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**Influence of low homogenization pressures on acid tolerance, bile tolerance, protease activity, and growth characteristics of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, *Streptococcus salivarius* ssp *thermophilus* ST-M5, and *Lactobacillus acidophilus* LA-K**

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**INFLUENCE OF LOW HOMOGENIZATION PRESSURES ON ACID TOLERANCE,  
BILE TOLERANCE, PROTEASE ACTIVITY, AND GROWTH CHARACTERISTICS  
OF *LACTOBACILLUS DELBRUECKII* SSP *BULGARICUS* LB-12, *STREPTOCOCCUS*  
*SALIVARIUS* SSP *THERMOPHILUS* ST-M5 AND *LACTOBACILLUS ACIDOPHILUS*  
LA-K**

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Submitted to the Graduate Faculty of the  
Louisiana State University and  
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in partial fulfillment of the  
requirements for the degree of  
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in  
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By  
Tanuja Muramalla  
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## ABSTRACT

*Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, *Streptococcus salivarius* ssp *thermophilus* ST-M5 and *Lactobacillus acidophilus* LA-K are dairy cultures widely used in the fermentation of dairy products. Homogenization is an essential process in dairy industry for making several products. High homogenization pressures (>50 MPa) are used to create stable emulsion, inactivate the pathogens and increase the protease activity of dairy cultures. Sparse literature is available on the influence of low homogenization pressures, and the effect of low homogenization pressures on beneficial characteristics of dairy cultures is not well understood. The objective of this study was to determine the effect of low homogenization pressures on acid tolerance, bile tolerance, protease activity and growth characteristic of the three dairy cultures. The cultures were individually inoculated in cool autoclaved skim milk (4° C) and homogenized for 5 continuous passes. The treatments were homogenization pressures of 3.45 MPa (500 psi), 6.90 MPa (1000 psi), 10.34 MPa (1500 psi) and 13.80 MPa (2000 psi). The control was the sample passed through homogenizer set at 0 MPa (0 psi). Growth and bile tolerance of samples were determined hourly for 10 hours of incubation. Acid tolerance was determined every 20 minutes for 120 minutes of incubation. Protease activity was determined at 0, 12 and 24 hours of incubation. The experimental design was repeated measurements on complete randomized block. Data were analyzed using proc mixed model of statistical analysis system (SAS). Differences of least square means were used to determine significant differences at  $p < 0.05$  for main effect (homogenization pressure) and interaction effect (homogenization pressure \* time). All low homogenization pressures improved acid tolerance of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 but had no beneficial effect on protease activity and had negative effect on growth and bile tolerance of the bacterium. Low homogenization pressure of 6.90 MPa (1000 psi) improved



the acid tolerance, bile tolerance, and protease activity but homogenization pressures had no effect on growth of *Streptococcus salivarius* ssp *thermophilus* ST- M5. Low homogenization pressures of 13.80 MPa (2000 psi), 6.90 MPa (1000 psi) improved acid tolerance, bile tolerance respectively of *Lactobacillus acidophilus* LA-K but had no effect on protease activity and growth of the bacterium. Some low homogenization pressures positively influenced some characteristics of yogurt culture bacteria and *Lactobacillus acidophilus* LA-K.

## CHAPTER 1: INTRODUCTION

### 1.1 Yogurt and its market trends:

Yogurt is defined as the food produced by culturing one or more of the optional dairy ingredients (cream, milk, partially skimmed milk, or skim milk, used alone or in combination) with a characterizing bacterial culture that contains the lactic acid-producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Code of federal regulations (21 CFR131.200), 2009). Chandan (1999) explained that the consumption of yogurt was enhanced in recent years, mainly because of its nutritional value and the beneficial health effects of yogurt cultures. *Lactobacillus acidophilus* is a probiotic bacterium widely used as an adjunct culture in approximately 80% of the yogurts being manufactured in the USA (Hutkins, 2006). Heller (2006) reported 3% increase in sales of cultured dairy products in USA from 2004 to 2005 making \$9.7 billion in the year 2005, of which 50% sales (\$4.9 billion) were contributed by yogurt. Dairy Facts (2009) reported 3.44 % increase in sales of yogurt from 2007 to 2008. Several studies reported yogurt cultures and *L. acidophilus* as probiotics (Guerrero et al., 1996; Reid, 2000; Guarner et al., 2005; Ahrens et al., 2007; Apostolidis et al., 2007; Kligler, 2008; Rodriguez et al., 2008).

### 1.2 Probiotics:

The term probiotics was first introduced by a Russian scientist Elie Metchnikoff. He predicted that the reason for long life of bulgarian peasants could be because of the consumption of fermented dairy products containing particular strains of *Lactobacillus* species (Douglas and Sanders, 2008). Probiotics are “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO, 2001). In general, Lactobacilli and Bifidobacteria species are the most widely known strains of probiotic bacteria. These lactic acid

bacteria have been reported to enhance immune response, exert antimutagenic and anticarcinogenic properties, and decrease the serum cholesterol levels (Manson et al., 1992). Probiotics contribute to the development of healthy immune system by sustaining the gastrointestinal stress factors such as acid and bile condition and by outnumbering the pathogens (Geier et al., 2007). Probiotics were grouped in the functional foods category as they offer a wide range of health benefits such as preventing cardiovascular diseases, obesity, allergy, colon cancer, inflammatory bowel syndrome, and other gastrointestinal diseases (Knorr, 1998).

### **1.2.1 Health benefits of probiotics:**

Yogurt culture bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* were reported to have various health benefits. For instance, yogurt containing these bacteria has been recommended by world health organization (1995) for the management of acute diarrheal disorders. Guarner et al., (2005) reported that consumption of yogurt containing these bacteria enhanced the immune system of immune-compromised people. Yogurt cultures have also proven to reduce antibiotic-associated diarrhea (Ahrens et al., 2007). In a recent study, it has been shown that probiotic preparations containing *Lactobacillus rhamnosus* GG or a combination of *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* were more effective compared to the oral rehydration solution in reducing the severity and duration of acute diarrhea in children (Kligler, 2008). Similarly, *L. acidophilus* has also been reported to treat diarrhea in children (Guerrero et al., 1996). Recently, Exopolysaccharide (EPS) produced by EPS-*Streptococcus thermophilus* has been reported to produce immune-stimulatory or antiulcer effects in mouse model of chronic gastritis (Rodriguez et al., 2008). EPS has also been reported to improve the texture of the fermented dairy products like yogurt and cheese (Jolly & Stingle, 2001).

Nun et al., (2005) reported that incidence rate of necrotizing enterocolitis (NEC), a disease commonly found in neonates (1-2 year old) was reduced by feeding probiotic mixture containing *Streptococcus thermophilus*, *Bifidobacterium infantis*, and *Bifidobacterium bifidus*. They opined that reduced incidence of NEC could be due to shifting of intestinal ecological balance from potential harmful to that of the beneficial microflora. They also explained that these probiotics, besides strengthening the intestinal mucosal layer and impeding the translocation of the pathogens, also produce certain proteins called bacteriocins capable of killing pathogenic bacteria. *L. acidophilus* has been reported to replace various pathogenic organisms in the intestine such as *Staphylococci* and *E. coli* leading to a healthy microbiological balance (Gordan et al., 1957; Fuller, 1977; Watkins and Miller., 1983). In another study, *L. acidophilus* has also been reported to be beneficial in treatment of urinary and vaginal tract infections (Reid, 2000). Previously, it has been reported that *L. acidophilus* increases the utilization of lactose in milk and decreases lactose intolerance, by the action of an intracellular enzyme  $\beta$ -galactosidase (Kim & Gilliland, 1983; Gilliland et al., 1985).

*L. bulgaricus* and *S. thermophilus* were also reported to decrease the risk of colorectal cancer *in vivo* (Young & Wolf, 1988; Balansky et al., 1999) and reduce proliferation of colon cancer cells *in vitro* (Baricault et al., 1995). It has been reported that administration of fermented milk containing *Lactobacillus acidophilus* to colon cancer patients for 6 weeks decreased colony counts of *Escherichia coli* and *Clostridium* spp in the feces (Lidbeck et al., 1991). *L. acidophilus* and *B. bifidum* have been proven to reduce incidence of cancer and proliferation of cancer cells in colon cancer patients (Shahani et al., 1983; Biasco et al., 1991). *L. acidophilus* deactivates enzymes  $\beta$ - glucoronidase, azoreductase and nitro reductase leading to lower proliferation of cancer cells (Goldin and Gorbach, 1984).

Ingestion of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* has been reported to be beneficial in diseases such as hyperglycemia and hypertension (Apostolidis et al., 2007). *L. acidophilus* has been shown to reduce serum cholesterol levels in pigs, infants, and human (Mann & Spoerry, 1974; Harrison and Peat, 1975; Gilliland et al., 1985), possibly by increased deconjugation of bile acids leading to reduced lipid absorption from the intestine (Eyssen, 1973). In a study, *Streptococcus thermophilus* has been shown to enhance cellular immune response against ovalbumin antigen in mice (Matsuzaki & Chin, 2000). Recently, a probiotic mixture containing *S. thermophilus* and *B. bifidum* has been proven to be successful in increasing the immunity of HIV infected children as suggested by increased CD4 (immune cells) counts (Trois et al, 2007).

### **1.3 Survival of cultures in gastrointestinal tract:**

#### **1.3.1 Acid and bile conditions:**

One of the basic requirements for a culture to be called probiotic is the ability to survive acid and bile conditions in the gastrointestinal tract (Dunne et al., 2001). Acids are produced in three inter-related acid phases in the stomach, namely cephalic, gastric and intestinal acid phases. The gastric acid is the most important contributor for acidic conditions in the stomach and is released in response to chemical effects of food and during bloating of stomach (Lloyd & Debas, 1994). Gastric acid is essentially an acidic solution containing 0.5% of hydrochloric acid and abundant amount of potassium chloride and sodium chloride which brings the stomach pH in the range of 1-2 (Lindstrom et al., 2001). The acidic condition in the stomach is essential for protein digestion, suppression of growth of acid sensitive pathogenic bacteria, and absorption of certain minerals such as calcium, iron and vitamin B12 (Soll, 2009). Bile juices are secreted in the liver and stored in the gall bladder. It is a yellowish green solution containing a mixture of bile acids,

cholesterol and phospholipids (Carey & Duane, 1994). Bile plays a critical role in fat digestion by solubilizing the fat through emulsification process. It also exerts antibacterial activity by dissolving the bacterial membranes (Begley et al., 2005).

Maintaining the functionality of bacterial cultures in the gastrointestinal tract conditions (acid and bile) has been one of the major challenges while developing probiotic products (Sandholm et al., 2002). Various strains of Lactobacilli (*L. acidophilus*, *L. casei*, and *L. bulgaricus*) and Bifidobacteria (*B. longum* and *pseudolongum*) have been shown to be acid and bile tolerant (Lankaputra & Shah, 1995; Shah & Jelen, 1990; Dune et al., 2001). Bezkorovainy, (2001) reported 20-40% survival of these bacteria in the stomach and intestine (Fig 1).

Acid tolerance of a microorganism has been described as the ability to survive during the passage (transit time of 30 – 120 minutes, depending on the type of strain) through the low pH (1.5–3) of the stomach (Lankaputra & Shah, 1995). Bile tolerance of a microorganism has been described as the ability to survive during the transit time (<3h, depending on the type of strain) through the high concentrations of bile acids (<1.5% (w/v)) in small intestine, and its subsequent colonization in the colon (Lankaputra & Shah, 1995).

### **1.3.2 Protease activity and growth:**

Lactic acid bacteria are gram positive bacteria with thick cell wall (Vasiljevic & Shah, 2008). They play vital role in the fermentation of dairy products such as yogurt and cheese (Gatti et al., 2004). Protease activity and viability of the cultures are two important characteristics that affect the texture, taste, and shelf life of the cultured dairy products (Soda, 1991). The enzymes present in the intra cellular components of bacteria are crucial in the proteolysis reaction in fermented products (Gatti et al., 2004).

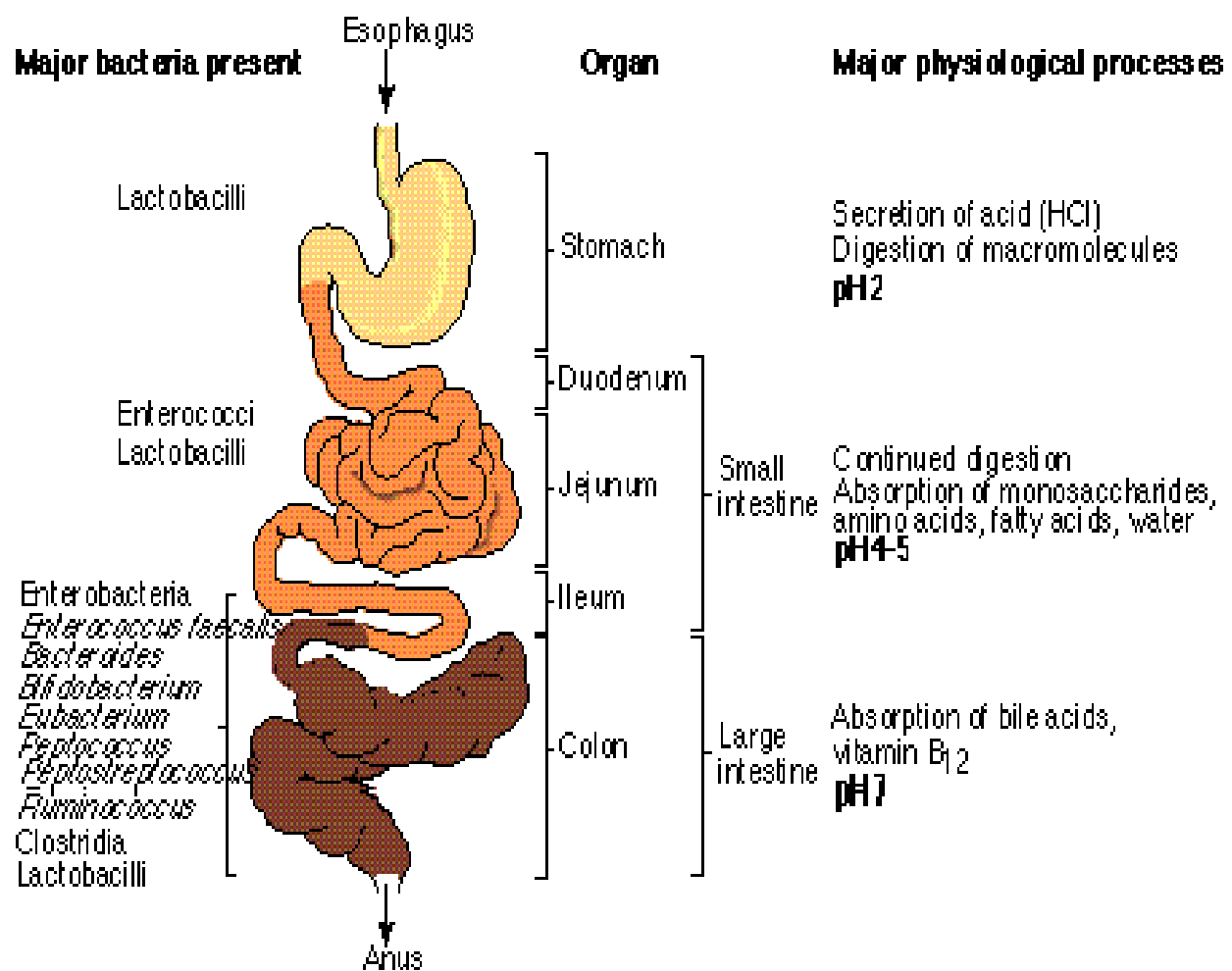


Figure 1. Survival of different types of micro organisms in the gastro intestinal tract ([www.cwx.prenhall.com](http://www.cwx.prenhall.com)).

Breakdown or disruption of the cell wall of the bacteria is an essential step to release the protease enzymes (Wilkinson & Kilcawley, 2005). Moreira et al., (2000) reported that protease enzymes produced by *L. bulgaricus* degrade the casein into peptides and amino acids which serve as growth factors for *S. thermophilus*. The carbon dioxide and formic acid produced by *S. thermophilus* stimulate the growth of *L. bulgaricus* (Moreira et al., 2000). *L. bulgaricus*, *L. acidophilus*, and *S. thermophilus* require different incubation conditions, enumeration methods and growth requirements for their propagation (Tharmaraj & Shah, 2003). Gatti et al., (2004)

reported that different strains of the lactic acid bacteria can be distinguishable depending on the type of growth kinetics, species, biotype and their number.

Growth and protease activity of dairy cultures are affected during processing and storage of foods due to high temperatures, high pH, and high osmotic pressures (Gardiner et al., 2000; Prasad et al., 2003; Talwalkar & Kailasapathy, 2003). Lanciotti et al., (2006) reported that osmotic shock (sudden change in solute concentration around the cell) could be responsible for the enhanced proteolytic activity of the homogenized (100 MPa/ 1, 45, 00 psi) lactic acid bacteria (caciotta cheese cultures) and the subsequent release of intracellular and cell wall proteases into the fluid. They suggested that at a pressure exceeding a threshold value, the specificity of the active sites of the enzymes can be altered by changing their configuration. At pressure levels between 50-100 MPa (7250 to 14500 psi), the secondary structures and water relationships of the protein were reported to be altered.

#### **1.4 Homogenization:**

‘Homogenization is a fluid mechanical process that involves the subdivision of particles or droplets into micron sizes, to create a stable dispersion or emulsion,’ (Diels and Michiels, 2006). Homogenization is conducted by a homogenizer (Fig 2). The basic function of a homogenizer is to break the big mass of particles into several small particles of micron size and prevent the formation of clusters (Middelberg, 1995). Typically, a homogenizer consists of a positive displacement pump and a homogenizing valve. The fluid is pumped into the homogenizing valve where it is passed through a small orifice under pressure. The pressure applied is controlled by adjusting the distance between valve and the valve seat in the homogenizer (Middelberg, 1995). The homogenization phenomenon actually takes place in the interval between the entry of the fluid into the gap between the valve and valve seat and exit from the valve seat. This process will



be completed in few microseconds and the homogenized fluid finally leaves the gap (orifice) like a radial jet (Middelberg, 1995).

There are three main mechanisms by which homogenization pressure exerts its action on fluid, namely: turbulence (Doulah & Hammond, 1975), cavitation (Deshimaru, 1994), and impingement (Kleinig and Middelberg, 1996). During the turbulent flow, vortices are formed as a result of deviation of the fluid from the surface shape of the tube. When the kinetic energy generated by the oscillatory motion of these vortices exceeds the strength of the cells in the fluid it leads to cell disruption (Doulah & Hammond, 1975). Second mechanism, cavitation, was explained in detail by Deshimaru, (1994). They stated that during the turbulent flow, difference between the local static pressure and the vapor pressure of the fluid increases leading to the formation of gas cavities. The cavity thus formed when encountered a high pressure region during the flow, collapses and causes vibration and noise leading to microbial cell disruption. Middelberg, (1995) found cavitation as the primary mechanism for cell disruption in homogenization process. Impingement is another mechanism of microbial cell disruption explained by Kleinig & Middelberg, (1996). They stated that impingement or impact with the solid surfaces is the pressure at the point of impact which also contributes to cell disruption. In conclusion, homogenization process causes various changes in the cell wall of microorganisms, leading to changes such as proteolysis, lipolysis, and glycolysis that can be used for industrial applications or for future studies with some beneficial bacteria like probiotics (Gatti et al., 2004; Vannini et al., 2004; Lanciotti et al., 2007).

Homogenization process is conducted for various purposes such as, creation of stable emulsions (Middelberg, 1995), inactivation of spoilage microorganisms and pathogens (Lanciotti et al., 1996; Vannini et al., 2004; Brinez et al., 2006; Diels and Michiels, 2006), release of certain

proteolytic enzymes from the intracellular components of beneficial microorganisms like dairy cultures and probiotic cultures (Wilkinson & Kilcawley, 2005), and for creating some desirable physical changes like texture, flavor, color in the product (Wuytack et al., 2002).

### **1.5 Factors affecting homogenization of micro organisms:**

#### **1.5.1 Temperature:**

Temperature effect is an important criterion that affects cell damage and acts synergistically with homogenization pressures (Floury et al., 2000). The rise in the temperature during high pressure homogenization is because of the dissipation of mechanical energy in the form of heat. The viscous stress caused by the high fluid velocity on the valves of homogenizer leads to the generation of heat (Floury et al., 2000). Recently, Bevilacqua et al., (2009) reported that counts of *Lactobacillus plantarum* and *Lactobacillus brevis* were reduced by 0.4 log units merely due to the rise in temperature during high homogenization process (150 MPa/21,750 psi).

#### **1.5.2 Type of micro organism:**

Gram positive bacteria were more resistant than gram negative bacteria when subjected to high homogenization pressure (> 50 MPa/ 7252 psi) (Vachon et al., 2002). High homogenization pressure kills the cells by mechanical destruction of cell integrity (Engler and Robinson, 1981; Moore et al., 1990). The cell wall composition of the gram positive bacteria with thick peptidoglycon layer of 40 layers has been reported to be more resistant to high pressures than the gram negative bacteria consisting of 1-5 layers of peptidoglycon chains. (Vachon et al., 2002; Wuytack et al., 2002). Pathanibul et al., (2009) explained that at pressure less than 250 MPa (36,259 psi) *E. coli* (gram negative bacteria) was inactivated by more than 5 log cycles where as *Listeria innocua* (gram positive bacteria) exhibited little or no inactivation. Geciova et al., (2002)

explained that yeast are more sensitive compared to gram positive bacteria in spite of former group having a thicker cell wall. This is because of relative differences in the size and cell wall composition. The cell wall of the yeast mainly contains enzymes where as the cell wall of the bacteria are made of structural component namely peptidoglycan (Engler and Robinson, 1981). Hence the resistance of yeast to high pressure (200 MPa/ 29007 psi) falls in between gram negative and gram positive bacteria (Tahiri et al., 2006).

### **1.5.3 Number of passes:**

Bevilacqua et al., (2009) stated that inactivation of lactic acid bacteria increase with increase in number of passes. They reported that lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus brevis*, and *Bifidobacterium coagulans*) were resistant to homogenization pressure (150 MPa/ 21,756 psi) after one pass, and when the number of passes was increased to three, the bacteria were reduced to 1 log cfu/ ml. In another study, Donsi et al.,(2009) explained that inactivation of *E. coli* subjected to homogenization pressure of (200 MPa/ 29007 psi) for one pass is equivalent to the inactivation at 100 MPa/ 14504 psi for 2 passes or 50 MPa/ 7252 psi for 6 passes.

### **1.5.4 Medium composition:**

Kheadr et al., (2002) explained the effect of high pressure (200 MPa/ 29007 psi) on the inactivation levels of endogenous flora in skim milk and whole milk and they found a greater reduction of flora in skim milk (4-5 log cycles) compared to whole milk (2 log cycles). Fat in the whole milk acted as protective layer. Moreover, complex formation of reduced casein protein and fat globules would also protect the bacteria in whole milk from cell damage (Kheadr et al., 2002). Contrasting this reasoning, Diels et al., (2005) proposed that inactivation was merely dependant on the fluid viscosity rather than product composition or water activity. They reported that inactivation of *E. coli* was same in food model and its corresponding polyethyleneglycol

(PEG) solution with same relative viscosity. Furthermore, they reported that as the relative viscosity of the buffer solution containing PEG was decreased from 4.9-1.0 centi poise, an increase in the inactivation of *E. coli* counts were observed (2 to 5 log cycles) at 300 MPa/ 43511 psi. Pathanibul et al., (2009) also supported that the medium composition does not affect the inactivation of *E.coli* at high pressure homogenization (250 MPa/ 36,259 psi). They compared the inactivation in apple juice and carrot juice and suggested that product composition does not influence the inactivation.

### **1.5.5 Pressure:**

The homogenization pressure was defined as the overall effect of compression in the intensifier (homogenizer), and the flow through the fittings and piping of the system that would lead to an effective stress on microbial population (Donsi et al., 2009). Bevilacqua et al., (2009) reported an increase in the log reduction of lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus brevis*, and *Bifidobacterium coagulans*) from 0.7 to 2.4 log cfu/ml on increasing the pressure from 50 to 150 MPa (7252 to 21,756 psi). Tahiri et al., (2006) also reported that *L. innocua* exhibited an increase in log reduction from 0 to 5 log cfu/ml when the pressure was increased from 250 - 350 MPa (36,259 - 50,763 psi). They reported that *E. coli* was completely inactivated at 350 MPa (50,763 psi) which showed a 5 log reduction at 250 MPa (36,259 psi). Recently, Donsi et al., (2009) reported that inactivation of *E. coli* had increased from (1 to 5 log cycles) upon increasing pressure from 100 to 300 MPa (14504 to 43511 psi).

Depending on the range of pressures applied; homogenization can be broadly classified into high homogenization pressures and low homogenization pressures. It is difficult to define the term high homogenization pressures precisely, however pressures higher than 50 MPa (7252 psi) were generally considered as high pressure (Vachon et al 2002; Vannini et al, 2004; Diels & Michiels,

2006; Tahiri et al, 2006). Several previous studies (Lanciotti et al.,1996; Vannini et al. 2004; Brinez et al. 2006; Diels and Michiels, 2006) had shown the effect of high homogenization pressures on the growth of pathogens and spoilage micro organisms, but very little is known about low homogenization pressures and its effects on bacteria.

Gatti et al., (2004) investigated the effect of low pressure on aminopetidase activity of lactic acid bacteria (*L. helveticus*, *L. delbrueckii* subsp *lactis* and *L. delbrueckii* ssp *bulgaricus*). They reported that pressure of 8.82 MPa (1280 psi) for 5-15 min caused increased enzymatic activity of these bacteria. Coskun, (2006) reported the effect of low homogenization pressure (30 MPa/ 4351 psi) on two types of lactic acid bacteria, mesophilic cultures (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp.*cremoris* and *Lactobacillus helveticus*) and thermophilic cultures (*Lb. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*). They concluded that low pressure homogenized (30 MPa or 4351 psi) mesophilic (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp.*cremoris*) bacteria exhibited higher proteolysis than low pressure homogenized (30 MPa or 4351 psi) thermophilic (*Lb. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) bacteria (Coskun, 2006). Productivity and viable counts of bacteria are both equally important for developing the probiotic foods industrially (Knorr, 1998). The pressure of 30 MPa (4351 psi) can still be considered as high pressures compared to pressures of 13.80 MPa (2000 psi) or less which are commonly used homogenization pressures in dairy industry (Pandolfe, 1982).

In the current study, several preliminary experiments were conducted to decide on range of low homogenization pressures and the number of passes to be applied on dairy cultures. Initially, maintaining a constant homogenization pressure of 1.72 Mpa/ 250 psi, *S. thermophilus* inoculated in sterile skim milk was subjected to 5, 10 and 15 passes (1 miute, 2 minutes, and 3

minutes respectively). No difference was observed in the viable counts of the bacterium treated to different passes. Hence we decided to continue the experiments with constant run time of 5 passes (1 minute). Secondly, *S. thermophilus* was subjected to different homogenization pressures of 1.72 MPa (250 psi), 2.59 MPa (375 psi), 3.45 MPa (500 psi), 5.17 MPa (750 psi), 6.90 MPa (1000 psi), 8.62 MPa (1250 psi), 10.34 MPa (1500 psi), 12.07 MPa (1750 psi), and 13.80 MPa (2000 psi) maintaining constant run time of 5 passes (1 minute). There was no difference in viable counts of *S. thermophilus* when subjected to pressures of 0 MPa (0 psi), 1.72 MPa (250 psi), and 2.59 MPa (375 psi). The homogenization pressure between 8.62 MPa (1250 psi) and 10.34 MPa (2000 psi) improved the growth of *S. thermophilus* than control (0 MPa/ 0 psi). Hence we decided the pressure range of 0 MPa (0 psi) to 13.80 MPa (2000 psi) with an increment of 3.45 MPa (500 psi) for this study.

Influence of low homogenization pressures on culture and probiotic bacteria are not clearly understood. The hypothesis was whether or not low homogenization pressures can improve the beneficial characteristics of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, *Streptococcus salivarius* ssp *thermophilus* ST-M5 and *Lactobacillus acidophilus* LA-K.

**The objectives were:**

1. To determine the effect of low homogenization pressures of 0 MPa (0 psi), 3.45 MPa (500 psi), 6.90 MPa (1000 psi), 10.34 MPa (1500 psi), and 13.80 MPa (2000 psi) on the acid tolerance of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, *Streptococcus salivarius* ssp *thermophilus* ST-M5 and *Lactobacillus acidophilus* LA-K
2. To study the effect of low homogenization pressures of 0 MPa (0 psi), 3.45 MPa (500 psi), 6.90 MPa (1000 psi), 10.34 MPa (1500 psi), and 13.80 MPa (2000 psi) on the bile

tolerance of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, *Streptococcus salivarius* ssp *thermophilus* ST-M5 and *Lactobacillus acidophilus* LA-K

3. To elucidate the effect of low homogenization pressures of 0 MPa (0 psi), 3.45 MPa (500 psi), 6.90 MPa (1000 psi), 10.34 MPa (1500 psi), and 13.80 MPa (2000 psi) on protease activity of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, *Streptococcus salivarius* ssp *thermophilus* ST-M5 and *Lactobacillus acidophilus* LA-K
4. To determine the effect of low homogenization pressures of 0 MPa (0 psi), 3.45 MPa (500 psi), 6.90 MPa (1000 psi), 10.34 MPa (1500 psi), and 13.80 MPa (2000 psi) on growth of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, *Streptococcus salivarius* ssp *thermophilus* ST-M5 and *Lactobacillus acidophilus* LA-K

## CHAPTER 2: MATERIALS AND METHODS

### 2.1 Experimental design:

Skim milk was sterilized by autoclaving at 121° C for 15 minutes. This sterile milk was cooled to 4°C and individually inoculated with 0.1% (v/v) *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, *Streptococcus salivarius* ssp *thermophilus* ST-M5 and *Lactobacillus acidophilus* LA-K (Chr. Hansen's Laboratory, Milwaukee, WI, USA). The treatments were homogenization pressures of 0 MPa (0psi), 3.45 MPa (500 psi), 6.90 MPa (1000 psi), 10.34 MPa (1500 psi) and 13.80 MPa (2000 psi). The control was the sample passed through homogenizer set at 0 MPa (0 psi). The homogenization pressures were randomized for all three replications.

The control and the treated (homogenized) samples were tested for acid tolerance, bile tolerance, protease activity and growth. Growth was determined by plating the homogenized cultures every hour for 10 hours. Bile tolerance of the cultures was determined by growing the homogenized cultures in presence of bile and plating every hour for 10 hours. Acid tolerance was determined by inoculating the homogenized cultures in the acidified broth and plating for every 20 minutes up to 120 minutes. Protease activity of the homogenized cultures was determined by measuring optical density (absorbance value) at 0, 12, and 24 hours of incubation of the samples. Three replications were conducted with replications as blocks. The design was repeated measurements on complete randomized block.

### 2.2 Homogenizer:

Gualin homogenizer (Manton-Gaulin Manufacturing Company, Inc., Everett, MA) was used for the experiment. It is a two stage homogenizer. First stage of the homogenizer break up the cells into smaller particles and second stage of the homogenizer prevent the cluster formation of the



cells (Middleberg, 1995). In the experiment first stage homogenizer was used for the entire pressures applied on the samples to maintain experimental design simple and avoid the combined effect of two stages of the homogenizer (Fig 3). The milk utilized in the experiment was sterile skim milk.

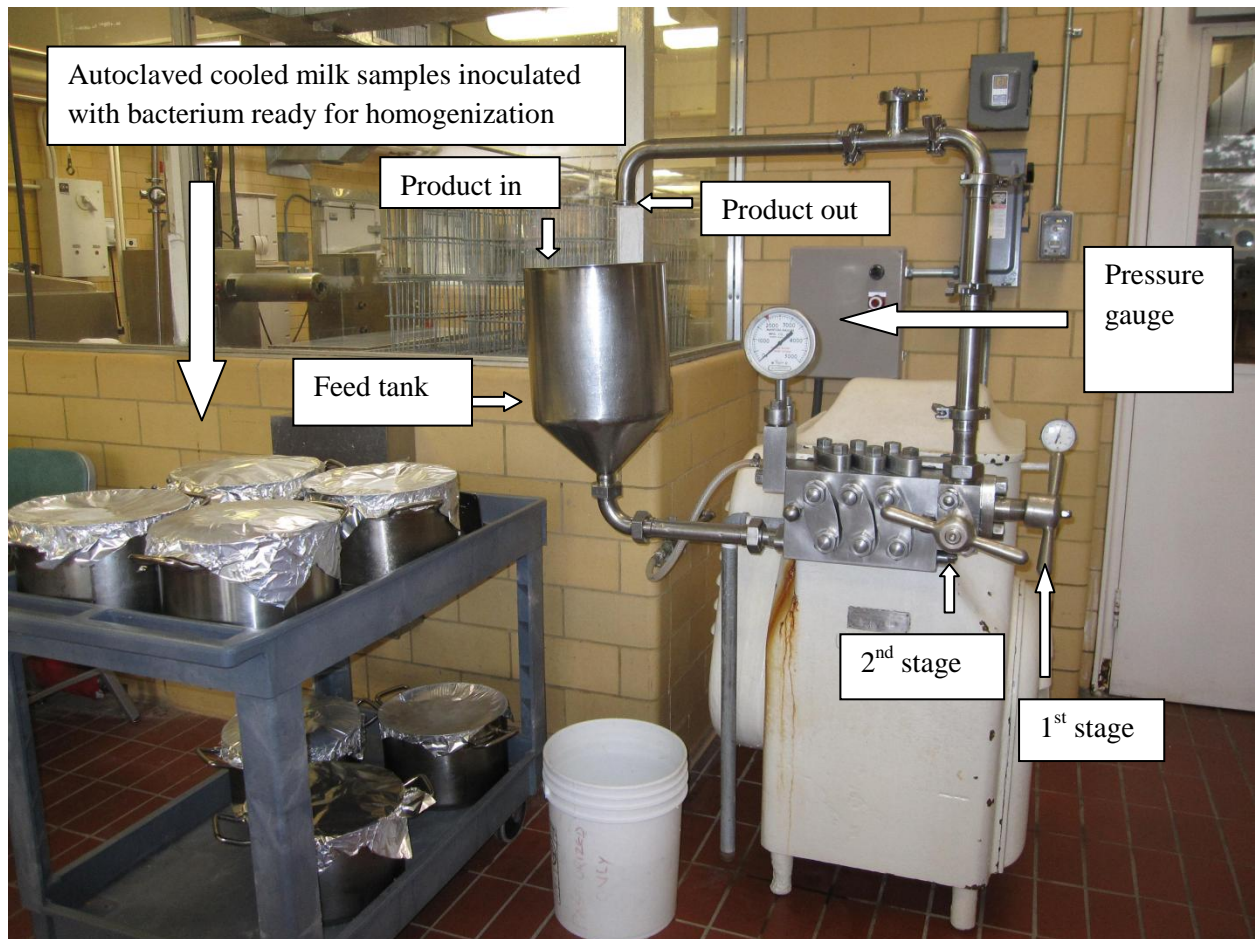


Figure 2. Homogenizer

## 2.3 Preparation of media:

### 2.3.1 *Streptococcus thermophilus* agar (ST agar):

The ST agar was prepared according to the method described by Dave and Shah, (1996). 10 g of Bacto™ Tryptone (Difco, Becton, Dickinson and company, Sparks, MD), 10 g of Sucrose

(Amresco, Solon, OH), 5 g of Bacto™ Yeast extract (Difco, Becton, Dickinson and company, Sparks, MD) and 2 g of Dipotassium Phosphate ( $K_2HP04$ ) (Fisher, Fair Lawn, NJ) was dissolved in 1 L distilled water. The pH of the solution was adjusted to  $6.8 \pm 0.1$  using 1 M HCl. 6 ml of 0.5 % bromocresol purple (Fisher, Fair Lawn, NJ) and 12 g of agar ((Fisher, Fair Lawn, NJ) was added to the medium. The medium was boiled and sterilized at  $121^\circ\text{C}$  for 15 min.

### 2.3.2 Lactobacilli MRS agar:

MRS agar was prepared according to the manufacturer instructions (Difco™, Becton, Dickinson and company, Sparks, MD).

### 2.3.3 pH modified MRS agar (pH 5.2-4.58):

The pH of MRS agar (Difco™, Becton, Dickinson and company, Sparks, MD) was adjusted to a pH of 5.2 to 4.58 using 1M HCl (Dave and Shah., 1996).

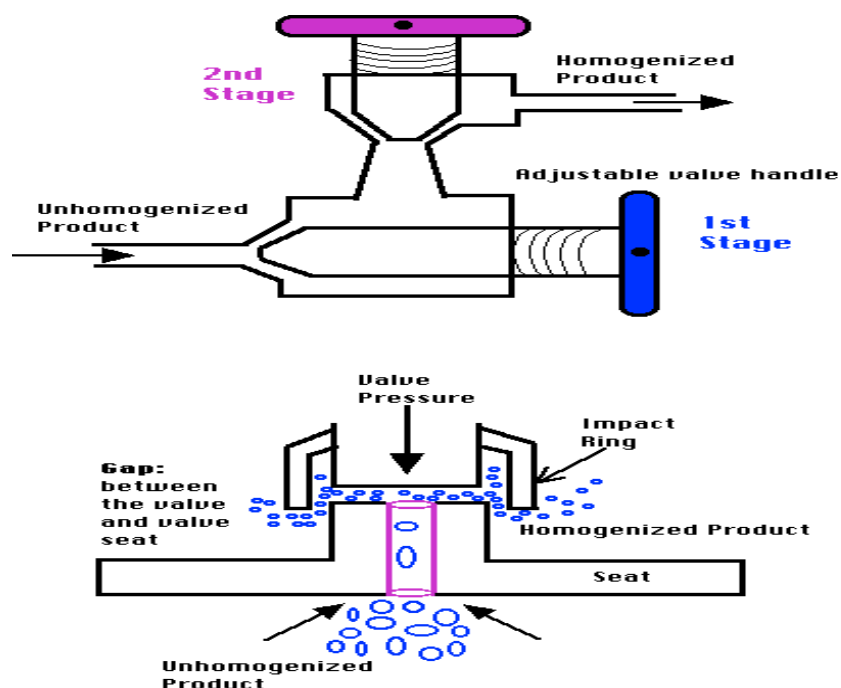


Figure 3. Homogenization phenomenon in a two stage homogenizer (<http://www.emt-india.net/process/dairy/img/homogenizer.gif>)

## **2.4 Analytical procedures:**

### **2.4.1 Acid tolerance:**

The acid tolerance of the three cultures was determined by the method proposed by Pereira and Gibson, (2002) with slight modifications. The control and homogenized samples were inoculated in acidified MRS broth (Difco™, Becton, Dickinson and company, Sparks, MD) previously adjusted to pH 2 using 1 N HCl. The inoculated acidified MRS broth were incubated at 37°C for *Lactobacillus acidophilus* LA-K, 43°C for *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 and 37°C for *Streptococcus salivarius* ssp *thermophilus* for 120 minutes. Every 20 minutes for 120 minutes, 1 ml of the inoculated broth was serially diluted in peptone water (0.1% wt/v) and pour plated. The cultures *Lactobacillus acidophilus* LA-K, *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 and *Streptococcus salivarius* ssp *thermophilus* ST-M5 were enumerated using Lactobacilli MRS agar, pH modified Lactobacilli MRS agar, and *Streptococcus thermophilus* agar respectively (Dave and Shah., 1996). The petriplates were incubated anaerobically at 37°C for 24 hours for *Lactobacillus acidophilus* LA-K, anaerobically at 43°C for 72 hours for *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 and aerobically at 37°C for 24 hours for *Streptococcus salivarius* ssp *thermophilus* ST-M5. After the incubation period the colonies were counted.

### **2.4.2 Bile tolerance:**

The bile tolerance was determined according to method proposed by Pereira and Gibson, (2002) with slight modifications. The bile tolerance of the three cultures was analyzed in MRS-THIO broth [MRS broth (Difco™, Becton, Dickinson and company, Sparks, MD)] supplemented with 0.3% (wt/v) oxgall (bovine bile) (USBiological, Swampscott, MA) and 0.2 % (wt/v) sodium thioglycolate (Acros Organics, Fair Lawn, NJ) . Oxgall was added to test bile tolerance of the

bacteria and sodium thioglycolate was used in the broth as oxygen scavenger. Control and homogenized cultures were inoculated 10% (v/v) separately in MRS-THIO broth and incubated at 37°C for *Lactobacillus acidophilus* LA-K, 43°C for *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 and 37°C for *Streptococcus salivarius* ssp *thermophilus* for 10 hours. Each hour for 10 hours, 1 ml of the inoculated broth was serially diluted in peptone water (0.1% wt/v) and pour plated. The cultures *Lactobacillus acidophilus* LA-K, *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 and *Streptococcus salivarius* ssp *thermophilus* ST-M5 were enumerated using Lactobacilli MRS agar, pH modified Lactobacilli MRS agar, and *Streptococcus thermophilus* agar respectively (Dave and Shah., 1996). The petriplates were incubated anaerobically at 37°C for 24 hours for *Lactobacillus acidophilus* LA-K, anaerobically at 43°C for 72 hours for *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 and aerobically at 37°C for 24 hours for *Streptococcus salivarius* ssp *thermophilus* ST-M5. After the incubation period the colonies were counted.

#### **2.4.3 Protease activity:**

The protease activity of three cultures was determined by *o*-phthaldialdehyde (OPA) spectrophotometric method proposed by Oberg et al., (1991) with slight modification. The control and homogenized samples were incubated at 40°C for 0, 12 and 24 hours. After incubation, 2.5 ml from each sample was mixed with 1 ml distilled water individually and was transferred into each of the test tubes containing 5 ml of 0.75N trichloro acetic acid (TCA) (Fisher Scientific) and the test tubes were vortexed. After setting at room temperature for 10 minutes the acidified samples were filtered through a whatman number 2 filter paper (Clifton, NJ). Duplicate aliquots from each TCA filtrate was analyzed by OPA testing using a spectrophotometer (Nicolet Evolution 100, Thermo Scientific; Madison, WI, USA). The OPA

final solution was prepared by combining the following reagents and diluting to a final volume of 50 ml with distilled water: 25 ml of 100 mM sodium borate (Fisher Scientific); 2.5 ml 20% (wt/wt) SDS (Fisher Scientific); 40 mg of OPA reagent (Alfa Aesar, Ward Hill, MA) dissolved in 1 ml methanol (Sigma); and 100  $\mu$ l of  $\beta$ -mercaptoethanol (Sigma). One hundred and fifty  $\mu$ l of each TCA filtrate was mixed with 3 ml of OPA reagent in a 3 ml cuvette, and the absorbance at 340 nm was read. Absorbance of the OPA final solution with the non inoculated sterile skim milk was subtracted from each sample reading. OPA reagent was used as a blank to calibrate the spectrophotometer.

#### **2.4.4 Growth:**

Growth of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, *Streptococcus salivarius* ssp *thermophilus* ST-M5 and *Lactobacillus acidophilus* LA-K was determined by the method proposed by Lin and Young, (2000) with slight modifications. Control and homogenized samples were inoculated 10% (v/v) separately into MRS broth (Difco™, Becton, Dickinson and company, Sparks, MD) which was previously autoclaved at 121 °C for 15 min with pH  $6.5 \pm 0.2$ . The inoculated broths were incubated at 37°C for *Lactobacillus acidophilus* LA-K, 43°C for *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 and 37°C for *Streptococcus salivarius* ssp *thermophilus* ST-M5 for 10 hours. Each hour for 10 hours, 1 ml of the inoculated broth was serially diluted in peptone water (0.1% wt/v) and pour plated. The cultures *Lactobacillus acidophilus* LA-K, *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 and *Streptococcus salivarius* ssp *thermophilus* ST-M5 were enumerated using Lactobacilli MRS agar, pH modified Lactobacilli MRS agar, and *Streptococcus thermophilus* agar respectively (Dave and Shah., 1996). The petriplates were incubated anaerobically at 37°C for 24 hours for *Lactobacillus acidophilus* LA-K, anaerobically at 43°C for 72 hours for *Lactobacillus delbrueckii* ssp

*bulgaricus* LB-12 and aerobically at 37°C for 24 hours for *Streptococcus salivarius* ssp *thermophilus* ST-M5. After the incubation period the colonies were counted.

## **2.5 Statistical analysis:**

Data were analyzed using proc mixed model of statistical analysis system (SAS). Differences of least square means were used to determine significant differences at  $p < 0.05$  for main effect (homogenization pressure) and interaction effect (homogenization pressure \* time). Data are presented as mean  $\pm$  standard error of the means. Significant differences were determined at  $\alpha = 0.05$ . Significant difference ( $p < 0.05$ ) among the homogenization pressures (3.45 MPa (500 psi), 6.90 MPa (1000 psi), 10.34 MPa (1500 psi), and 13.80 MPa (2000 psi)) and the control (0 psi) was analyzed using Tukey's adjustment.

## CHAPTER 3: RESULTS AND DISCUSSION

### 3.1 Acid tolerance:

#### 3.1.1 *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12:

The viability of bacterium subjected to different low homogenization pressures when incubated in acid conditions has been expressed as log cfu/ml (Fig 4A). The bacterium was incubated in acid condition (pH 2) for 120 minutes and viable counts were determined every 20 minutes. There was a significant ( $p<0.05$ ) interaction between the homogenization pressures and time (minutes) (Table 1). In control, the bacterium exhibited a significant ( $p<0.05$ ) decrease in viable counts every 20 minutes and the viable count was zero after 60 minutes of incubation (Fig 4A). In general, the viable counts of bacterium subjected to various pressures were significantly ( $p<0.05$ ) higher at every 20 minutes of incubation when compared to control and this trend was observed throughout 120 minutes of incubation (Table 2). More importantly, bacterium subjected to pressures of 13.80 MPa (2000 psi) and 10.34 MPa (1500 psi) exhibited almost no decline in the viable counts throughout 120 minutes of incubation period (Fig 4A).

Homogenization pressures had a significant ( $p<0.05$ ) effect on acid tolerance of the bacterium (Table 1). The acid tolerance of the bacterium subjected to pressures of 3.45 MPa (500 psi), 6.90 MPa (1000 psi), 10.34 MPa (1500 psi) and 13.80 MPa (2000 psi) was significantly ( $p<0.05$ ) higher than the control (0 MPa/ 0 psi) (Table 3). There also exists a significant ( $p<0.05$ ) difference among acid tolerances of the bacterium subjected to various homogenization pressures (Table 3). The bacterium homogenized at pressures of 13.80 MPa (2000 psi) and 10.34 MPa (1500 psi) was found to exhibit significantly ( $p<0.05$ ) higher acid tolerance compared to the bacterium homogenized at pressures of 6.90 MPa (1000 psi) and 3.45 MPa (500 psi). Furthermore, the bacterium subjected to pressure of 13.80 MPa (2000 psi) was found to be

significantly ( $p<0.05$ ) more acid tolerant than the bacterium homogenized at 10.34 MPa (1500 psi). These results indicate that the acid tolerance of the bacterium subjected to pressure of 13.80 MPa (2000 psi) was significantly ( $p<0.05$ ) highest followed by 10.34 MPa (1500 psi), 3.45 MPa (500 psi) and 6.90 MPa (1000 psi). The acid tolerance of the control (0 psi/ 0 MPa) was significantly ( $p<0.05$ ) the lowest.

### **3.1.2 *Streptococcus salivarius* ssp *thermophilus* ST-M5:**

The acid tolerance expressed as log cfu/ml at different low homogenization pressures is shown in fig 4B. The interaction between the homogenization pressures and time (minutes) was not significant ( $p>0.05$ ). The homogenization pressures had a significant ( $p<0.05$ ) effect (Table 1). Acid tolerance of the bacterium subjected to different homogenization pressures was significantly ( $p<0.05$ ) higher than the control (0 MPa/0 psi) (Table 3). Acid tolerance of the bacterium subjected to pressure of 3.45 MPa (500 psi) was significantly ( $p<0.05$ ) higher than the acid tolerance of bacterium subjected to pressure of 6.90 MPa (1000 psi) (Table 3). The homogenized culture subjected to different pressures exhibited viable counts after 120 minutes of incubation in acid conditions (Fig 4B). The acid tolerance of the bacterium subjected to the pressure of 3.45 MPa (500 psi) was significantly ( $p<0.05$ ) highest followed by 13.80 MPa (2000 psi), 10.34 MPa (1500 psi), and 6.90 MPa (1000 psi). The acid tolerance of control (0 MPa/ 0 psi) was significantly ( $p<0.05$ ) the lowest.

### **3.1.3 *Lactobacillus acidophilus* LA-K.**

The acid tolerance expressed as log cfu/ml at different low homogenization pressures is shown in fig 4C. The interaction between homogenization pressures and time (minutes) was not significant ( $p>0.05$ ). The homogenization pressures had a significant effect ( $p<0.05$ ) on acid tolerance (Table 1). The acid tolerance of the bacterium subjected to homogenization pressure of 13.80



MPa (2000 psi) was significantly ( $p<0.05$ ) higher compared to the control (0 psi) (Table 3). The pressures of 13.80 MPa (2000 psi) and 10.34 MPa (1500 psi) resulted in significantly ( $p<0.05$ ) higher acid tolerance of the bacterium compared to 3.45 MPa (500 psi) and 6.90 MPa (1000 psi) (Table 3). The pressure of 13.80 MPa (2000 psi) significantly ( $p<0.05$ ) increased acid tolerance of the bacterium compared to 10.34 MPa (1000 psi) (Table 3). Homogenized culture subjected to different pressures as well as the control (0 psi) were acid tolerant until the end of the 120 minutes of incubation in acid conditions, however, there was a significant ( $p<0.05$ ) decrease in the viable counts after each incubation time interval of 20 minutes. The acid tolerance of *Lactobacillus acidophilus* LA-K subjected to pressure of 13.80 MPa (2000 psi) was significantly ( $p<0.05$ ) highest followed by 10.34 MPa (1500 psi), 6.90 MPa (1000 psi) and 3.45 MPa (500 psi). The acid tolerance of control (0 psi) was significantly ( $p<0.05$ ) the lowest.

Previously, it has been shown that acid tolerance is strain dependant (Tuomola et al., 2001). Liong & Shah, (2005) observed that *Lactobacillus acidophilus* exhibited more acid tolerance than *L. casei*. They reported that *L. acidophilus* counts decreased by 1.72 log cycles when incubated in acid conditions of pH 2 for 120 minutes, whereas, *L. casei* counts were decreased by 3.04 log cycles. Mean log reduction of the viable counts of the homogenized cultures obtained by subtracting counts at 120 minutes from 0 minutes in acidic conditions of pH 2 at different homogenization pressures are reported in table 4. In table 4, a higher number means high bacterial death and lower number means low bacterial death. Comparing the viability of the bacteria from 0 MPa (psi) to 13.80 MPa (2000 psi) i.e comparing 3.87 to 4.21 for *L. acidophilus* indicates a decrease in viability; comparing 6.52 to 1.6 for *L. bulgaricus* indicates an increase in viability; and comparing 10.25 to 4.29 for *S. thermophilus* also indicates an increase in viability. These findings indicate that increase in homogenization pressure from 0 MPa/ 0 psi (control) to

13.80 MPa (2000 psi) resulted in increased acid tolerance of the yogurt cultures (*Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 and *Streptococcus salivarius* ssp *thermophilus* ST-M5). Coskun, (2006) found that homogenization of lactic acid bacterial cultures (*Lactococcus lactis* ssp *lactis*, *Lactococcus lactis* ssp *cremoris*, *Lactobacillus delbrueckii* ssp *bulgaricus* and *Streptococcus thermophilus* and *Lactobacillus helveticus*) to a pressure of 30 MPa (4351 psi) decreased the acid production of the cultures because of cell damage.

Table 1. Pr > F of homogenization pressure, time and their interaction for acid tolerance of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, *Streptococcus salivarius* ssp *thermophilus* ST-M5 and *Lactobacillus acidophilus* LA-K

Effect	<i>L. bulgaricus</i>	<i>S. thermophilus</i>	<i>L. acidophilus</i>
	Pr > F	Pr > F	Pr > F
Pressure	<.0001	<.0001	0.0117
Time	<.0001	<.0001	<.0001
Pressure * Time	<.0001	0.4497	0.7587

Time = Incubation period of 120minutes

Table 2. Pr>F of acid tolerance of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 at various pressures compared to control (0 psi/ 0 MPa)

Pressure	Time interval of 20 min during incubation period of 120 minutes					
	20 min	40 min	60 min	80 min	100min	120 min
500 psi	<0.0001	0.0059	0.0005	<0.0001	<0.0001	1.0
1000 psi	0.0110	0.4162	0.1064	<0.0001	<0.0001	0.4162
1500 psi	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
2000 psi	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Shah & Jelen, (1990) reported that at pH 1.5 *Lactobacillus* ssp *bulgaricus* and *Lactobacillus acidophilus* proved to be more acid resistant than *Streptococcus thermophilus* strains. Dunne et al., (2001) reported that *Lactobacillus acidophilus* was more acid resistant at pH's 1.2 and 2.5 for 30 minutes, compared to *Bifidobacterium* species which did not tolerate the acid conditions after 5 min. In our study, *Lactobacillus acidophilus* LA-K and *Lactobacillus delbrueckii* ssp

*bulgaricus* LB-12 were found to be more acid tolerant compared to *Streptococcus salivarius* ssp *thermophilus* ST-M5 at 0 MPa/ 0 psi as indicated by log reduction value of 3.87, 6.52 and 10.25 log cfu/ml, respectively (Table 4). Surprisingly, *Lactobacillus delbrueckii* ssp *bulgaricus* exhibited better acid tolerance compared to *Lactobacillus acidophilus* LA-K and *Streptococcus salivarius* ssp *thermophilus* after homogenization at 13.80 MPa (2000 psi) as indicated by their log reduction values of 1.6, 4.21 and 4.29 log cfu/ml, respectively.

Table 3. Least Square Means for acid tolerance of bacteria as influenced by homogenization pressures

Pressure	<i>L. bulgaricus</i>	<i>S. thermophilus</i>	<i>L. acidophilus</i>
	LSmean	LSmean	LSmean
Control	1.3041 <sup>D</sup>	2.4349 <sup>C</sup>	6.7120 <sup>B,C</sup>
500 psi	3.6596 <sup>C</sup>	4.9003 <sup>A</sup>	6.7707 <sup>C</sup>
1000 psi	3.1824 <sup>C</sup>	3.8766 <sup>B</sup>	6.9816 <sup>C</sup>
1500 psi	4.6502 <sup>B</sup>	4.4275 <sup>A,B</sup>	7.1730 <sup>B</sup>
2000 psi	5.3328 <sup>A</sup>	4.6179 <sup>A,B</sup>	7.3474 <sup>A</sup>

<sup>ABCD</sup> LSMeans with same letter within the column are not significantly different

Table 4. Mean log reduction of the viable counts of the homogenized cultures obtained by subtracting counts at 120 minutes from 0 minutes in acidic conditions of pH 2 at different homogenization pressures

Pressure	<i>L. bulgaricus</i>	<i>S. thermophilus</i>	<i>L. acidophilus</i>
	Log cfu/ml	Log cfu/ml	Log cfu/ml
Control	6.52	10.25	3.87
500 psi	6.62	4.91	4.26
1000 psi	4.56	6.41	4.44
1500 psi	2.25	4.46	4.52
2000 psi	1.6	4.29	4.21

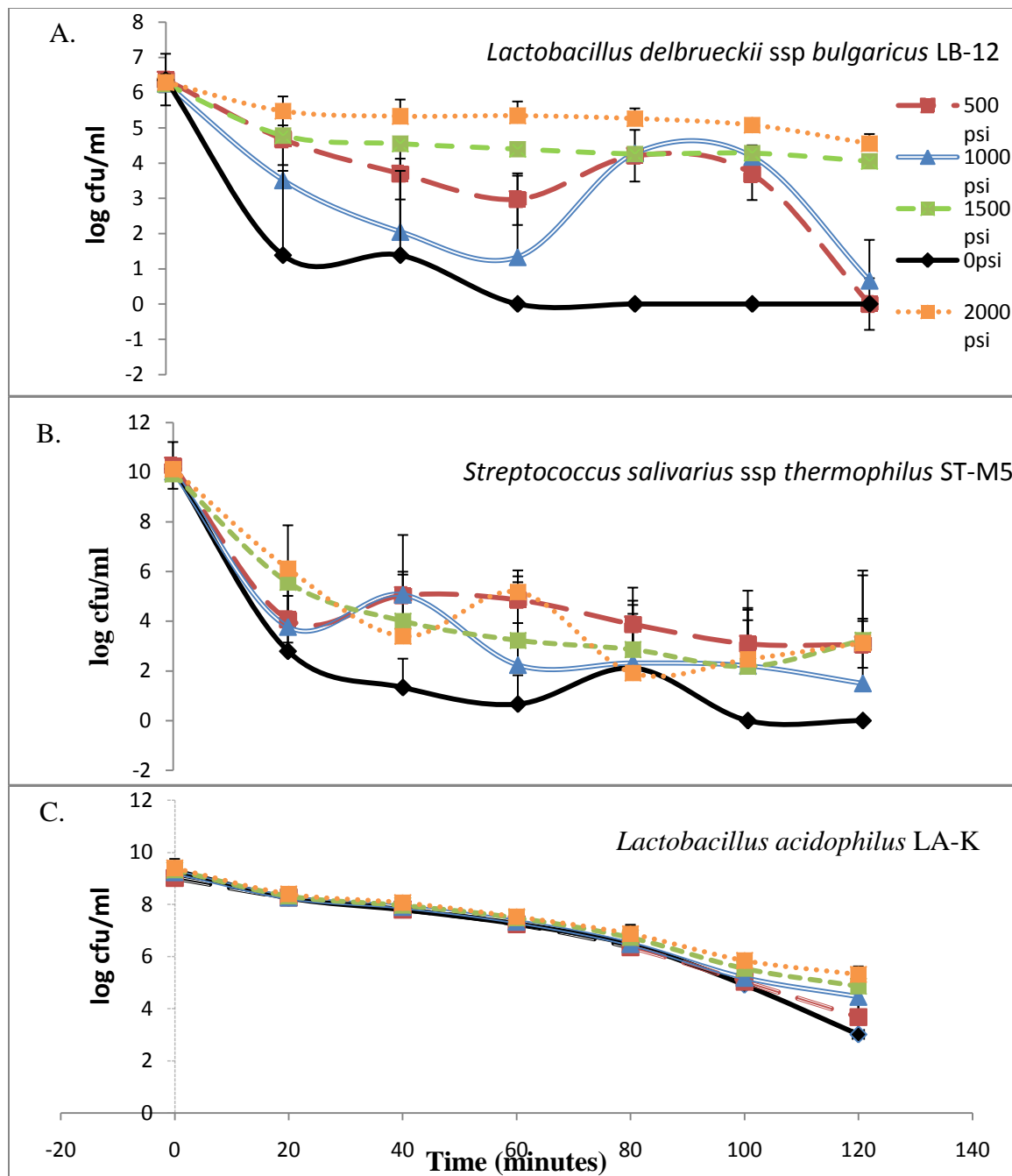


Figure 4. Acid tolerance of homogenized cultures of (A.) *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, (B.) *Streptococcus salivarius* ssp *thermophilus* ST-M5 and (C.) *Lactobacillus acidophilus* LA-K at different pressure

### **3.2 Bile Tolerance:**

#### **3.2.1 *Lactobacillus delbrueckii ssp bulgaricus* LB-12:**

The bile tolerance expressed as log cfu/ml at different low homogenization pressures is shown in fig 5A. The interaction between homogenization pressures and time was not significant ( $p>0.05$ ) (Table 5). The homogenization pressures had a significant ( $p<0.05$ ) effect on bile tolerance of the bacterium (Table 5). The bile tolerance of the bacterium subjected to pressure of 0 MPa/ 0 psi (control) was significantly ( $p<0.05$ ) higher than bile tolerance of the bacterium subjected to different homogenization pressures (Table 6). The bile tolerance of homogenized culture subjected to the pressures of 3.45 MPa (500 psi), 6.90 MPa (1000 psi) was significantly ( $p<0.05$ ) higher than the bile tolerance of bacterium subjected to pressure of 13.80 MPa (2000 psi) (Table 6). *Lactobacillus delbrueckii ssp bulgaricus* LB-12 was found to be bile tolerant throughout 10 hours of incubation in bile conditions (Fig 5A). However, the bacterium subjected to different low homogenization pressures as well as control (0 psi) exhibited a significant ( $p<0.05$ ) decline in viable counts at the end of 10 hours of incubation (Table 7). The bile tolerance of *Lactobacillus delbrueckii ssp bulgaricus* LB-12 subjected to pressure of 0 MPa (0 psi) was significantly ( $p<0.05$ ) highest followed by 3.45 MPa (500 psi), 6.90 MPa (1000 psi), and 10.34 MPa (1500 psi) (Table 6). The bile tolerance of *Lactobacillus delbrueckii ssp bulgaricus* LB-12 subjected to pressure of 13.80 MPa (2000 psi) was significantly ( $p<0.05$ ) the lowest (Table 6).

#### **3.2.2 *Streptococcus salivarius ssp thermophilus* ST-M5:**

The bile tolerance expressed as log cfu/ml at different low homogenization pressures is shown in fig 5B. The interaction between homogenization pressure and time was not significant ( $p>0.05$ ) whereas, homogenization pressures had a significant ( $p<0.05$ ) effect on bile tolerance of the bacterium (Table 5). The bile tolerance of *Streptococcus salivarius ssp thermophilus* ST-M5

subjected to the homogenization pressures of 3.45 MPa (500 psi), 6.90 MPa (1000 psi), 10.34 MPa (1500 psi) and 13.80 MPa (2000 psi) was significantly ( $p<0.05$ ) higher than the control (0 MPa/ 0 psi) (Table 6). There also exists a significant ( $p<0.05$ ) difference among bile tolerance of the bacterium subjected to different homogenization pressures (Table 6). The bile tolerance of the homogenized culture subjected to pressures of 13.80 MPa (2000 psi) and 6.90 MPa (1000 psi) was significantly ( $p<0.05$ ) higher than the bile tolerance subjected to pressures of 3.45 MPa (500 psi) and 10.34 MPa (1500 psi) (Table 6). The bacterium exhibited no decrease in viable counts comparing 0 hour and 10 hours of incubation in bile conditions (Fig 5B). The bile tolerance of the bacterium subjected to homogenization pressures of 13.80 MPa (2000 psi) and 6.90 MPa (1000 psi) was significantly ( $p<0.05$ ) highest followed by 10.34 MPa (1500 psi) and 3.45 MPa (500 psi) (Table 6). The bile tolerance of *Streptococcus salivarius* ssp *thermophilus* ST-M5 subjected to pressure of 0 MPa (0 psi) (control) was significantly ( $p<0.05$ ) the lowest (Table 6).

### **3.2.3 *Lactobacillus acidophilus* LA-K:**

The bile tolerance expressed as log cfu/ml at different homogenization pressures is shown in the fig 5C. The interaction between homogenization pressures and time was not significant ( $p>0.05$ ) (Table 5). The homogenization pressures had a significant ( $p<0.05$ ) effect on bile tolerance of the bacterium (Table 5). The bile tolerance of the bacterium subjected to homogenization pressure of 6.90 MPa (1000 psi) was significantly ( $p<0.05$ ) higher than bile tolerance of the bacterium subjected to pressure of 0 MPa/ 0 psi (control) and 13.80 MPa (2000 psi) (Table 6). *Lactobacillus acidophilus* LA-K exhibited good tolerance to the bile conditions with an increase in viable counts of the homogenized culture during 10 hours of incubation in bile conditions (Table 7). The bile tolerance of *Lactobacillus acidophilus* LA-K subjected to pressure of 6.90

MPa (1000 psi), 3.45 MPa (500 psi), 10.34 MPa (1500 psi) was significantly ( $p<0.05$ ) highest followed by control (0 psi) and 13.80 MPa (2000 psi) (Table 6).

Table 5. Pr > F of homogenization pressure, time and their interaction for bile tolerance of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, *Streptococcus salivarius* ssp *thermophilus* ST-M5, and *Lactobacillus acidophilus* LA-K

Effect	<i>L. bulgaricus</i>	<i>S. thermophilus</i>	<i>L. acidophilus</i>
	Pr > F	Pr > F	Pr > F
Pressure	<.0001	0.0007	0.0085
Time	<.0001	<.0001	<.0001
Pressure * Time	0.7941	0.8608	0.2665

Time = Incubation period of 10 hours.

Table 6. Least Square Means for bile tolerance of bacteria as influenced by homogenization pressures

Pressure	<i>L. bulgaricus</i>	<i>S. thermophilus</i>	<i>L. acidophilus</i>
	Lsmean	Lsmean	Lsmean
Control	8.9573 <sup>A</sup>	10.5959 <sup>C</sup>	8.9662 <sup>B,C</sup>
500 psi	8.8413 <sup>B</sup>	10.7277 <sup>B</sup>	8.9916 <sup>A,B,C</sup>
1000 psi	8.8456 <sup>B</sup>	10.7895 <sup>A</sup>	9.0186 <sup>A</sup>
1500 psi	8.8301 <sup>B,C</sup>	10.7130 <sup>B</sup>	8.9958 <sup>A,B</sup>
2000 psi	8.7359 <sup>C</sup>	10.8130 <sup>A</sup>	8.9569 <sup>C</sup>

<sup>ABC</sup> LSMeans with same letter within the column are not significantly different

In the present study, we found that *L. acidophilus* exhibited similar growth patterns in the presence or absence of bile acids (Fig 5C and Fig 7C). Similar findings were reported by Liong & Shah, (2005) who studied the bile tolerance of different strains of *Lactobacillus* species and found that *L. acidophilus* exhibited similar growth pattern in the presence or absence of bile acid (Oxgall). In the present study, *L. acidophilus* showed highest bile tolerance followed by *S. thermophilus* while *L. bulgaricus* exhibited least bile tolerance. We found that homogenization pressures had significant ( $p<0.05$ ) effects on bile tolerance of these bacteria. Homogenization

pressures significantly decreased ( $p<0.05$ ) the bile tolerance of *L. bulgaricus* whereas the pressures of 6.90 MPa (1000 psi) and 13.80 MPa (2000 psi) significantly ( $p<0.05$ ) improved bile tolerance of *S. thermophilus*. Shah & Jelen, (1990) attributed increased bile tolerance of *L. acidophilus* to its rigid cell wall. Our results indicated that the bile tolerance of *L. acidophilus* increased by increasing the homogenization pressure from 0 MPa (0 psi) to all low pressures studied namely 3.45 MPa (500 psi), 6.90 MPa (1000 psi), 10.34 MPa (1500 psi) and 13.80 MPa (2000 psi) (Table 7). These results indicated that in addition to rigid cell wall (Shah & Jelen, 1990), there could be other factors responsible for increased bile tolerance of *L. acidophilus* when subjected to homogenization pressures.

Table 7. Mean log difference in the viable counts of the homogenized cultures obtained by subtracting viable log cfu/ml between 0 hour and 10 hours of incubation in the presence of bile acid (Oxgall)

Pressure	<i>L. bulgaricus</i>	<i>S. thermophilus</i>	<i>L. acidophilus</i>
	Decrease in log cfu/ml	Difference in log cfu/ml	Increase in log cfu/ml
0 psi (control)	0.439	0.081(decrease)	0.747
500 psi	0.830	0.148 (decrease)	0.876
1000 psi	0.813	0.021 (increase)	0.967
1500 psi	0.663	0.020 (decrease)	0.948
2000 psi	0.567	0.013 (increase)	0.787

### 3.3 Protease activity:

#### 3.3.1 *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12:

The protease activity expressed as OD (absorbance values) at different homogenization pressures is shown in the fig 6A. The interaction between homogenization pressures and time was not significant ( $p>0.05$ ) (Table 8). The homogenization pressures also did not have a significant ( $p>0.05$ ) effect on the protease activity (Table 8).



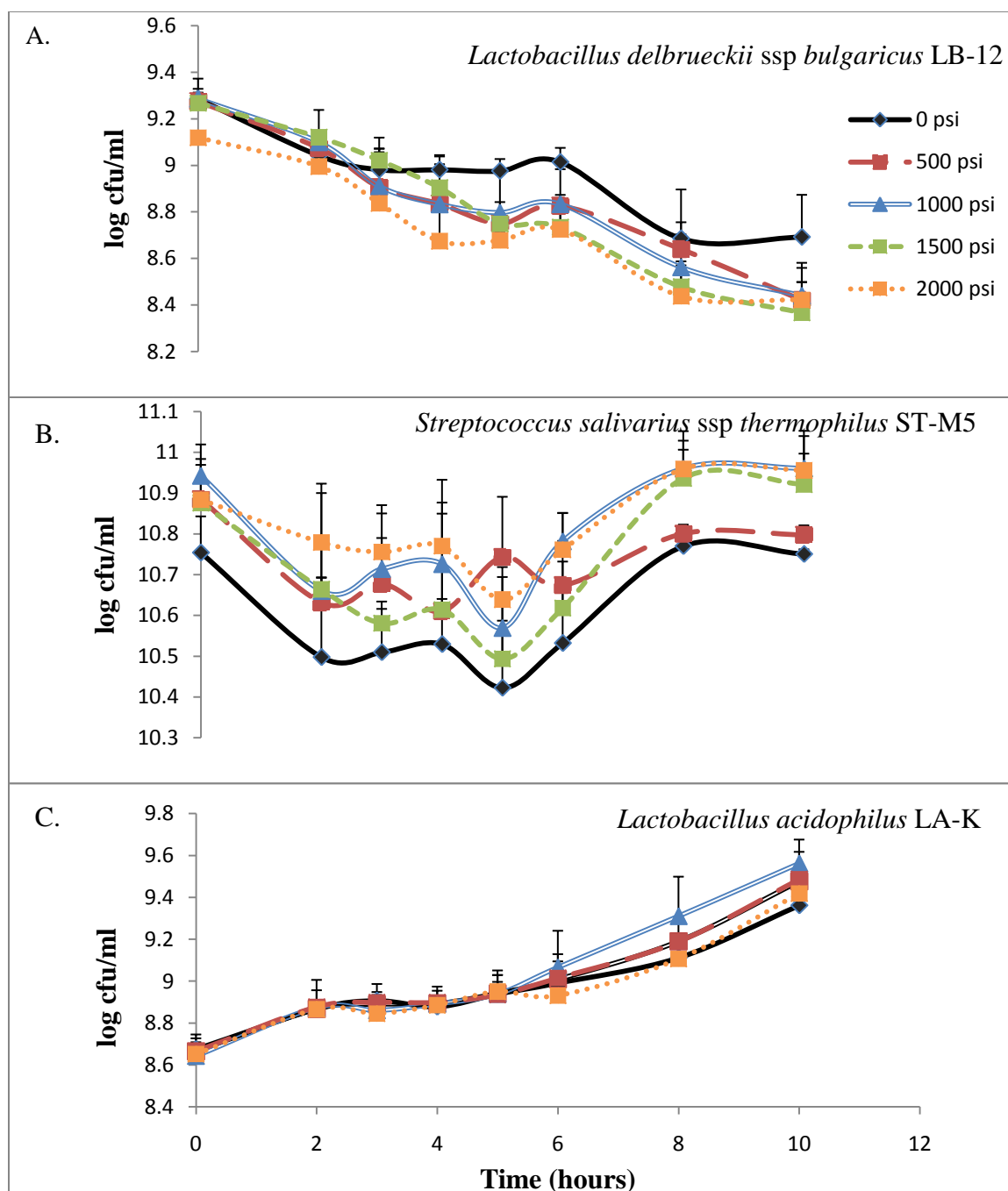


Figure 5. Bile tolerance of homogenized cultures of (A.) *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, (B.) *Streptococcus salivarius* ssp *thermophilus* ST-M5 and (C.) *Lactobacillus acidophilus* LA-K at different pressures

### **3.3.2 *Streptococcus salivarius* ssp *thermophilus* ST-M5:**

The protease activity expressed as OD (absorbance values) at different homogenization pressures is shown in fig 6B. The interaction between homogenization pressures and time was not significant ( $p>0.05$ ) (Table 8). The homogenization pressures had a significant ( $p<0.05$ ) effect on protease activity of the bacterium (Table 8). The protease activity of bacterium subjected to homogenization pressure of 6.90 MPa (1000 psi) and 10.34 MPa (1500 psi) was significantly ( $p<0.05$ ) higher than the control (0 MPa/ 0 psi) (Table 9). The protease activity of the homogenized culture subjected to the pressure of 6.90 MPa (1000 psi) and 10.34 MPa (1500 psi) was significantly ( $p<0.05$ ) higher than protease activity of bacterium subjected to pressures of 3.45 MPa (500 psi) and 13.80 MPa (2000 psi) (Table 9). The homogenization pressures of 6.90 MPa (1000 psi) and 10.34 MPa (1500 psi) had significantly ( $p<0.05$ ) improved the protease activity of *Streptococcus salivarius* ssp *thermophilus* ST-M5.

### **3.3.3 *Lactobacillus acidophilus* LA-K:**

The protease activity expressed as OD (absorbance values) at different homogenization pressures is shown in fig 6C. The interaction between the homogenization pressures and time was not significant ( $p>0.05$ ) (Table 8). The homogenization pressures did not have any significant ( $p>0.05$ ) effect on the bacterium (Table 8).

In the present study, low homogenization pressures did not result in any significant ( $p>0.05$ ) change in protease activity of *L. bulgaricus*. Previously, Gatti et al., (2004) reported decrease in aminopeptidase activity of *L. delbrueckii* subsp *bulgaricus* after subjecting to 8.82 MPa (1280 psi) using a pressure cell. Probably the differences in how the pressures are applied using a pressure cell versus homogenizer can explain the difference in findings.

Table 8. Pr > F of homogenization pressure, time and their interaction for protease activity of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, *Streptococcus salivarius* ssp *thermophilus* ST-M5, and *Lactobacillus acidophilus* LA-K

Effect	<i>L. bulgaricus</i>	<i>S. thermophilus</i>	<i>L. acidophilus</i>
	Pr > F	Pr > F	Pr > F
Pressure	0.4339	0.0004	0.3330
Time	<.0001	0.0004	<.0001
Pressure * Time	0.7139	0.3871	0.4436

Time = Incubation period of 24 hours

Table 9. Least Square Means for protease activity of bacteria as influenced by homogenization pressures

Pressure	<i>L. bulgaricus</i>	<i>S. thermophilus</i>	<i>L. acidophilus</i>
	Lsmean	Lsmean	Lsmean
Control	0.3540 <sup>A</sup>	0.02544 <sup>B</sup>	0.07106 <sup>A</sup>
500 psi	0.3161 <sup>A</sup>	0.03128 <sup>B</sup>	0.05372 <sup>A</sup>
1000 psi	0.3152 <sup>A</sup>	0.04583 <sup>A</sup>	0.05750 <sup>A</sup>
1500 psi	0.3063 <sup>A</sup>	0.04472 <sup>A</sup>	0.06528 <sup>A</sup>
2000 psi	0.3519 <sup>A</sup>	0.02656 <sup>B</sup>	0.05956 <sup>A</sup>

<sup>AB</sup>LSMeans with same letter within the column are not significantly different

Table 10. Difference in protease activity of homogenized cultures determined by subtracting the absorbance values at 0 hour from 12<sup>th</sup> hour at different homogenization pressures

Pressure	<i>L. bulgaricus</i>	<i>S. thermophilus</i>	<i>L. acidophilus</i>
	OD (Absorbance values)	OD (Absorbance values)	OD (Absorbance values)
Control	0.546	-0.0042	0.062
500 psi	0.458	0.022	0.0543
1000 psi	0.477	0.01	0.0434
1500 psi	0.492	0.016	0.06
2000 psi	0.548	0.02	0.052

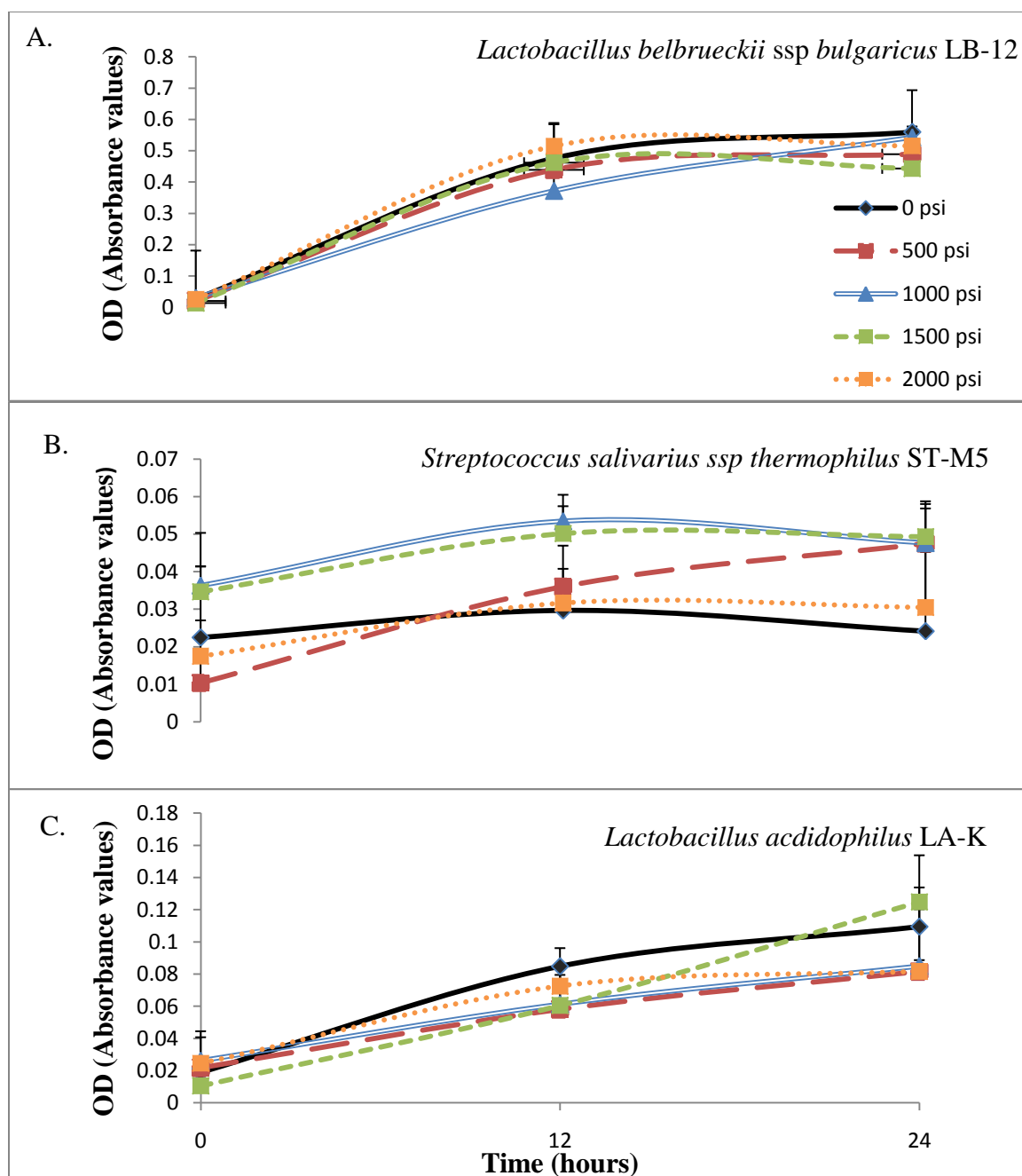


Figure 6. Protease activity of the homogenized cultures of (A.) *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, (B.) *Streptococcus salivarius* ssp *thermophilus* ST-M5, and (C.) *Lactobacillus acidophilus* LA-K at different pressures

There was no significant ( $p>0.05$ ) effect of low homogenization pressures on protease activity of *L. acidophilus* and *L. bulgaricus* but homogenization pressures of 6.90 MPa (1000 psi) and 10.34 MPa (1500 psi) significantly ( $p<0.05$ ) improved the protease activity of *S. thermophilus* (Table 8). Coskun (2006) also stated that addition of attenuated (homogenized) cultures subjected to the homogenization pressure of 30 MPa (4351 psi) enhanced the proteolytic activity of the lactic acid bacteria (*L. lactis*, *L. cremoris*, *L. bulgaricus*, *S. thermophilus*). Difference in protease activity of homogenized cultures determined by subtracting the absorbance values at 0 hour from 12<sup>th</sup> hour at different homogenization pressures were shown in table 10. In table 10, a positive number indicates an increase in protease activity, negative number indicates decrease in the protease activity and higher the number high is the protease activity. In our experiments *L. bulgaricus* exhibited the highest protease activity after 12 hours of incubation compared to *L. acidophilus* and *S. thermophilus*. *S. thermophilus* exhibited least protease activity (Table 10). This is in accordance to the results reported by Shah & Jelen, (1990) that *L. bulgaricus* exhibited high  $\beta$ - galactosidase activity compared to *S. thermophilus* and *L. acidophilus*.

### **3.4 Growth:**

#### **3.4.1 *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12:**

Growth of the bacterium expressed as log cfu/ml at different homogenization pressures is shown in fig 7A. The interaction between the homogenization pressures and time was not significant ( $p>0.05$ ), however, the homogenization pressures had a significant ( $p<0.05$ ) effect on growth of the bacterium (Table 11). The homogenization pressures of 6.90 MPa (1000 psi), 10.34 MPa (1500 psi) and 13.80 MPa (2000 psi) resulted in significantly ( $p<0.05$ ) decreased bacterial growth compared to control (0 psi), whereas, the homogenization pressure of 3.45 MPa (500 psi) did not result in significant change in growth compared to control (Table 12). Furthermore, the

growth of homogenized cultures subjected to pressures of 3.45 MPa (500 psi), 6.90 MPa (1000 psi) and 10.34 MPa (1500 psi) were significantly ( $p<0.05$ ) higher than growth of bacterium subjected to pressure of 13.80 MPa (2000 psi) (Table 12). The logarithmic phase of the bacterium was not observed during 10 hours of incubation in any of the pressures under study including the control (Fig 7A). The growth of the bacterium in the control was significantly ( $p<0.05$ ) highest and growth of the bacterium subjected to homogenization pressure of 13.80 MPa (2000 psi) was significantly ( $p<0.05$ ) the lowest.

#### **3.4.2 *Streptococcus salivarius* ssp *thermophilus* ST-M5:**

Growth of the bacterium expressed as log cfu/ml at different homogenization pressures is shown in the fig 7B. The interaction between homogenization pressures and time was not significant ( $p>0.05$ ) (Table 11). The homogenization pressures also did not have a significant ( $p>0.05$ ) effect on growth of the bacterium (Table 11). The bacterium subjected to different homogenization pressures under study including the control exhibited lag phase for 3 hours, logarithmic phase during 3 to 8 hours of incubation and stationary phase thereafter (Fig 7B).

#### **3.4.3 *Lactobacillus acidophilus* LA-K:**

Growth of the bacterium expressed as log cfu/ml at different homogenization pressures is shown in the fig 7C. The interaction effect between the homogenization pressures and time was not significant ( $p>0.05$ ) (Table 11). The homogenization pressures also did not have a significant ( $p>0.05$ ) influence on the growth (Table 11). In the control, significant ( $p<0.05$ ) increase in the viability of the bacterium was observed after 3 hours of incubation period. In the bacterium subjected to pressure of 3.45 MPa (500 psi) and 13.80 MPa (2000 psi) significant ( $p<0.05$ ) increase in the viable bacterial counts was observed after 6 hours. In the bacterium subjected to pressure of 6.90 MPa (1000 psi) and 10.34 MPa (1500 psi) significant ( $p<0.05$ ) increase in

viable counts of the bacterium was observed after 5 hours. The bacterium was still in the logarithmic phase after 10 hours of incubation for all pressures studied and the control.

Table 11. Pr > F of homogenization pressure, time, and their interaction for growth of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, *Streptococcus salivarius* ssp *thermophilus* ST-M5, and *Lactobacillus acidophilus* LA-K

Effect	<i>L. bulgaricus</i>	<i>S. thermophilus</i>	<i>L. acidophilus</i>
	Pr > F	Pr > F	Pr > F
Pressure	<.0001	0.2186	0.3334
Time	0.0292	<.0001	<.0001
Pressure * Time	0.9296	0.6221	0.2758

Time = Incubation period of 10 hours.

Table 12. Least Square Means for growth of bacteria as influenced by homogenization pressures

Pressure	<i>L. bulgaricus</i>	<i>S. thermophilus</i>	<i>L. acidophilus</i>
	LSmean	LSmean	LSmean
Control	9.3496 <sup>A</sup>	11.7817 <sup>B</sup>	9.0801 <sup>B</sup>
500 psi	9.3103 <sup>B,A</sup>	11.8667 <sup>A,B</sup>	9.1353 <sup>A</sup>
1000 psi	9.2658 <sup>B</sup>	11.9395 <sup>A</sup>	9.1038 <sup>A,B</sup>
1500 psi	9.3002 <sup>B</sup>	11.9389 <sup>A</sup>	9.1004 <sup>A,B</sup>
2000 psi	9.1721 <sup>C</sup>	11.8788 <sup>A,B</sup>	9.0937 <sup>A,B</sup>

<sup>ABC</sup>LSMeans with same letter within the column are not significantly different

Table 13. Difference in the viable counts of cultures homogenized at different pressures obtained by subtracting viable log cfu/ml counts at 0 hour from those at 10 hours of incubation.

Pressure	<i>L. bulgaricus</i>	<i>S. thermophilus</i>	<i>L. acidophilus</i>
	Log cfu/ ml	Log cfu/ ml	Log cfu/ ml
Control	-0.117	1.764	0.737
500 psi	0.0796	1.787	0.68
1000 psi	-0.011	1.706	0.964
1500 psi	0.038	1.848	0.827
2000 psi	-0.099	1.819	0.698

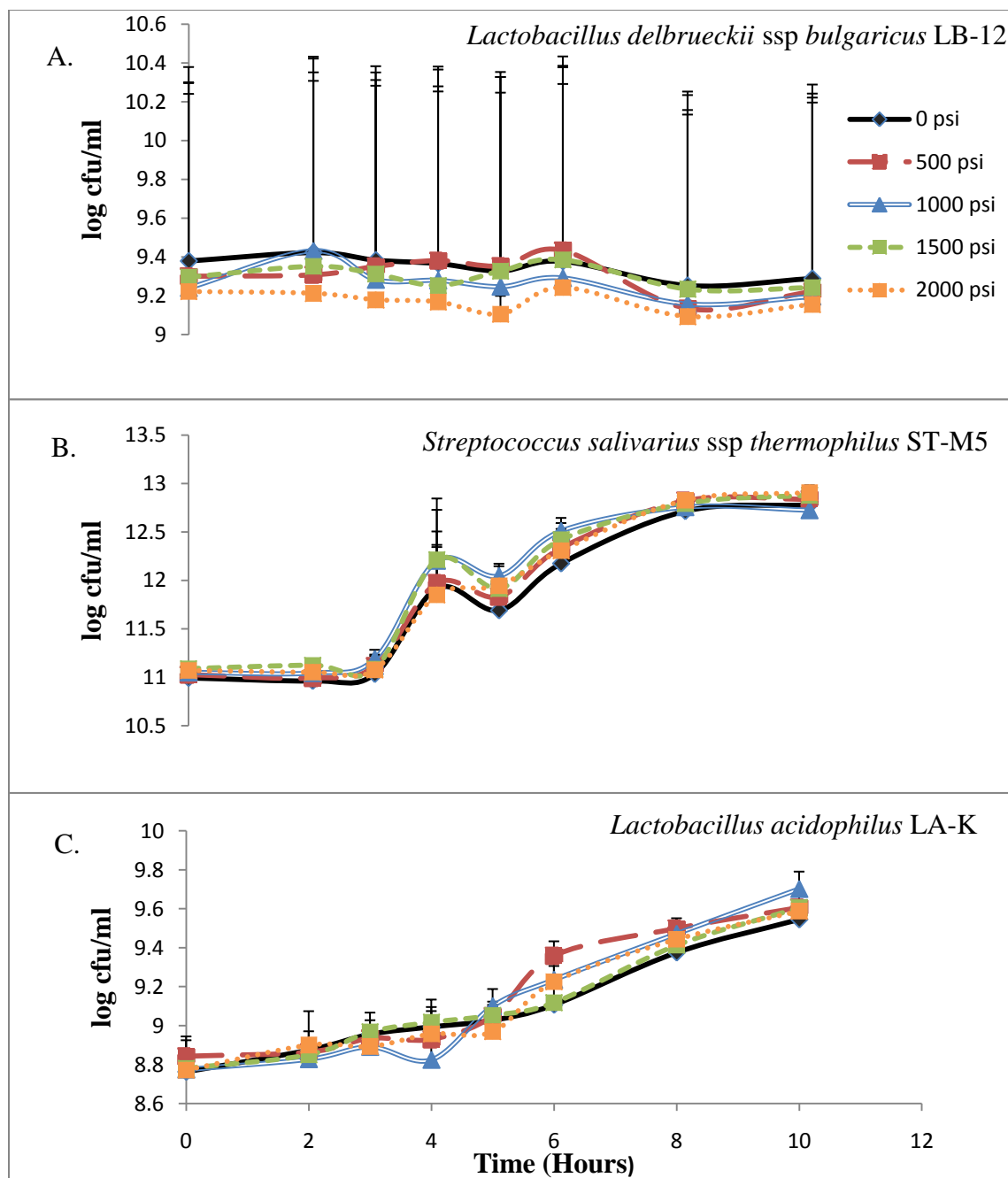


Figure 7. Growth of homogenized cultures of (A.) *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12, (B.) *Streptococcus salivarius* ssp *thermophilus* ST-M5, and (C.) *Lactobacillus acidophilus* LA-K at different pressures



Simova et al., (2006) analyzed the growth profile of *S. thermophilus* T15 and *L. bulgaricus* HP1 inoculated individually in autoclaved reconstituted skim milk and reported that growth reached exponential phase in the first 5 hours and reached stationary phase in 8-12 hours. In the present study, we found that though the growth of *S. thermophilus* in control (0 psi) reached exponential phase after 3 hours, *L. bulgaricus* did not reach exponential phase during the 10 hours of incubation. The difference in results could be because *L. bulgaricus* used in the present study were pure frozen cultures inoculated in cool (4°C) autoclaved skim milk, whereas, the culture mentioned by Simova et al., (2006) were pre-incubated for 5.5 hours before inoculation. Another possible reason could be that the homogenization treatment might have delayed the logarithmic phase of the bacterium in the present study. Moreover, Shah et al., (2008) reported that *L. bulgaricus* was the most sensitive bacterium among the three bacterial cultures exhibiting least viability when subjected to high pressures of 480 MPa (69,618 psi). The results indicated that rate of growth of the bacterium *L. acidophilus* was delayed on increasing the homogenization pressures.

All low homogenization pressures significantly ( $p < 0.05$ ) decreased the growth of *L. bulgaricus*. The homogenization pressures did not have significant ( $p > 0.05$ ) effect on growth of *L. acidophilus* and *S. thermophilus*. *L. acidophilus* exhibited significant ( $p < 0.05$ ) increase in the viable bacterial counts after 6 hours but did not reach stationary phase during 10 hours of incubation. This is in accordance with results suggested by Liong & Shah, (2005) who stated that growth of the bacterium *L. acidophilus* was found predominant in the first 9 – 15 hours after which it reached a stationary phase. Difference in the viable counts of cultures homogenized at different pressures obtained by subtracting viable log cfu/ml counts at 0 hour from those at 10 hours of incubation are reported in table 13. In table 13, a positive number indicates the bacterial

growth, a negative number indicates the bacterial death and higher the positive number high is the growth rate of the culture and high is the resistance to low pressure homogenization. Among the three bacterial cultures, *L. bulgaricus* was least resistant followed by *L. acidophilus* while *S. thermophilus* exhibited highest resistance to low homogenization pressures as determined by difference in viable counts of the bacterium at 0 hour from 10 hours (Table 13). Similar observations were noted by Shah et al., (2008) who subjected cultures to very high pressures using an ultra high pressure press and studied culture growth at a single time point after high pressure treatment. They reported that *S. thermophilus* was the most resistant bacterium followed by *S. thermophilus* while *L. bulgaricus* was the least resistant when subjected to a pressure of 480 MPa (69618 psi) (Shah et al., 2008).

## CHAPTER 4: CONCLUSIONS

Yogurt cultures (*Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 and *Streptococcus salivarius* ssp *thermophilus* ST-M5) and *Lactobacillus acidophilus* LA-K were subjected to different low homogenization pressures of 3.45 MPa (500 psi), 6.90 MPa (1000 psi), 10.34 Mpa (1500 psi) and 13.80 MPa (2000 psi) and four important characteristics namely acid tolerance, bile tolerance, protease activity and growth of each bacterium were studied.

All the low homogenization pressures under study had significantly ( $p<0.05$ ) improved the acid tolerance of yogurt cultures. The homogenization pressure of 13.80 MPa (2000 psi) had significantly ( $p<0.05$ ) improved the acid tolerance of *Lactobacillus acidophilus* LA-K. Low homogenization pressures had greatly increased the acid tolerance of the yogurt cultures compared to *Lactobacillus acidophilus* LA-K.

Among the three dairy cultures, *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 was least tolerant to bile conditions followed by *Streptococcus salivarius* ssp *thermophilus* ST-M5 and *Lactobacillus acidophilus* LA-K. Low homogenization pressures significantly ( $p<0.05$ ) decreased the bile tolerance of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12. The homogenization pressure of 13.80 MPa (2000 psi) and 6.90 MPa (1000 psi) had significantly ( $p<0.05$ ) increased the bile tolerance of *Streptococcus salivarius* ssp *thermophilus* ST-M5 and *Lactobacillus acidophilus* LA-K respectively.

*Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 exhibited highest protease activity followed by *Lactobacillus acidophilus* LA-K and *Streptococcus salivarius* ssp *thermophilus* ST-M5. Low homogenization pressures did not significantly ( $p>0.05$ ) improve the protease activity of *Lactobacillus acidophilus* LA-K and *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12. The

homogenization pressures of 6.90 MPa (1000 psi) and 10.34 MPa (1500 psi) significantly ( $p<0.05$ ) increased the protease activity of *Streptococcus salivarius* ssp *thermophilus* ST-M5.

Growth of *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 was significantly ( $p<0.05$ ) decreased by subjecting the culture to various low homogenization pressures. The homogenization pressures did not have a significant ( $p>0.05$ ) influence on growth of *Lactobacillus acidophilus* LA-K and *Streptococcus salivarius* ssp *thermophilus* ST-M5. *Lactobacillus delbrueckii* ssp *bulgaricus* LB-12 was in lag phase throughout 10 hours of incubation. *Lactobacillus acidophilus* LA-K had exhibited lag and log phase during the 10 hours of incubation. Growth curve of *Streptococcus salivarius* ssp *thermophilus* ST-M5 exhibited lag, log and stationary phase proving to be most resistant bacterium to low homogenization pressures among the three.

Some low homogenization pressures positively influenced some characteristics of yogurt culture bacteria and *Lactobacillus acidophilus* LA-K. Depending upon the improvement in the characteristics of a culture bacterium desired, low homogenization pressure can selectively be used.

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