higher than soymilk with the absence of lactose (8.13 log CFU/mL and 6.36 log CFU/mL respectively).

They stated that probiotic bacteria are traditionally grown in lactose rich dairy foods such as yogurt; hence the growth is better in the presence of this carbohydrate. The highest counts of *Streptococcus thermophilus* ST-M5 were observed in days 14 and 28 of storage (Table 12). At day 21 there was a significant decrease in counts compared to days 14, 28 and 35. However, the counts stayed within the same log CFU/mL (Table 12). Studies have shown that *Streptococcus thermophilus* survive well in yogurt throughout the shelf life (Hamann and Marth, 1984; Rohm *et. al.*, 1990; Akalin *et. al.*, 2004). Mean log difference in the viable counts of *Streptococcus thermophilus* ST-M5 was obtained by subtracting log CFU/mL of day 1 from day 35 of storage. A high number indicates higher bacterial. The bacteria survival was higher for lactose added samples compared to control. The bacterial survival was the highest for 1% w/w added lactose compared to the rest (Table 13).

Table 11. Least Square Means (Log CFU/mL) for growth of *Streptococcus thermophilus* ST-M5 as influenced by added lactose concentrations.

Added Lactose Concentration (%)	<i>Streptococcus thermophilus</i> ST-M5
	LS Means
Control	9.33 ^b
One	9.46 ^{ab}
Three	9.44 ^{ab}
Five	9.49 ^a

^{ab} LSMeans with different letter within the column are significantly different.

3.3.2 *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12

The growth characteristics of *Lactobacillus bulgaricus* LB-12 as influenced by added lactose concentration over storage of 35 days is shown in Figure 3B. Treatment*day interaction effect and treatment effect were not significant ($P>0.05$).

The day effect was significant ($P<0.05$) (Table 10). Viable counts of *Lactobacillus bulgaricus* LB-12 decreased for all treatments over storage period (Figure 3B). This behavior agrees with the results reported by Venkatesh *et. al.* (1993) who found that when fermentations were carried out with 60 g/liter of lactose in the medium, specific growth rates for *Lactobacillus delbrueckii* ssp. *bulgaricus* increased to a maximum and then decreased. Donkor *et. al.* (2006) reported that the growth of *Lactobacillus delbrueckii* ssp. *bulgaricus* declined from 7 to 6 log CFU/g in yogurt during storage period of 28 days.

Table 12. Least Square Means (Log CFU/mL) for growth of *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12 as influenced by the storage period of 35 days.

Storage Period (Days)	<i>Streptococcus thermophilus</i> ST-M5	<i>Lactobacillus bulgaricus</i> LB-12
	LS Means	
1	9.20 ^c	8.38 ^a
7	9.28 ^c	8.19 ^{bc}
14	9.62 ^a	8.38 ^a
21	9.38 ^{bc}	8.18 ^c
28	9.58 ^a	7.21 ^d
35	9.53 ^{ab}	6.34 ^e

^{ab} LSMeans with different letter within the column are significantly different.

Table 13. Mean log difference in the viable counts of *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12 as influenced by added lactose concentration.

Added Lactose Concentration (%)	<i>Streptococcus thermophilus</i> ST-M5	<i>Lactobacillus bulgaricus</i> LB-12
	Log CFU/mL	
Control	0.15	1.65
One	0.54	2.31
Three	0.25	2.11
Five	0.38	2.09

The highest counts of *Lactobacillus bulgaricus* LB-12 were observed at days 1 and 14 of storage (Table 12). Mean log difference in the viable counts of *Lactobacillus bulgaricus* LB-12 was obtained by subtracting log CFU/mL of day 35 from day 1 of storage. A low number indicates lower bacterial death. The bacterial death was the lowest for control compared to the rest (Table 13). The bacterial survival was lower for lactose added samples compared to control.

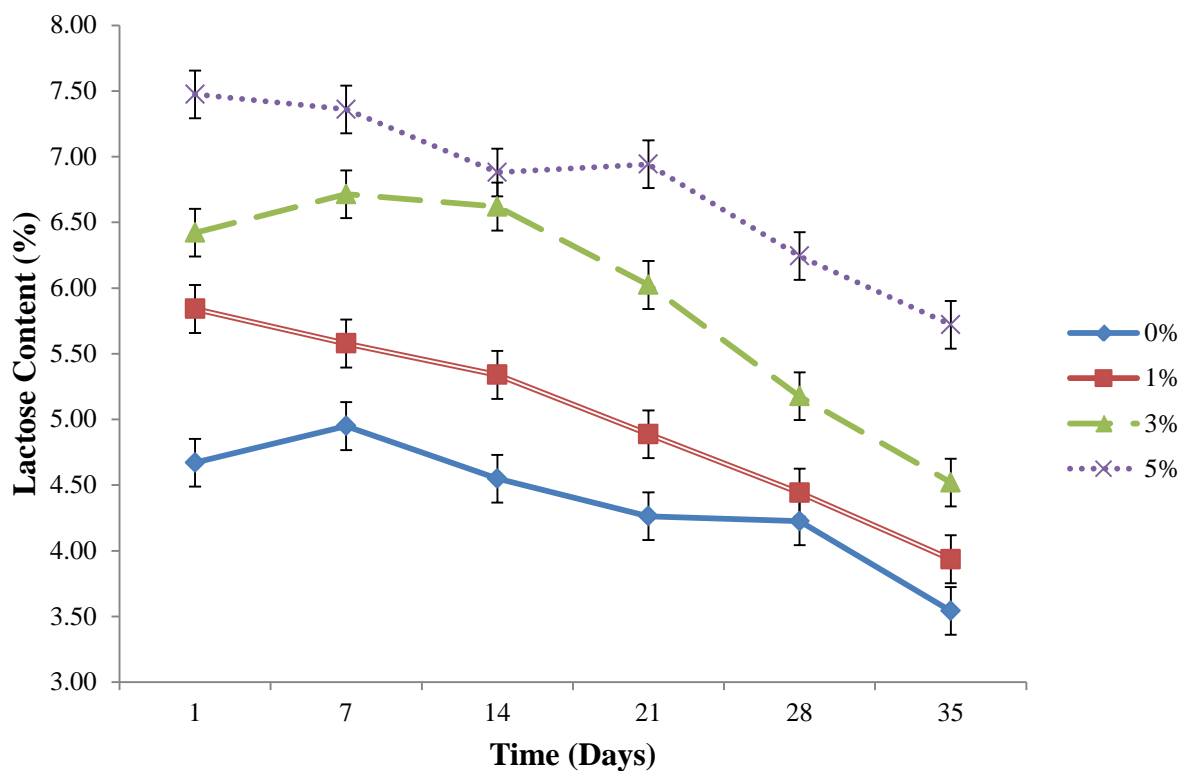


Figure 4. Lactose Content of yogurts as influenced by lactose addition over storage period of 35 days.

3.4 LACTOSE CONTENT

Lactose content of yogurts as influenced by lactose addition over storage of 35 days is shown in Figure 4. Treatment*day interaction effect was not significant ($P>0.05$) while treatment effect and day effect were significant ($P<0.05$) (Table 10).

Table 14. Least Square Means for lactose content of yogurts as influenced by added lactose concentrations.

Added Lactose Concentration (%)	Lactose Content
	LS Means
Control	4.37 ^d
One	5.01 ^c
Three	5.92 ^b
Five	6.77 ^a

^{ab} LSMeans with different letter within the column are significantly different.

Table 15. Least Square Means for lactose content of yogurts as influenced by the storage period of 35 days.

Storage Period (Days)	Lactose Content
	LS Means
1	6.11 ^a
7	6.16 ^a
14	5.85 ^{ab}
21	5.53 ^b
28	5.03 ^c
35	4.42 ^d

^{ab} LSMeans with different letter within the column are significantly different.

Lactose content decreased for all treatments (Figure 4). As expected treatment containing 5% w/w added lactose had the highest lactose content (Table 14). The highest lactose content was observed at days 1 and 7 of storage period (Table 15).

Also as expected lactose content steadily decreases from day 21 onwards over the rest of the storage time (Table 15). Calvo *et. al.* (1999) found a reduction in lactose concentration of yogurt after storage period of 7 days. They attributed the loss of lactose to the fact that lactic acid bacteria not only produce lactic acid from lactose but also flavor compounds and polysaccharides during storage (Calvo *et. al.*,1999).

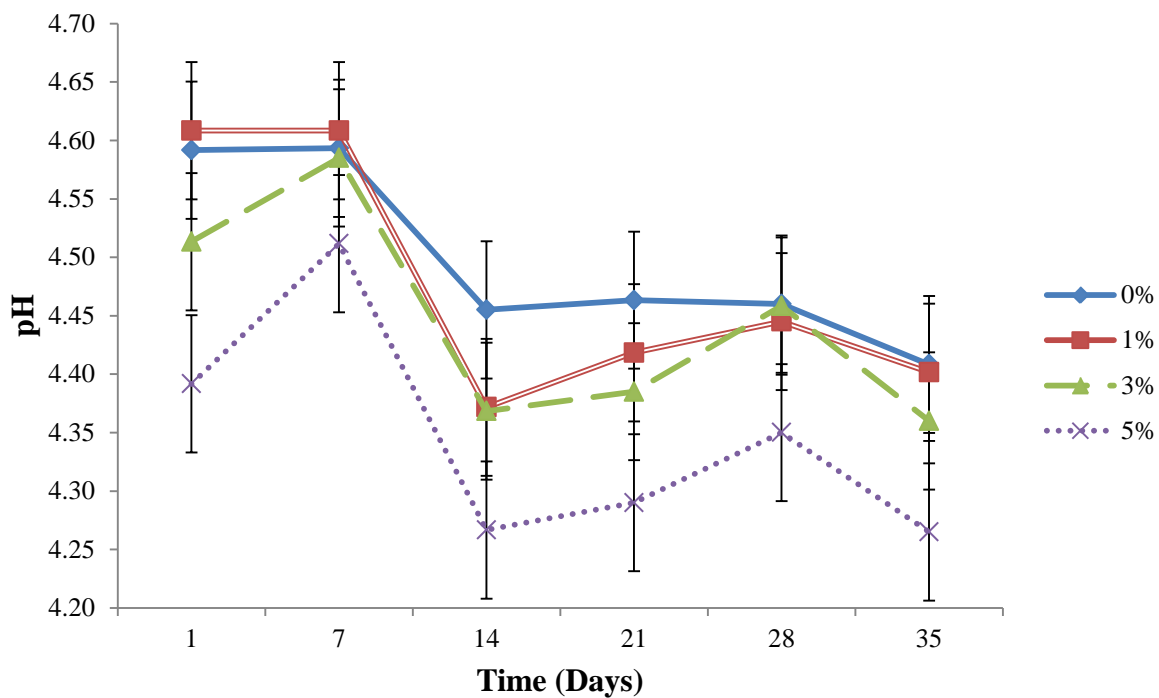


Figure 5. Lactose Concentration of yogurts as influenced by added lactose levels over storage period of 35 days.

3.5 pH

The pH of yogurts as influenced by added lactose concentration over storage of 35 days is shown in Figure 5.

Table 16. Least Square Means for pH of yogurts as influenced by added lactose concentrations.

Added Lactose Concentration (%)	pH
	LS Means
Control	4.50 ^a
One	4.48 ^a
Three	4.45 ^a
Five	4.35 ^b

^{ab}LSMeans with different letter within the column are significantly different.

Table 17. Least Square Means for pH of yogurts as influenced by the storage period of 35 days.

Storage Period (Days)	pH
	LS Means
1	4.53 ^{ab}
7	4.58 ^a
14	4.37 ^c
21	4.39 ^c
28	4.43 ^{bc}
35	4.36 ^c

^{ab}LSMeans with different letter within the column are significantly different.

Treatment*day interaction effect was not significant ($P>0.05$) while treatment effect and day effect were significant ($P<0.05$) (Table 10). The pH values decreased for all treatments at day 35 compared to day 1 (Figure 5). According to Damin *et. al.* (2009) a decrease in pH during storage is expected as result of the metabolic activity of starter cultures.

Treatments containing 5% w/w added lactose had the lowest pH (Table 16). Calvo *et. al.*, (1999) incorporated CO₂ in yogurt manufacture and reported a drop in pH values possibly due to CO₂ content. The more lactose present, the higher the production of lactic acid by the starter cultures. According to Venkatesh *et. al.* (1993) at high pH most of the lactic acid is formed due to a growth-associated mechanism and the growth curve has a short stationary growth phase. On the contrary, at low pH most of the lactic acid produced is nongrowth-associated (Venkatesh *et. al.*, 1993). The lowest pH values were obtained from day 14 onwards (Table 17).

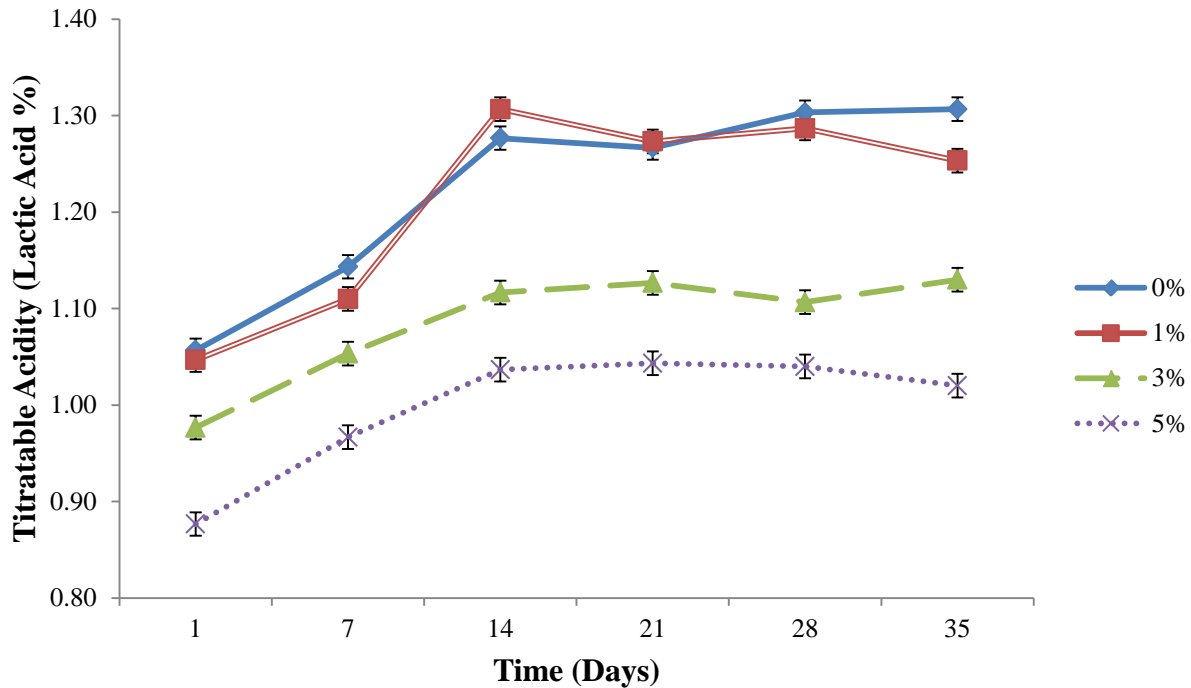


Figure 6. Titrateable Acidity (TA) of yogurts as influenced by added lactose levels over storage period of 35 days.

3.6 TITRATABLE ACIDITY (TA)

The Titratable Acidity (TA) of yogurts as influenced by added lactose concentration over storage of 35 days is shown in Figure 6. Treatment*day interaction effect, treatment effect and day effect were significant ($P<0.05$) (Table 10).

Table 18. Least Square Means for Titratable Acidity (TA) of yogurts as influenced by added lactose concentrations over storage period of 35 days.

Added Lactose Concentration (%)	Titratable Acidity					
	Time (Days)					
	1	7	14	21	28	35
Control	1.06 ^{CDEF}	1.14 ^B	1.28 ^A	1.27 ^A	1.30 ^A	1.31 ^A
One	1.05 ^{DEF}	1.11 ^{BCD}	1.31 ^A	1.27 ^A	1.29 ^A	1.25 ^A
Three	0.98 ^{GH}	1.05 ^{CDEF}	1.12 ^{BC}	1.13 ^B	1.11 ^{BCDE}	1.13 ^B
Five	0.88 ^I	0.97 ^H	1.04 ^{FG}	1.04 ^{DEFG}	1.04 ^{EFG}	1.02 ^{FGH}

^{ABC} LSMeans with diferent letter within the table are significantly different.

In general, TA at day 35 was higher compared to days 1 and 7 for treatments of lactose at 1 and 3% w/w (Table 18, Figure 6). Treatments containing 3 and 5% w/w added lactose had lower TA values compared to 0 and 1% w/w added lactose, except at day 7 for 3% w/w added lactose (Table 18). This phenomenon can be explained through the fact that whey separation cause by lactose hydrolysis leads to slow rate of acid production in yogurt (Nagaraj *et. al.*, 2009).As lactose is hydrolyzed by lactic acid bacteria the amount of lactic acid production increased.

This behavior may be due to the availability of more quantity of easily fermentable sugar (glucose) which is required for the faster growth of starters (Knanagaeva *et. al.*, 1980; Baeve, 1981; Whalen *et. al.*, 1988). Fan *et. al.* (2008) reported that changes in titratable acidity do not necessarily have an effect on pH values.

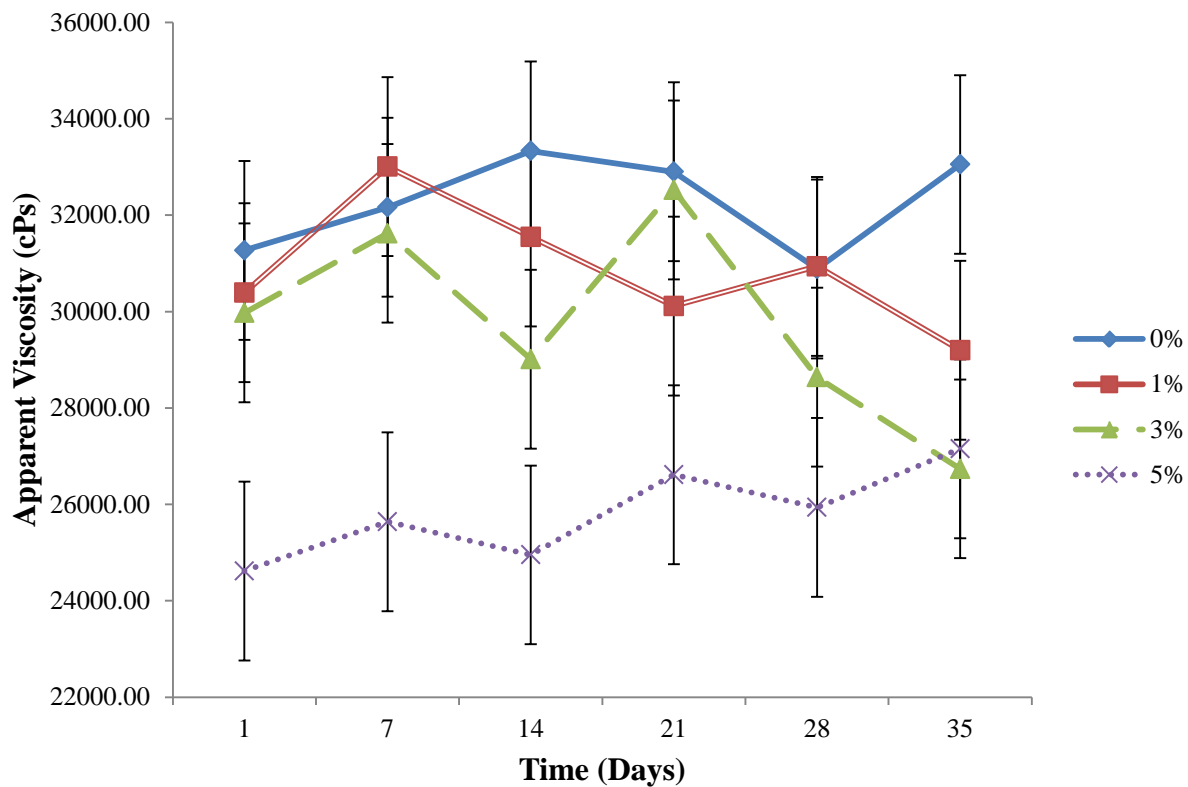


Figure 7. Apparent Viscosity of yogurts as influenced by added lactose levels over storage period of 35 days.

3.7 APPARENT VISCOSITY

The apparent viscosity of yogurts as influenced by lactose addition over storage of 35 days is shown in Figure 7. Treatment*day interaction effect and day effect were not significant ($P>0.05$) while treatment effect was significant ($P<0.05$) (Table 10).

Table 19. Least Square Means for Apparent Viscosity of yogurts as influenced by added lactose concentrations.

Added Lactose Concentration (%)	Apparent Viscosity
	LS Means
Control	32267.00 ^a
One	30866.00 ^a
Three	29751.00 ^a
Five	25820.00 ^b

^{ab} LSMeans with different letter within the column are significantly different.

Lactose at 5% w/w had the lowest apparent viscosity values compared to 0, 1 and 3% w/w added lactose (Table 19). This was because of the higher amount of whey released. Weaker body and texture of yogurt may be due to higher amount of whey separation which reduced the viscosity when lactose is hydrolyzed (Hilgendorf, 1981; Davood *et. al.*, 1982; Wilson *et. al.*, 2003).

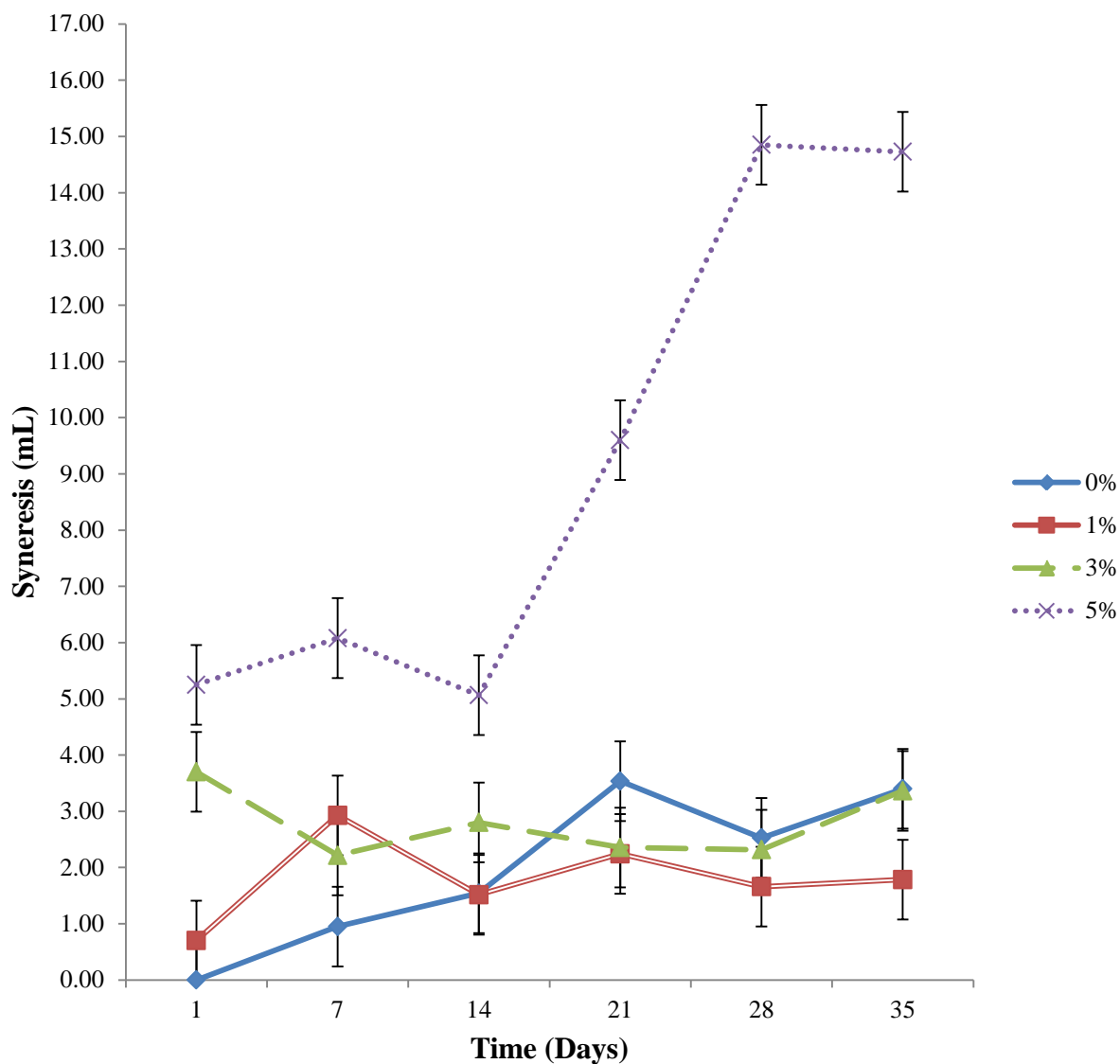


Figure 8. Syneresis of yogurts as influenced by added lactose levels over storage period of 35 days.

3.8 SYNERESIS

The syneresis of yogurts as influenced by lactose addition over storage of 35 days is shown in Figure 8. Treatment*day interaction effect, treatment effect and day effect were significant ($P<0.05$) (Table 10).

Table 20. Least Square Means for Syneresis of yogurts as influenced by added lactose concentrations over storage period of 35 days.

Added Lactose Concentration (%)	Syneresis					
	Time (Days)					
	1	7	14	21	28	35
Control	0.00 ^E	0.95 ^E	1.54 ^{DE}	3.54 ^{CDE}	2.53 ^{CDE}	3.40 ^{CDE}
One	0.70 ^E	2.93 ^{CDE}	1.52 ^{DE}	2.24 ^{CDE}	1.66 ^{DE}	1.79 ^{DE}
Three	3.70 ^{CDE}	2.22 ^{DE}	2.80 ^{CDE}	2.36 ^{CDE}	2.32 ^{CDE}	3.36 ^{CDE}
Five	5.25 ^{CD}	6.08 ^{BC}	5.06 ^{CD}	9.60 ^B	14.85 ^A	14.73 ^A

^{ABC} LSMeans with different letter within the table are significantly different.

Treatment containing 5% w/w added lactose had significantly the highest syneresis values compared to 0, 1 and 3% w/w added lactose during storage period at day 7 and from day 21 onwards (Table 20). Nagaraj *et. al.* (2009) reported that as the degree of lactose hydrolysis increased the amount of whey separation increased ($P < 0.05$). Difference in the amount of whey released was visually evident for 5% w/w added lactose treatment.

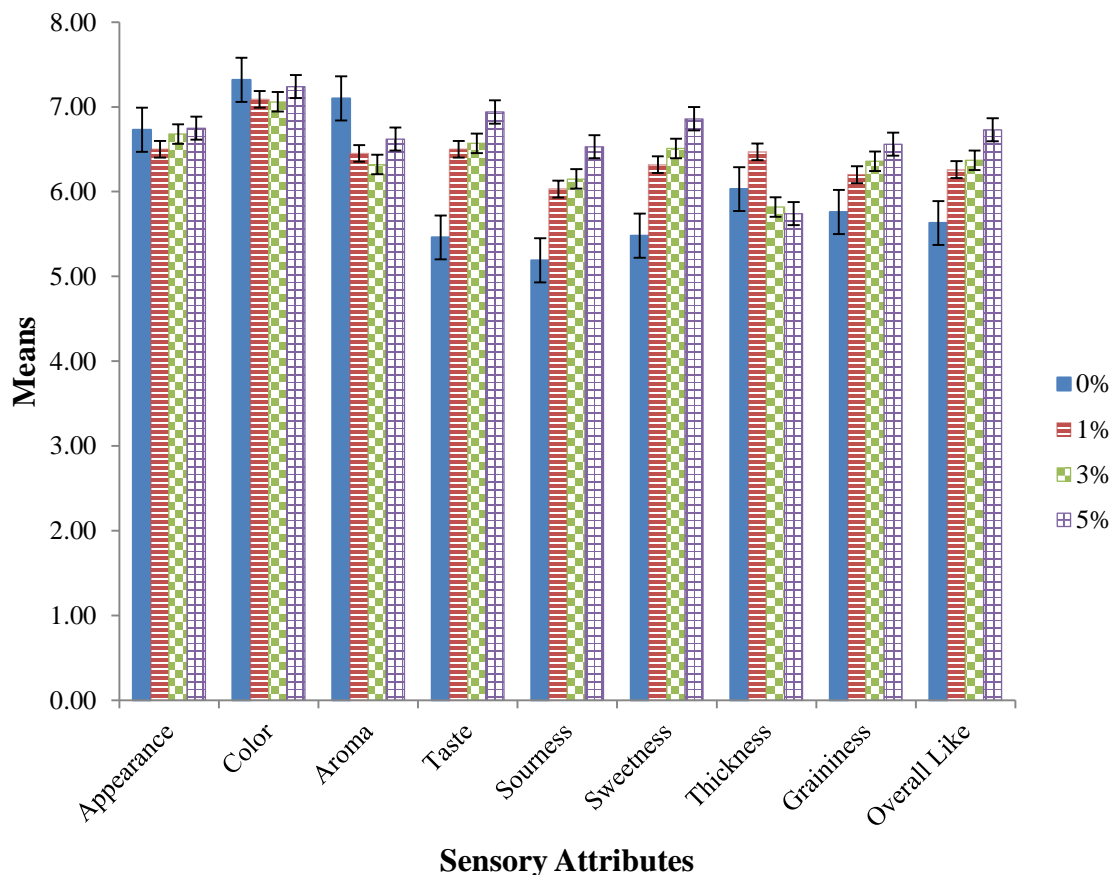


Figure 9. Means for sensory attributes of blueberry yogurt as influenced by lactose addition.

3.9 SENSORY STUDY

Means for all tested attributes (appearance, color, aroma, taste, sourness, sweetness, thickness, graininess, and overall liking) are shown in Figure 9. Probabilities for fixed effect of sensory attributes are shown in Table 21.

Samples containing 1% w/w added lactose had higher scores for thickness compared to 5% w/w added lactose. Control samples had lower scores for graininess compared to 5% w/w added lactose (Table 22). The overall linking scores indicated that samples containing added lactose were preferred over control (Table 22).

Table 21. Probability > F Value (Pr > F) for fixed effect of sensory attributes of yogurts containing 0, 1, 3 and 5% w/w added lactose.

Effect	Appearance	Color	Aroma	Taste	Sourness	Sweetness	Thickness	Graininess	Overall Liking
Treatment	0.6258	0.3587	0.0011	<0.0001	<0.0001	<0.0001	0.0217	0.0095	0.0001

Table 22. Means for sensory properties of yogurts as influenced by added lactose concentrations.

Added Lactose Concentration (%)	Sensory Attributes								
	Appearance	Color	Aroma	Taste	Sourness	Sweetness	Thickness	Graininess	Overall Like
Control	6.73 ^a ± 1.48	7.32 ^a ± 1.14	7.10 ^a ± 1.46	5.46 ^b ± 1.93	5.19 ^b ± 1.93	5.48 ^b ± 1.79	6.03 ^{ab} ± 1.84	5.76 ^b ± 1.81	5.63 ^b ± 1.85
One	6.50 ^a ± 1.56	7.09 ^a ± 1.26	6.45 ^b ± 1.40	6.50 ^a ± 1.81	6.03 ^a ± 1.70	6.32 ^a ± 1.63	6.47 ^a ± 1.71	6.20 ^{ab} ± 1.68	6.26 ^{ab} ± 1.85
Three	6.68 ^a ± 1.48	7.06 ^a ± 1.14	6.32 ^b ± 1.61	6.57 ^a ± 1.55	6.15 ^a ± 1.58	6.51 ^a ± 1.49	5.82 ^{ab} ± 1.84	6.36 ^{ab} ± 1.79	6.37 ^a ± 1.70
Five	6.75 ^a ± 1.43	7.24 ^a ± 1.22	6.62 ^{ab} ± 1.35	6.94 ^a ± 1.43	6.53 ^a ± 1.61	6.86 ^a ± 1.33	5.74 ^b ± 1.85	6.56 ^a ± 1.63	6.73 ^a ± 1.53

^{ab} Means with different letter within the column are significantly different.

Lactose addition did not appear to significantly ($P>0.05$) impact appearance and color of yogurts (Table 21). Similar results were reported by Nagaraj *et. al.* (2009) who studied lactose hydrolysis on enzymatically hydrolyzed yogurts. Aroma and taste are the most important sensory characteristics of yogurt (Routray and Mishra, 2011). Control and 5% w/w added lactose were preferred for the attribute of aroma compared to 1 and 3% w/w added lactose (Table 22). For taste, sourness and sweetness samples containing added lactose were preferred over control (Table 22).

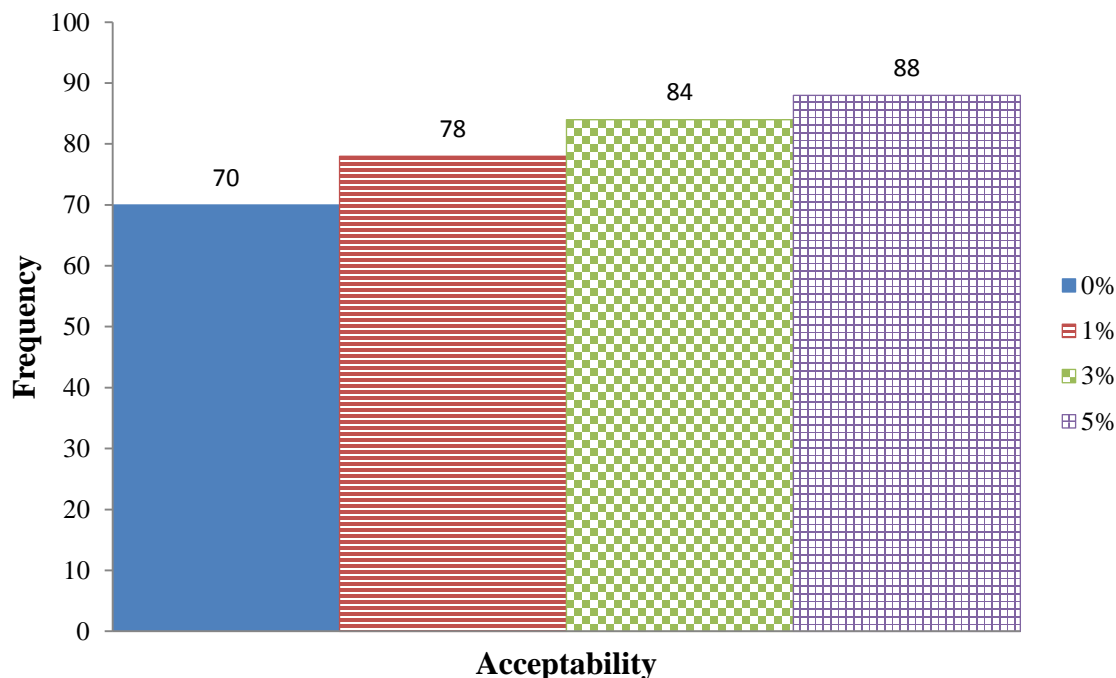


Figure 10. Frequency for acceptability of blueberry yogurt as influenced by lactose addition.

Yogurt acceptability frequency values are shown in Figure 10. Added lactose yogurts had a greater acceptability compared to control yogurts. The consumer acceptability of yogurts increased as lactose addition increased. Yogurts containing 5% w/w added lactose led to higher acceptability (88%) compared to control (70%). This is probably due to a better palatability given by the sweetness of lactose. According to Nagaraj *et. al.* (2009) when lactose is hydrolyzed in yogurt it resulted in increased digestibility, sweeter taste, and better mouth feel.

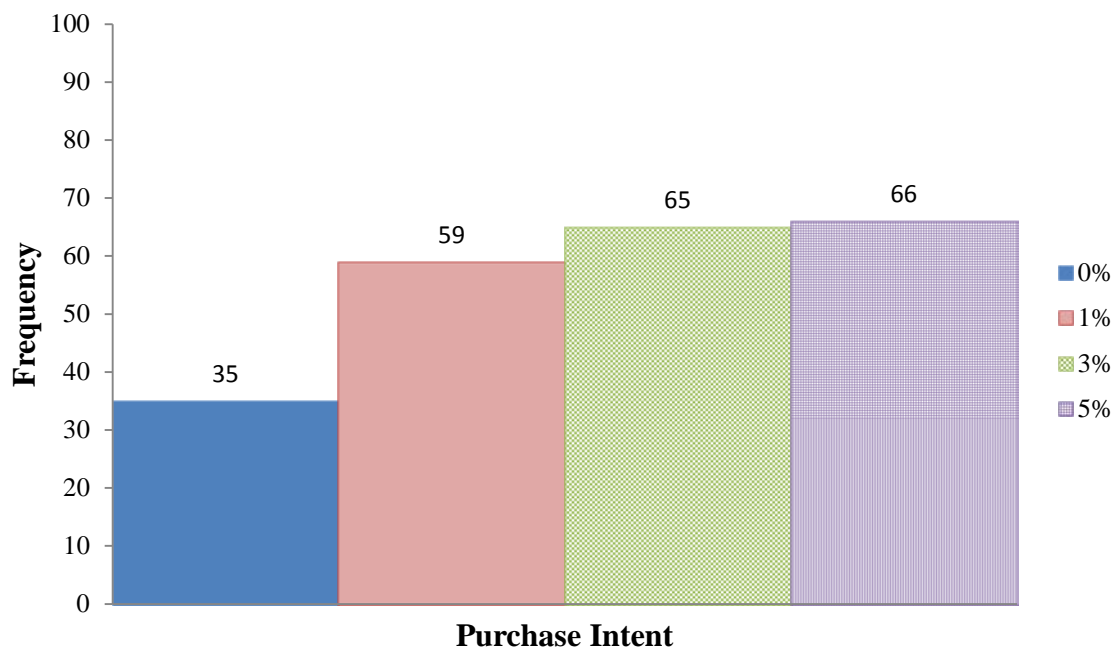


Figure 11. Frequency for purchase intent of blueberry yogurt as influenced by lactose addition.

Yogurt purchase intent frequency values are shown in Figure 11. Added lactose yogurts had greater purchase intent values compared to control yogurts. Purchase intent increased as lactose addition increased. Yogurts containing 5% w/w added lactose led to higher purchase intent (66%) compared to control (35%).

CHAPTER 4: CONCLUSIONS

Results obtained in this research showed that added lactose had a positive effect on probiotic properties of yogurt starter bacteria and yogurt characteristics. Added lactose at 3 and 5% w/w showed the highest acid tolerance for *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12 at 120 minutes of incubation. Added lactose at 5% w/w showed the highest bile tolerance for *Lactobacillus bulgaricus* LB-12 compared to the rest of treatments and for *Streptococcus thermophilus* ST-M5 added lactose at 1% w/w at 2 hours of incubation showed the highest bile tolerance. Growth of *Streptococcus thermophilus* ST-M5 significantly increased by lactose addition at 5% w/w in yogurt. Lactose addition did not have a significant effect on growth of *Lactobacillus bulgaricus* LB-12 in yogurt. Treatments containing 5% w/w added lactose showed the highest lactose content during storage period and had the lowest pH values. Treatment containing 5% added lactose showed the lowest viscosity values compared to the rest; and also the highest syneresis values over storage period of 35 days. Level of added lactose had no effect on appearance and color of blueberry yogurt. Scores for aroma were higher for control and 5% w/w added lactose. Samples containing added lactose showed higher scores for taste, sourness and sweetness. Lactose addition contributed to higher scores for overall liking. Also the acceptability of yogurts and purchase intent markedly increased with the addition of lactose.

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APPENDIX A. CONSENT FORM FOR CONSUMER STUDY

RESEARCH CONSENT FORM

APPROVED BY
LSU AG CENTER
IRB AS HE-13-6
ON 4-18-2013

I, _____, agree to participate in the research entitled "Influence of added lactose on the sensory characteristics of yogurt" which is being conducted by the School of Animal Sciences at Louisiana State University, phone number (225)-578-4411.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated in my school. I can withdraw my consent at any time without penalty of loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. A total of at least 100 people will participate in this research. For this particular research, about a 10 minute participation will be required.

The following points have been explained to me:

1. In any case, it is my responsibility to report prior to participation to the investigators any allergies I may have.
2. The reason for the research is to gather information on the acceptance of yogurts with added lactose. The benefits that I may expect from it are a satisfaction that I have contributed to solution and evaluation of problems relating to such examinations.
3. The procedures are as follows: Coded samples of resistant starch yogurts will be placed in front of me and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.
4. Participation entails minimal risks: The only risk that can be envisioned is an allergic reaction to milk and lactose intolerance. However, because it is known to me beforehand what type of food to be tested, the situation can normally be avoided.
5. The results of this participation will be confidential and will not be released in any individual identifiable form without my prior consent unless required by law.
6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me and all my questions have been answered. I understand that additional questions regarding the study should be directed to the investigators. In addition, I understand that research at Louisiana State University, which involves human participation, is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Michael Keenan, Chairman, Institutional Review Board, (225) 578 1708, mkeenana@agcenter.lsu.edu

Kayamush J. Anyane
Signature of Investigator

Date: 4/17/2013

Signature of Participant

Witness: Behannis Mena

APPENDIX B. QUESTIONNAIRE FOR CONSUMER STUDY

Sample # _____

Date _____

- Please evaluate the product and mark the score [✓] that best reflects your feeling about the product.
- Between the samples, you are required to drink water and eat unsalted cracker to clean your palate.

1. How would you rate the overall **APPEARANCE** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

2. How would you rate the **COLOR** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

3. How would you rate the **AROMA** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

4. How would you rate the **TASTE** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

5. How would you rate the **SOURLINESS** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

6. How would you rate the **SWEETNESS** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

7. How would you rate the **TEXTURE (THICKNESS)** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

8. How would you rate the **TEXTURE (GRAININESS)** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

9. OVERALL, how would you **"LIKE"** this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
[]	[]	[]	[]	[]	[]	[]	[]	[]
1	2	3	4	5	6	7	8	9

10. Is this product **ACCEPTABLE**? Yes [] No []

11. Would you **BUY** this product if it were commercially available? Yes [] No []

VITA

Behannis Jasmin Mena Chalas was born in La Vega, Dominican Republic, on August, 1988. In August, 2006 she graduated with an Associate's degree on Dairy Products Processing from Maximo Gomez Polytechnic Institute in Bani, Dominican Republic. In fall 2010 she received her Bachelor of Science degree in Food Science and Technology from Escuela Agrícola Panamericana, Zamorano University in Tegucigalpa, Honduras. As a requirement for graduation at Zamorano University, she participated in an internship program in the School of Animal Sciences/Dairy Science Division at Louisiana State University, where she performed research on probiotics characteristics of yogurt culture bacteria. Before becoming a graduate student in the School of Animal Sciences//Dairy Science Division at Louisiana State University in the fall of 2011, she worked as Projects Coordinator for Agroindustry at World Vision International – FIME, Inc. in Santo Domingo, Dominican Republic. At this position, she had the opportunity to support producers through personalized training and promote mechanisms for rural development, income and education improvement. While a graduate at Louisiana State University, she participated in leading positions at various student clubs such as: LSU Food Science Club, Graduate Student Association (GSA) and Zamorano Agricultural Society (ZAS). Currently, she is a candidate for Master's degree of Dairy Foods Technology from Louisiana State University and Agricultural Mechanical College in August 2013.