IMPACT OF A PLANT EXTRACT ON THE VIABILITY OF YOGURT STARTER AND PROBIOTIC CULTURES IN NONFAT YOGURT

by

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Abstract

Yogurt starter and probiotic bacteria have been reported to confer health benefits to the consumer; however, to confer these health benefits, yogurt and probiotic bacteria should be live and present at the recommended concentration of 6 to 8 log cfu g^{-1}. Cegemett® Fresh (Cognis Nutrition & Health, Monheim, Germany) is a plant extract that possesses antioxidant properties. This research was divided into two experiments. The objective of experiment-I was to investigate the effect of plant extract supplementation on the redox potential (Eh) and the viability of starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) in nonfat yogurt. Five yogurt samples [non-supplemented, supplemented with 0.5 or 1.0% (w/v) plant extract, or supplemented with 0.014 or 0.028% (w/w) L-cysteine.HCl] were prepared, stored at 5 ºC for 50 days and analyzed weekly. *L. bulgaricus* counts in supplemented yogurts were > 6 log cfu mL^{-1} for additional 7 to 21 days compared with non-supplemented yogurt; however, *S. thermophilus* counts in all yogurts were > 6 log cfu mL^{-1} throughout the storage. Overall, Eh of plant extract supplemented yogurts was similar to non-supplemented yogurt during storage; therefore the improvement in *L. bulgaricus* viability cannot be attributed to the Eh alone. The objective of experiment-II was to investigate the effect of plant extract supplementation on the buffering ability of the yogurt mix, and on the viability of starter and probiotic (*Bifidobacterium animalis* ssp. *animalis* and *Lactobacillus acidophilus*) cultures in nonfat yogurt stored at 5 ºC for 50 days. Yogurts were formulated with 0.5% (w/v) plant extract, 0.25% (w/v) sodium acetate or no supplement, fermented with starter cultures and *B. animalis*, *L. acidophilus* or both probiotics, and analyzed weekly. Yogurt mixes supplemented with plant extract and sodium acetate had greater buffering capacities compared with non-supplemented yogurt mix. *L. bulgaricus* and *L. acidophilus* counts in supplemented yogurts were > 6 log cfu mL^{-1} for additional 7 to 35 days compared with non-supplemented yogurts. *S. thermophilus* and *B. animalis* counts were not affected by supplementation. These results suggested that greater buffering capacity could improve the viability of *L. bulgaricus* and *L. acidophilus* in probiotic yogurt during storage.

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Dedication

I would like to dedicate this thesis to my father, Mr. Michael K.; mother, Mrs. Martha Michael; and sister, Ms. Mona Michael.

CHAPTER 1 - Introduction

Refrigerated yogurts dominate the U.S. fermented milk market. U.S. consumers like less tart and sharp yogurt products; therefore fruit and flavored yogurt products are more popular than plain yogurt (Gilliland, 1998). Many national and international companies have introduced European-style yogurt products in the U.S. market in the past few years. Examples would be Agro-Farma Inc., Stonyfield, Greek Gods, Serra Natural Foods Ltd. and Cedar's Mediterranean Foods Inc., who have all tried to capture the U.S. market with 'Greek Yogurt', while the Icelandic Milk & Skyr Corp. and Wallaby Yogurt Co. have introduced 'Skyr', an Icelandic dairy product, and Australian-style organic yogurt, respectively (Roberts, 2009).

Yogurt, a nutrient-dense food, is one of the most popular fermented milk products worldwide. Over the years, the beneficial health effects of yogurt have been attributed to the yogurt nutrients, the yogurt starter bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) and if added, probiotic bacteria. However, to confer health benefits the yogurt starter and probiotic bacteria should be present at the recommended concentration of 6 to 8 log cfu g^{-1} at the time of consumption (Ross, Desmond, Fitzgerald & Stanton, 2005; Vasiljevic & Shah, 2008). In most yogurt products, culture viability typically declines during refrigerated storage due to a variety and possibly a combination of changes in the environmental conditions, such as decreased pH with increased oxygen tension and redox potential (Eh), as well as hydrogen peroxide accumulation (Dave & Shah, 1997a; Dave & Shah, 1997c; Donkor, Henriksson, Vasiljevic & Shah, 2006; Lourens-Hattingh & Viljoen, 2001; Sarkar, 2008; Vasiljevic et al., 2007).

But yogurt is not without controversy in the U.S. In 2007, a lawsuit was settled by Pinkberry® for falsely claiming the presence of live yogurt bacteria in their frozen yogurt. In their settlement, they donated $750,000 to the Los Angeles Regional Food Bank and Paras Los Niños (Hayes, 2007; Steinhauer, 2008). The following year, the state of California filed a lawsuit against Dannon® claiming that the advertisements for 'Activia' were misleading, as the health benefits advertised were not proven. In an out-of-court settlement, Dannon® agreed to establish a $35 million fund to reimburse customers who purchased 'Activia' or 'DanActive' products since their introduction into the U.S. market in 2006 and 2007, respectively with a maximum

compensation of $100 to an individual customer (Oppenheim, 2009; USA Today, 2009). In addition, Dannon® agreed to improve their label as well as to provide the scientific names of the bacteria used in these fermented milks.

An obvious challenge for yogurt manufacturers and researchers is to develop a strategy that will extend the viability of starter and probiotic bacteria in yogurts. As the yogurt starter and probiotic bacteria are facultative or obligate anaerobes, approaches such as supplementing yogurts with antioxidants such as cysteine or ascorbic acid to reduce the oxygen content (Bari et al., 2009; Dave & Shah, 1997a; Dave & Shah, 1997b) or with prebiotics such as inulin, fructooligosaccharides (FOS) or β-glucan to provide an additional carbon or energy source (Akalin, Gönç, Ünal & Fenderya, 2007; Aryana, Plauche & Nia, 2007; Oliveira, Perego, Converti & De Oliveria, 2009; Vaseiljevic et al., 2007) have been evaluated and reported to improve the viability of yogurt starter and probiotic bacteria in fermented milk products.

Cegemett® Fresh (Cognis, Nutrition & Health, Monheim, Germany) is a plant extract prepared from an oleoresin mixture [consisting of olive, garlic, onion and citrus extract, and uses sodium acetate (~ 50%) as a carrier] that possesses antioxidant properties. The combined effect of oxygen-reduced and buffered environment exerted by the plant extract in yogurt may enhance the viability of yogurt starter and probiotic bacteria in yogurt. Plant extract (~ \$10 kg^{-1}) being a cheaper supplement compared to cysteine, ascorbic acid or inulin (ranging from ~ \$90 to over \$1000 kg^{-1}; alfa.com, 2010; ajiaminoacids.com, 2010), may be a more economical option for improving the viability of yogurt starter and probiotic bacteria.

Thus, this research was conducted to better understand the changes in the yogurt environment, both during fermentation and storage, to help design strategies for prolonging the viability of starter and probiotic (*Bifidobacterium animalis* ssp. *animalis* and *Lactobacillus acidophilus*) bacteria. This may provide an opportunity for manufacturers and/or distributors to market active culture yogurt products, claiming various health benefits, with a longer shelf life.

CHAPTER 2 - Literature review

2.1 Fermented milk products

Fermented milk products have been an important part of the human diet from the time humans started domesticating animals. Fermentation of milk preserves the nutrients for a longer period of time, which otherwise would deteriorate at a faster rate. Fermented milk products are consumed as staple foods, snacks, drinks and desserts. The diversity of fermented milk products can be attributed to the variety of starter cultures used in the fermentation, the addition of different ingredients (such as sugar, salt, condiments and fruits) and the application of additional preservation techniques (such as freezing, concentrating and drying; Chandan, 2006a). Approximately 400 different fermented milk products are consumed globally (Chandan, 2006a). The worldwide consumption of fermented dairy foods was more than 17.8 billion kg in 2005 (van Hylckama Vlieg & Hugenholtz, 2007). Yogurt, cultured buttermilk, acidophilus milk, cultured/sour cream, kefir, dahi, shrikhand and Scandinavian ropy milk are some of the popular fermented milk foods consumed in different parts of the world (Chandan, 2006a; Tamime & Robinson, 1999d).

Lactic acid bacteria are predominately used as the starter culture in fermented milk products but in some products yeasts and/or molds may be included (Chandan, 2006a). Conversion of lactose into lactic acid is the most significant phenomenon during the fermentation process (IFM, 2004); however the different flavor compounds generated by the starter cultures contribute to the diversity of end products. Table 2.1 presents some typical starter cultures used to manufacture fermented milk products.

Table 2.1 Starter cultures used in the manufacture of some commercial fermented milks

Product	Primary microorganism(s)	Secondary/optional microorganism(s)	Major function of culture
Yogurt	*Lb. delbrueckii* ssp. *bulgaricus, S. thermophilus*	*Lb. acidophilus, Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium infantis, Lb. casei, Lb. lactis, Lb. rhamnosus, Lb. helveticus, Lb. reuteri*	Acidity, texture, aroma, flavor, probiotic
Cultured butter milk and sour cream	*Lc. lactis* ssp. *lactis, Lc. lactis* ssp. *cremoris, Lc. lactis* ssp. *lactis* var. *diacetylactis*	*Leuc. lactis, Leuc. mesenteroides* ssp. *cremoris*	Acidity, flavor, aroma
Probiotic fermented milks	*S. thermophilus, Lb. acidophilus, Lb. reuteri, Lb. rhamnosus GG, Lb. johnsoni, Lb. casei, Bifidobacterium longum, Bifidobacterium bifidus*	*Lc. lactis* ssp. *lactis, Lc. lactis* ssp. *cremoris*	Acidity, flavor, probiotic
Kefir	*Lc. lactis* ssp. *lactis, Lc. lactis* ssp. *cremoris, Lb. delbrueckii* ssp. *bulgaricus, Lb. delbrueckii* ssp. *lactis, Lb. casei, Lb. helveticus, Lb. brevis, Lb. kefir, Leuc. mesenteroides, Leuc. dextranicum*	*Kluyveromyces marxianus* ssp. *marxianus, Torulaspora delbrueckii, Saccharomyces cerevisiae, Candida kefir, Acetobacter aceti*	Acidity, aroma, flavor, gas(CO_2), alcohol, probiotic
Koumiss	*Lb. delbrueckii* ssp. *bulgaricus, Lb. kefir, Lb. lactis Saccharomyces lactis, Saccharomyces cartilaginosus, Mycoderma* spp.	*Acetobacter aceti*	Acidity, alcohol, flavor, gas (CO_2)

Adapted from Chandan (2006a)

2.2 Yogurt

In the U.S., per capita consumption of refrigerated yogurt has increased from 2.63 to 5.22 kg and yogurt sales increased from 714 to 1577 million kg from 1997 to 2007 (ERS, 2009). Yogurt popularity can be attributed to its nutrient density as well as its beneficial health effects (McKinley, 2005). In the early part of the 20th century, Metchnikoff's theory "The Prolongation of Life" advocated the beneficial health effects of yogurt consumption that propelled both the popularity of yogurt in Europe and research studies in this field (Lourens-Hattingh & Viljoen, 2001; Vasiljevic & Shah, 2008).

In the U.S., yogurt production is overseen by the U.S. Food and Drug Administration (FDA) and according to Code of Federal Regulation (CFR), "Yogurt is a food product produced by culturing cream, milk, partially skim milk or skim milk, used alone or in combination, with lactic acid bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and should not have titratable acidity less than 0.9% expressed as lactic acid" (CFR 131.200, 2009). Other ingredients such as sweeteners, flavoring agents, color additives and stabilizers can be added to yogurt during manufacturing; however, yogurt mix should be pasteurized prior to the inoculation with yogurt starter cultures or addition of flavoring ingredients (CFR 131.200, 2009).

The National Yogurt Association (NYA) is a national non-profit organization representing manufacturers and marketers of live and active culture yogurt products, and suppliers to the yogurt industry, with the aim of sponsoring research for live and active culture yogurt and serving as an information source to the public (NYA, 2009). On February 18th, 2000, NYA submitted a petition to the FDA to amend some of the standards regarding yogurt products so as to set a minimum concentration of live bacteria that should be present in the products labeled as 'yogurt', and pasteurized yogurts should specify on the label that the product does not contain live bacteria (NYA, 2009; Roberts, 2009). In the U.S., NYA has established a voluntary program according to which, refrigerated yogurts displaying the "Live & Active Cultures" seal on the containers should have ≥ 8 log cfu g^{-1} yogurt bacteria (i.e. sum of *S. thermophilus* and *L. bulgaricus* counts) at the time of manufacture (NYA, 2009). Because this seal program is voluntary for yogurt manufacturers, yogurt products without this seal may also contain viable yogurt bacteria but not necessarily at the recommended levels. Yogurt manufactures and/or distributors can get the approval from the NYA seal program committee to place the "Live &

Active Cultures" seal on the product's label by submitting a laboratory performance report from a state or USDA-certified independent laboratory stating that the product samples meet the NYA seal program criteria (NYA, 2009). Apart from having ≥ 8 log cfu g^{-1} yogurt bacteria in the yogurt sample at the time of manufacture, the NYA seal program requires that the yogurt sample should pass the "culture activity test" at the end of the stated shelf life. In order to pass the culture activity test, rehydrated and pasteurized 12 % non-fat dry milk should have an increase of ≥ 1 log cfu g^{-1} in total yogurt bacteria count when inoculated with 3 % of the yogurt sample and fermented at 43 °C for 4 h (NYA, 2009).

2.3 Brief overview of yogurt manufacturing

The two main types of yogurts are "set" and "stirred" depending upon the method of production (Varnam & Sutherland, 2001a; Tamime & Deeth, 1980). Set yogurt is fermented directly in the retail containers, whereas stirred yogurt is fermented in bulk and the gel is stirred before cooling and subsequently packaged (Tamime & Deeth, 1980). Yogurt can also be classified as plain, flavored, frozen or dried. Although there are some variations in the method employed for manufacturing various yogurts, the basic principle is same for all. Figure 2.1 presents the various steps involved in the manufacturing of stirred-style yogurt.

2.3.1 Yogurt mix

The first step in yogurt manufacturing is the formulation and standardization of yogurt mix. Standardization can be done by skimming whole milk, blending skim milk and whole milk, or using standardizing centrifuges (Varnam & Sutherland, 2001a). Protein content of yogurt mix plays an important part in the final consistency, texture and flavor of yogurt. Generally, protein content and/or total solids of the yogurt mix is increased during the standardization process and this can be achieved by supplementing yogurt mix with skim milk powder, whole milk powder, or whey powder (Tamime & Deeth, 1980; Varnam & Sutherland, 2001a). Yogurt mixes vary in total solids from 9 to 30% (Tamime & Deeth, 1980), but commercially yogurt mixes with total solids of ~ 15% predominate (Varnam & Sutherland, 2001a). Stabilizers are added to the yogurt mix to enhance consistency, texture, mouthfeel and to decrease syneresis. Carboxymethyl cellulose, xanthan, pectin, gum arabic and gum guar are typical stabilizers used in yogurt manufacturing (Chandan & O'Rell, 2006a; Tamime & Robinson, 1999a). In the U.S., yogurt mix is often supplemented with natural or artificial sweeteners such as sucrose, invert sugar, fructose,

glucose, aspartame and acesulfame-K (Chandan & O'Rell, 2006a; Tamime & Robinson, 1999a; Varnam & Sutherland, 2001a). All ingredients are mixed well before proceeding to the next steps.

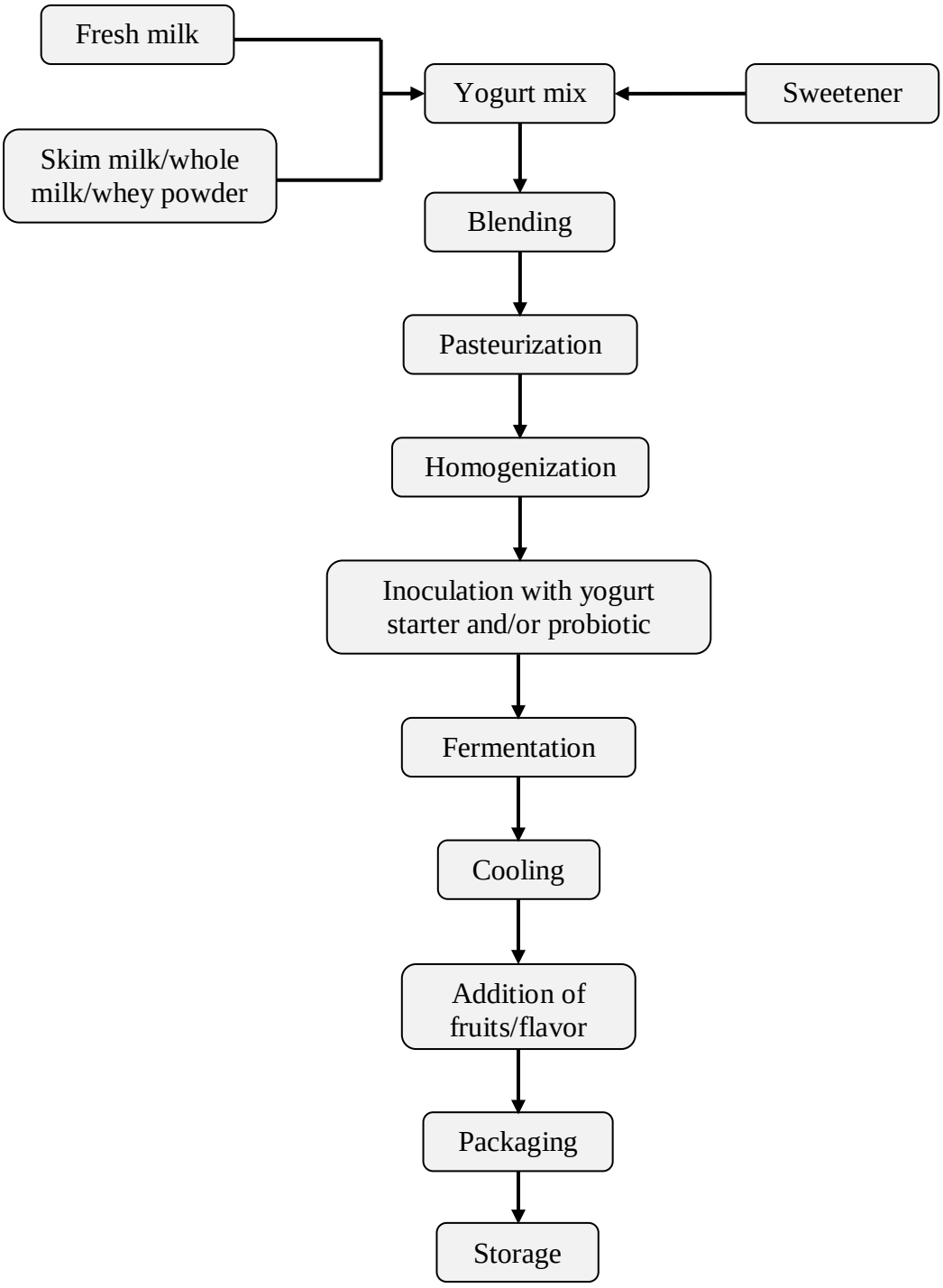

Figure 2.1 Stirred yogurt manufacturing flowchart

2.3.2 Pasteurization

Commercially, yogurt mix is pasteurized at 85 ºC for 30 min or 90 to 95 ºC for 5 to 10 min (Tamime & Deeth, 1980). Pasteurizing the mix destroys any pathogenic microflora and inactivates most milk enzymes. As pasteurization eliminates most of the competitive microorganisms in the yogurt mix, it allows the desirable growth of yogurt bacteria during fermentation. Pasteurization expels the dissolved oxygen in the yogurt mix creating reduced conditions (Chandan & O'Rell, 2006b). This reduced redox potential (Eh) in yogurt mix creates a favorable environment for the yogurt bacteria during fermentation (Vasiljevic & Shah, 2008). Thermal denaturation of whey protein induces sulfhydryl group exposure and further contributes to the decreased Eh of the mix (Chandan & O'Rell, 2006b; Tamime & Robinson, 1999a). Denatured whey proteins increase the water binding capacity and viscosity, and decrease syneresis in the yogurt.

2.3.3 Homogenization

Homogenization of yogurt mix is done at 55 to 80 ºC and 10 to 20 MPa (Chandan & O'Rell, 2006b). Homogenization reduces the fat globules size and distributes the fat globules uniformly in the yogurt mix; therefore, homogenization prevents cream separation in the mix during fermentation. Homogenization denatures some whey proteins and as a result additional sulfhydryl groups are exposed (Tamime & Deeth, 1999a). Homogenization increases the fat-casein interactions as the newly formed smaller fat globules are coated by casein micelles, and increases whey protein-casein interactions (Tamime & Deeth, 1980). Homogenization improves yogurt consistency and mouth feel, and decreases syneresis (Chandan & O'Rell, 2006b; Tamime & Deeth, 1980; Tamime & Robinson, 1999a).

2.3.4 Fermentation

Fermentation is the heart of yogurt manufacturing. Pasteurized yogurt mix is cooled to the incubation temperature (40 to 45 ºC; Tamime & Robinson, 1999), inoculated with yogurt starter cultures (i.e. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) and transferred to the fermentation vat (for stirred yogurt) or retail cups (for set yogurt). For probiotic yogurt, probiotic cultures are inoculated with the starter cultures. As these temperatures may exceed the optimum growth temperatures for probiotic cultures, lower incubation temperatures (~ 37 ºC) are used for the fermentation of probiotic yogurt (Lourens-Hattingh &

Viljoen, 2001). Conversion of milk lactose into lactic acid by the bacteria is the most significant change that occurs during fermentation. Other metabolites such as acetaldehyde, acetone, diacetyl, formic acid, acetic acid and propionic acid are also produced during fermentation and contribute to the characteristic yogurt flavor (Chandan & O'Rell, 2006b). The yogurt gel is formed as a result of destabilization of the casein micelles as a direct result of the pH decrease during fermentation. Destabilization of casein micelles begins at pH 4.9 when calcium phosphate becomes soluble and converts to the ionic form. At pH 4.6, calcium phosphate is completely dissolved and the casein micelles are at their isoelectric pH. Hence, casein micelles aggregate forming the yogurt gel (Chandan, 2006b). Proteolysis during yogurt fermentation plays a vital role in the survivability of yogurt bacteria and production of flavor compounds. Proteolysis during yogurt fermentation is initiated by extracellular proteinase of *L. bulgaricus* that hydrolyzes casein to oligopeptides, which are subsequently converted into individual amino acids and used by yogurt bacteria (Serra, Trujillo, Guamis & Ferragut, 2009). Proteolysis results in the production of essential amino acids and improves the nutritive profile (by improving bioavailability of amino acids) of the yogurt (Chandan & O'Rell, 2006b). Typically, fermentation is stopped at pH 4.6 or titratable acidity of 0.9 (% lactic acid) (CFR 131.200, 2009).

2.3.5 Cooling and packaging

Yogurt bacteria have limited activity at < 10 ºC (Tamime & Robinson; 1999a); therefore the objective of cooling is to arrest the metabolic activity of yogurt bacteria once the desired pH of yogurt is achieved (Chandan & O'Rell, 2006b; Tamime & Deeth, 1980). Stirred yogurt is then packaged in the retail cups, predominately plastic (high-impact polystyrene) containers with metal foil seals or plastic 'snap-on' lids (Varnam & Sutherland, 2001a).

2.3.6 Storage and transportation

Yogurt should be maintained at < 10 ºC during storage and transportation to arrest various biological and biochemical reactions (Tamime & Robinson, 1999a); however in the U.S., yogurt should be maintained at ≤ 7 ºC throughout storage and transportation (PMO, 2007). Generally, yogurt has a shelf life of 4 to 7 weeks (Chandan & O'Rell, 2006b); however, yogurts with live and active cultures should have counts of the individual yogurt and probiotic bacteria ≥ 6 log cfu g^{-1} until the end of stated shelf life (Lourens-Hattingh & Viljoen, 2001).

2.4 Nutrition and health benefits of yogurt

Yogurt is an excellent source of milk nutrients such as protein, calcium, phosphorous, riboflavin, folate, niacin, magnesium and zinc (Buttriss, 1997; McKinley, 2005). Yogurt provides all essential amino acids, and minerals and vitamins in bioavailable forms (McKinley, 2005; Shah, 2006a). Consuming 150 g of fruit yogurt can fulfill 26% of the daily calcium requirement of an adult and 41% of the daily calcium requirement of a 5 year old child (McKinley, 2005). Yogurt is often supplemented with milk solids and therefore is a good source of protein. Consumption of 200 to 250 mL of yogurt can fulfill the minimum daily animal protein requirement (15 g) of an individual (Tamime & Robinson, 1999c).

Lactose intolerant individuals can digest lactose more easily in yogurt rather than in milk (McKinley, 2005; Salminen, Playne & Lee, 2004; de Vrese et al., 2001). The exact reason for this alleviation of lactose intolerance is not clear but various theories have been proposed. Gilliland and Kim (1984) and Lerebours et al. (1989) showed that the presence of live bacteria in yogurt was important for the alleviation of lactose intolerance, whereas some studies showed no difference between the lactose intolerance alleviation of heat-treated yogurt (i.e. with no live bacteria) and yogurt containing live bacteria (Hove et al., 1999). Vesa et al. (1996) proposed that due to the higher viscosity of yogurt, its slower passage through the gastrointestinal tract may improve the absorption and digestion of lactose in the colon.

Regular consumption of yogurt has been reported to stimulate the natural gut microflora, as the lysed cells of the yogurt starter bacteria release some growth factors (such as vitamins) that are beneficial for the growth of natural gut microflora (Tamime & Robinson, 1999b). Yogurt has been reported to possess anti-carcinogenic activities (Sarkar, 2008; Shah, 2006a). Some studies with rats and mice have shown that consumption of yogurt inhibited certain types of tumors (Tamime & Robinson, 1999b); however *L. bulgaricus* was more effective than *S. thermophilus* towards tumor inhibition (Sakar, 2008). Consumption of yogurt is reported to decrease the risk of breast cancer and exocrine pancreatic cancer (Sarkar, 2008). Certain strains of *L. bulgaricus* and *S. thermophilus* have been reported to reduce cholesterol levels during an *in vitro* study (Dilmi-Bouras, 2006) and inclusion of probiotic strains in yogurt enhances the cholesterol reduction (Sarkar, 2008).

2.5 Yogurt starter cultures

By definition, yogurt starter cultures consist of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, and both bacteria belong to the family of lactic acid bacteria (LAB). Yogurt starter cultures convert lactose into lactic acid and produce various flavor compounds, and contribute to the characteristic yogurt flavor and texture. The growth and survival of yogurt starter cultures during fermentation and storage depend upon the ratio and strains of *S. thermophilus* and *L. bulgaricus* in the inoculum, time and temperature of incubation and storage, presence of undesirable microflora and their enzymes, and availability of nutrients (Cais-Sokolińska and Pikul, 2004). In the U.S., the FDA has mandated that both *S. thermophilus* and *L. bulgaricus* be used in all types of yogurts; however, additional probiotic cultures can be added (CFR 131.200, 2009).

2.5.1 Streptococcus thermophilus

S. thermophilus is a Gram-positive, non-motile, catalase-negative, homofermentative**,** facultative anaerobe with a spherical/ovoid shaped-cell (Chandan & O'Rell, 2006b; Singleton & Sainsbury, 1987). Optimum growth temperature for S. *thermophilus* is 37 ºC but it can grow within the temperature range of 20 to 52 ºC (Chandan & O'Rell, 2006b; Frank & Hassan, 1998). Optimum pH for growth of *S. thermophilus* is 6.5 and growth stops at pH 4.2 to 4.4 (Davis, 1975; Frank & Hassan, 1998). *S. thermophilus* can ferment glucose, fructose, mannose, sucrose and lactose; and produces L (+) lactic acid (Chandan & O'Rell, 2006b). *S. thermophilus* degrades lactose into galactose and glucose with β-galactosidase (EC 3.2.1.23) but can only utilize glucose with the exception of very few strains that can also utilize galactose (Chandan and O'Rell, 2006b).

2.5.2 Lactobacillus delbrueckii ssp. *bulgaricus*

L. bulgaricus is a Gram-positive rod, catalase-negative, non-motile, homofermentative, anaerobic/aerotolerant organism (Chandan & O'Rell, 2006b; Frank & Hassan, 1998). *L. bulgaricus* degrades lactose into glucose and galactose by β-galactosidase (EC 3.2.1.23) and utilizes only glucose to produce D (-) lactic acid; thus, galactose accumulates in the medium (Chandan & O'Rell, 2006b; Tamime & Robinson, 1999b). *L. bulgaricus* can utilize lactose, glucose and fructose and some strains can ferment galactose (Chandan & O'Rell 2006b; Tamime & Robinson, 1999b). *L. bulgaricus* can grow within the temperature range of 22 to 52 ºC;

however, the optimum growth temperature of *L. bulgaricus* is 45 ºC but yogurt fermentation is done at 43 ºC in order to accommodate the optimum temperature of *S. thermophilus* (Chandan & O'Rell 2006b). Optimum pH for the growth of *L. bulgaricus* is 5.8 and growth stops at pH 3.5 to 3.8 (Davis, 1975; Frank & Hassan, 1998). The high proteolytic activity of *L. bulgaricus* liberates the amino acids that can support the growth of the weakly proteolytic *S. thermophilus* and probiotics such as *L. acidophilus* and bifidobacteria (Frank & Hassan, 1998; Vasiljevic & Shah, 2008).

2.5.3 Symbiotic behavior of yogurt starter cultures

The growth of starter bacteria, *S. thermophilus* and *L. bulgaricus*, in the yogurt during fermentation is a classic example of symbiotic behavior, and as a result produces greater acid and flavor compounds than either strain used individually (Chandan & O'Rell, 2006b; Glass & Bishop, 2007). Although *S. thermophilus* and *L. bulgaricus* can grow independently, these bacteria utilize each other's metabolites resulting in faster growth. *L. bulgaricus* exhibits greater proteolytic activity and produces peptides and a few amino acids (especially valine) from casein (Chandan & O'Rell, 2006b; Frank & Hassan, 1998; Glass & Bishop, 2007), which stimulates the growth of *S. thermophilus*. Thus, *S. thermophilus* grows faster during the early stages of the fermentation and is the main producer of lactic acid (Glass & Bishop, 2007). As *S. thermophilus* utilizes oxygen and produces carbon dioxide and formic acid, growth of *L. bulgaricus* is stimulated and *L. bulgaricus* dominates during later stages of fermentation (Chandan & O'Rell, 2006b; Glass & Bishop, 2007). Growth of *S. thermophilus* is inhibited at pH 4.4 to 4.2 but *L. bulgaricus* can grow until pH 3.5 to 3.8 (Glass & Bishop, 2007). *L. bulgaricus* is the primary producer of flavor compounds (acetaldehyde, acetone, acetoin and diacetyl) during yogurt fermentation (Glass & Bishop, 2007; Singleton & Sainsbury, 1987).

2.6 Probiotics

The word "probiotic" originated from the Greek, meaning "for life" (Shah, 2006b; Schrezenmeir & de Vrese, 2001). According to FAO/WHO, probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host (Vasiljevic & Shah, 2008; Moriya et al., 2006). Approximately, 30% of the world's population buys probiotic dairy foods on a regular basis; and in 2008, the global probiotic food market was over $15.7 billion, which was > 18% of the global functional foods market

(MarketResearch.com, 2009). Fermented milk and other dairy foods are the most common vehicles for delivery of probiotics in the food industry, although new products have been introduced such as candy bars, cereals, juices and cookies (Sanz, 2007; usprobiotics.org, 2009).

The important criteria for the selection of probiotics are that the bacteria should be non-pathogenic, non-toxic, acid tolerant, bile tolerant, viable and present in sufficient quantity during consumption, survive passage through the gastrointestinal tract, colonize at the target site, and withstand the processing conditions (Gibson & Roberfroid, 1995; McKinley, 2005; Shah, 2006b; Vasiljevic & Shah, 2008). Gut microflora maintain the normal intestinal function and resist disease-causing microorganisms; however, lifestyle, dietary patterns and consumption of pharmaceutical products (such as antibiotics) alter the natural gut microflora (Fooks & Gibson, 2002; McKinley, 2005). Consumption of probiotic yogurt can help to restore the natural gut microflora (Fooks & Gibson, 2002).

To confer health benefits the recommended concentration of probiotics in the yogurt range from 6 to 8 log cfu g^{-1} (Güler-Akın & Akın, 2007; Vasiljevic & Shah, 2008; Vasiljevic et al., 2007). Beneficial health effects of probiotics are strain specific. Even strains of the same species will not exert the same health benefits (Schrezenmeir & Vrese, 2001); hence, studies done on one strain cannot be extrapolated to a related strain. Lactic acid bacteria of the genera *Lactobacillus* and *Bifidobacterium* are the most commonly used probiotics in the food industry. Within these genera, *L. acidophilus, L. casei, L. rhamnosus, L. reuteri, L. plantarum, L. johnsonii, B. animalis, B. longum, B. bifidum, B. breve, B. lactis* and *B. infantis* are the most commonly used species (McKinley, 2005; Ouwehand, Salminen & Isolauri, 2002; Vasiljevic & Shah, 2008). Although there is disagreement as to whether yogurt starter cultures are considered to be probiotics or not (because some studies suggested their poor *in vitro* survival), yogurt starter cultures fulfill all criteria (as mentioned above) to be considered as probiotics (Lomax & Calder, 2009; Guarner et al., 2005; Salminen, Lahtinen, & Gueimonde, 2005) and have been reported to confer health benefits (Dzida, 2009a; Dzida, 2009b; McKinley, 2005; Sarkar, 2008; Strnad & Babus, 1997). Some of the established and potential health benefits of *Lactobacillus* spp. and *Bifidobacterium* spp. are presented in Table 2.2.

Table 2.2 Established and potential health benefits of *Lactobacillus* spp. and *Bifidobacterium* spp.

Probiotics strain	Associated health benefits	References
L. acidophilus LB	Increases *Helicobacter pylori* eradication rate	Canducci et al. (2000)
	Decreases duration of non-rotavirus diarrhea	Liévin-Le et al. (2007)
	Decreases duration of rotavirus diarrhea	McKinley (2005)
L. acidophilus La5	Reduces duration of antibiotic related diarrhea	Ouwehand et al. (2002)
L. acidophilus 299v	Relieves irritable bowel syndrome	Ouwehand et al. (2002)
	Reduces LDL-cholesterol	
L. casei Shirota	Reduces risk of bladder cancer	Salminen et al. (2005)
L. johnsonii La1	Increases *Helicobacter pylori* eradication rate	Felley et al. (2001)
B. animalis DN-173 010	Prevents carcinogenesis	Tavan et al. (2002)
B. bifidum	Reduces the risk of infection from food borne pathogens	Lourens-Hattingh & Viljoen (2001)
B. breve	Reduces symptoms of inflammatory bowel disease	Ouwehand et al. (2002)
B. lactis BB-12	Reduces incidence of traveler's diarrhea	Ouwehand et al. (2002)
	Alleviates symptoms of food allergies	Salminen (2001)
B. longum	Prevents carcinogenesis	Marks (2004)
B. longum BB536	Prevents carcinogenesis	Zsivkovits et al. (2003)

2.6.1 Lactobacillus acidophilus

L. acidophilus is a Gram-positive rod, non-motile, non-spore-forming and catalase negative (some exceptions may be catalase-positive; Frank & Shah, 1998; Vasiljevic & Shah, 2008). *L. acidophilus* can be microaerophilic, aerotolerant or anaerobic and occurs naturally in the gastrointestinal tracts of humans and animals, and the human mouth and vagina (Shah, 2006b). *L. acidophilus* are mostly obligate homofermenters, with some exceptions of facultative heterofermenters that produce equimolar amounts of lactic acid, carbon dioxide and ethanol (Shah, 2006b). *L. acidophilus* requires carbohydrates as well as nucleotides, amino acids and vitamins for growth. Most strains of *L. acidophilus* can utilize cellobiose, fructose, galactose, glucose, lactose, maltose, salicin, sucrose and aesculine; however, *L. acidophilus* can utilize sucrose more efficiently than lactose (Shah, 2006b). Optimum temperature and pH range for the growth of *L. acidophilus* are 35 to 40 ºC and 5.5 to 6.0, respectively (Shah, 2006b). Growth of *L. acidophilus* will cease at > 45^{o}C or pH < 3.6 (Shah, 2006b).

2.6.2 Bifidobacterium spp.

Bifidobacteria are the major saccharolytic bacteria found in the human colon, and constitute 95% and 25% of total microbial gut population in newborns and adults, respectively (Gibson & Roberfroid, 1995). Bifidobacteria are Gram-positive, rod shaped, obligate anaerobes that are non-gas-forming, non-motile and catalase-negative with a bifurcated morphology (Vasiljevic & Shah, 2008; Shah, 2006b). Bifidobacteria produce acetic acid and lactic acid in the molar ratio of 3:2 (Vasiljevic & Shah, 2008). *Bifidobacterium* spp. of human origin can utilize galactose, lactose and usually fructose (Shah, 2006b). Optimum temperature and pH ranges of bifidobacteria are 37 to 41 ºC and 6.0 to 7.0, respectively. Bifidobacteria cannot grow at < 25 ºC or > 45 º C and pH < 4.5 and > 8.5 (Shah, 2006b). *Bifidobacterium animalis* ssp. *lactis* is the most acid tolerant species of bifidobacteria (Vasiljevic & Shah, 2008; Sanz, 2007).

2.7 Passage of probiotics through the gastrointestinal tract and their mechanism action

The human gastrointestinal tract (GIT) consists of the oral cavity, esophagus, stomach, small intestine and large intestine (colon). Probiotics when ingested through the mouth are first transferred to the stomach through the esophagus. The first barrier probiotics have to pass is the

low stomach pH (1 to 4) (Kailasapathy & Chin, 2000). Survival of probiotics through the stomach depends upon the pH, passage time and probiotic strains (Bezkorovainy, 2001). Morelli (2007) and Kailasapathy and Chin (2000) discussed that *L. acidophilus* is more tolerant to gastric juice acidity than the yogurt starter bacteria and *Bifidobacterium* spp. When exiting the stomach probiotics are introduced to the small intestine, and bile salts (0.5 to 2%) are the harshest obstacle to probiotic survivability (Kailasapathy & Chin, 2000). Bezkorovainy (2001) discussed that probiotics survival through the small intestine depends upon the bile salt concentration, exposure time and probiotic strains. Finally, probiotics are transferred to the colon. Bezkorovainy (2001) discussed that although *in vitro* studies have shown the successful adhesion and colonization of probiotics in the colon, no clear evidence of adhesion and colonization has been obtained from *in vivo* studies. Therefore, it was concluded that ingested probiotic strains recovered from the feces during the feeding studies were present only if probiotics were consumed but strains disappeared from the feces when probiotic consumption was discontinued. Although probiotics were not able to colonize in the human intestines, the metabolic activity of probiotics during the passage through the GIT provided the beneficial health effects to the host. Survivability of various probiotic strains through the GIT has been reported to vary from 10 to 25% (Bezkorovainy, 2000; Hove et al., 1999). Various techniques, such as using acid and bile resistant probiotic cultures, microencapsulation of probiotic cultures, usage of prebiotics in probiotic food matrix, greater protein levels to increase the buffering capacity (for yogurt) and genetic modification of probiotic bacteria, have been reported to increase the survivability of probiotics through the passage of the GIT (Bezkorovainy, 2001; Gibson et al., 2004; Gibson & Roberfroid, 1995; Kailasapathy & Chin, 2000; Ross et al., 2005; Sheehan et al., 2007; Sleator & Hill, 2006).

Probiotics influence the host's microflora and health in more than one way, and various possible mechanisms that have been hypothesized are discussed briefly as follows (Lomax & Calder, 2009; Ng et al., 2009):

1. Probiotics compete with pathogenic bacteria for nutrients and adhesion sites in the intestines and hence reduce the possibility of survival of pathogenic bacteria.
2. Probiotics produce antimicrobial peptides and bacteriocins against the pathogenic bacteria.

3. Probiotics produce short chain fatty acids from carbohydrate fermentation in the colon. Production of these acids makes the environment harsh for the survivability of pathogenic bacteria and also provides nutritional benefits to colonocytes.
4. Probiotics increase mucus production and this enhances the barrier functions of the colon.
5. Probiotics modulate the immune function through direct interaction with the mucosal immune system.

2.8 Prebiotics

The term "prebiotic" was first used by Gibson and Roberfroid (1995), and is defined as a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species in the colon and thus improves host health." According to Gibson et al. (2004) an ingredient can be considered as a prebiotic if "it resists gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption; is fermented by the intestinal microflora; and stimulates selectively the growth and/or activity of intestinal bacteria associated with health and wellbeing." Prebiotics are generally non-digestible oligosaccharides and fructooligosaccharides (FOS) in particular (Gibson & Roberfroid, 1995).

Fructooligosaccharides and inulin are naturally present in garlic, onion, artichoke and asparagus (Gibson & Roberfroid, 1995). Fructooligosaccharides and inulin can improve bioavailability of minerals such as calcium, magnesium and iron (Aryana et al., 2007). Inulin boosts the body's natural defense and provides health benefits related to dietary fiber (Aryana et al., 2007). Larch arabinogalactan is a prebiotic fiber that protects against gastrointestinal diseases, enhances immune system, and possesses mitogenic, antimutagenic, gastroprotective and antimicrobial properties (Cueva & Aryana, 2007).

Prebiotic-supplemented fermented milk products have been reported to have improved yogurt and probiotic bacteria viability during fermentation and storage (Bruno, Lankaputhra & Shah, 2002; Vasiljevic et al., 2007). The term "symbiotic" is used to describe a product containing both probiotics and prebiotics, with the ultimate goal of conferring the health benefits to the host (Sanz, 2001).

2.9 Viability of yogurt starter and probiotic bacteria in yogurt

The viability of starter and probiotic bacteria in yogurt during fermentation and storage depends upon intrinsic and extrinsic factors. Intrinsic factors are the physical and chemical properties of the food product itself, and include nutrients, acidity/pH, redox potential, water activity and presence of antimicrobial or prebiotic compounds (IFT, 2003). Extrinsic factors consist of the environmental factors in which food product has been manufactured and stored; for example, storage temperature, time, humidity and atmosphere (IFT, 2003).

2.9.1 Intrinsic factors

2.9.1.1 Nutrients

All microorganisms require certain nutrients to grow and maintain their viability; and the basic nutrients required by microorganisms are water, carbohydrates, proteins, vitamins and minerals (IFT, 2003). Sufficient nutrients in the yogurt mix are necessary to support the growth and sustain the viability of yogurt starter and probiotic bacteria. Milk does not contain a sufficient quantity of free amino acids, and probiotic bacteria have low proteolytic activity; therefore probiotic cultures are usually used in combination with yogurt starter bacteria, which have greater proteolytic activity (Shihata & Shah, 2000). Various vitamins and amino acids that *S. thermophilus* cannot synthesize but require for growth include: niacin, pantothenic acid, pyridoxine, biotin, nitroflavin, isoleucine, leucine, valine and histidine; whereas *L. bulgaricus* and *L. acidophilus* require pantothenic acid, niacin, nitroflavin, isoleucine, leucine, valine and histidine (Frank & Hassan, 1998). *Bifidobacterium* spp. are less fastidious compared with *Streptococcus thermophilus* and *Lactobacillus* spp. (Van der Meulen, Adriany, Verbrugghe & Vuyst, 2006).

2.9.1.2 Acidity / pH

At the end of fermentation (0.9% lactic acid; CFR 131.200, 2009), the yogurt pH is ~ 4.5 (Tamime & Robinson, 1999a), but post-fermentation acidification during storage can reduce the yogurt pH to 4.2. *S. thermophilus* and bifidobacteria cannot grow below pH 4.2 (Glass & Bishop, 2007) and 4.5 (Marks, 2004), respectively; whereas *L. bulgaricus* and *L. acidophilus* can continue to grow until pH decreases to 3.5 (Glass & Bishop, 2007) and 3.6 (Shah, 2006b),

respectively. Therefore, *Bifidobacterium* spp. shows poor viability in refrigerated yogurt at low pH compared to *S. thermophilus*, *L. bulgaricus* and *L. acidophilus*.

2.9.1.3 Redox potential (Eh)

Redox potential is the measure of a substance's ability to gain or lose electrons, and is expressed in mV. In milk, the Eh depends upon various factors such as dissolved oxygen, ascorbic acid, riboflavin, cystine-cysteine transformation and pH (Chandan, 2006b). The Eh of raw milk varies from +200 to +300 mV, whereas for pasteurized milk the Eh was reported as + 182 mV (Bolduc et al., 2006). In general, the desirable Eh range for the growth of various microorganisms is +300 to +500 mV for aerobes, -100 to +300 mV for facultative anaerobes, and -250 to +100 mV for obligate anaerobes (Ray, 2004). *S. thermophilus* is a facultative anaerobe, whereas *L. bulgaricus*, *L. acidophilus* and *B. animalis* are obligate anaerobes; therefore, the lower Eh ($\leq$ +300 mV) of yogurt may improve the viability of these microorganisms (Vasiljevic & Shah, 2008).

2.9.1.4 Water activity (a_w)

The water required for the growth of microorganisms is defined in terms of water activity, which is the ratio of water vapor pressure of the food substrate to the vapor pressure of pure water at the same temperature (IFT, 2003). Bacteria cannot grow if the water activity of the media is below 0.85 (Bell et al., 2005). Water activity of milk is 0.98 (Varnam & Sutherland, 2001b); therefore a_w has no inhibitory effect on the yogurt and probiotic bacteria during fermentation; however, supplementing yogurt mix with high amounts of sugar (10 g 100 g^{-1}) could reduce a_w to < 0.85 and inhibit yogurt starter and probiotic bacteria during fermentation (Tamime & Robinson, 1999a).

2.9.1.5 Presence of inhibitory factors

Post-pasteurization contamination of yogurt mix with spoilage or pathogenic microorganisms can slow starter and/or probiotics growth (Glass & Bishop, 2007). Prolonged usage of yogurt and probiotic cultures makes them susceptible to phage attack and results in the loss of acid production ability (Frank & Hassan, 1998). Excessive hydrogen peroxide production by *L. bulgaricus* during yogurt fermentation decreases the viability of the hydrogen peroxide sensitive *S. thermophilus*, *L. acidophilus* and *B. animalis* during storage (Dave & Shah, 1997b;

Dave & Shah, 1997c). Dave and Shah (1997c) reported that on day 0, the concentration of hydrogen peroxide in yogurts manufactured with yogurt starter bacteria, *L. acidophilus* and bifidobacteria was ≥ 8.80 µg mL^{-1} compared with < 3.00 µg mL^{-1} for fermented milk manufactured with *S. thermophilus*, *L. acidophilus* and bifidobacteria. They attributed this greater hydrogen peroxide production to *L. bulgaricus* and reported less counts of *S. thermophilus* (≤ 8.50 log cfu g^{-1}) and *L. acidophilus* (≤ 5.35 log cfu g^{-1}) on day 35 compared with the counts (> 8.84 log cfu g^{-1} and > 5.92 log cfu g^{-1}, respectively) in fermented milks without *L. bulgaricus*. Presence of antibiotics such as penicillin and cloxacillin in yogurt mix also inhibits the growth of yogurt starter and probiotic bacteria (Frank & Hassan, 1998).

2.9.2 Extrinsic factors

2.9.2.1 Storage temperature and time

In the U.S., yogurt is maintained at ≤ 7 ºC during storage (PMO, 2007). At this temperature yogurt and probiotic bacteria have limited activity and produce limited amounts of lactic acid; therefore cold storage delays the decline/death phase of the bacteria. Ainaz et al. (2008) reported an increase in titratable acidity from 0.90 to ~ 1.1% from day 1 to 21 in yogurt manufactured from pasteurized skim milk supplemented with 2% skim milk powder and inoculated with yogurt starter bacteria, *L. acidophilus* and *B. lactis*; and the total *L. acidophilus* and *B. lactis* counts decreased from ~ 8.5 log cfu mL^{-1} to ~ 5.5 log cfu mL^{-1}. Viljoen, Lourens-Hattingh, Ikalafeng and Peter (2003) studied the spoilage of yogurts obtained from different commercial manufacturers, and reported that yogurt stored at 25 ºC for 30 days had yeast counts of 5 to 6 log cfu g^{-1} compared with 3 to 4 log cfu g^{-1} for yogurts stored at 5 ºC for 30 days. Mortazavian, Ehsani, Mousavi, Sohrabcvandi and Reinheimer (2006b) reported that yogurt manufactured with yogurt starter bacteria, *L. acidophilus* and *B. lactis* BB-12 and stored for 20 days at 2 ºC had greater *L. acidophilus* counts (6.47 log cfu mL^{-1}) compared with the yogurts stored at 5 or 8 ºC (< 5.80 log cfu mL^{-1}); whereas *B. lactis* counts were greater in yogurts stored at 8 ºC (6.15 log cfu mL^{-1}) compared with 2 or 5 ºC (≤ 5.80 log cfu mL^{-1}).

2.9.2.2 Storage atmosphere

Storage atmosphere plays a vital role in the viability of probiotic bacteria during storage. *Bifidobacterium* spp. and *L. acidophilus* lack electron transport chains and catalase enzymes;

therefore hydrogen peroxide is produced from the oxygen and accumulates within their cells, hence killing them (Vasiljevic & Shah, 2008). Production of hydrogen peroxide by *L. bulgaricus* in the presence of oxygen also acts as an anti-microbial against probiotics (Vasiljevic & Shah, 2008). Yogurt is generally packaged in high-impact polystyrene (HIPS) containers, an oxygen permeable material (Talwalkar et al., 2004) which has oxygen and carbon dioxide permeability of 1,600 and 10,000 cm^3 m^{-2} day^{-1} bar^{-1}, respectively (Massey, 2003). Dave and Shah (1997c) reported that on day 35, yogurt stored in glass bottles had greater *L. acidophilus* (5.70 log cfu g^{-1}) and bifidobacteria (6.94 log cfu g^{-1}) counts compared with the counts in yogurts stored in plastic cups (3.00 and 6.47 log cfu g^{-1}, respectively); and *L. bulgaricus* counts in yogurt stored in glass bottles were > 5 log cfu $g^{-1}$for 5 days more than yogurts stored in the plastic cups. They attributed the lower viability of these bacteria to the greater dissolved oxygen (9 ppm) as a result of air permeability through the plastic cups compared with the glass bottles (8.5 ppm). On the other hand, Talwalkar et al. (2004) reported less dissolved oxygen in yogurt stored in Nupak™ (< 4.29 ppm), a polyester-based gas barrier, compared with yogurt stored in high-impact polystyrene (~ 58 ppm) on day 42, but no significant differences were observed in *Bifidobacterium lactis* CSCC 1912 and *L. bulgaricus* CSCC 2409 counts. They concluded that oxygen might be the significant factor for *Bifidobacterium* spp. viability in yogurt during storage but the viability could be strain specific.

2.10 Addition of prebiotics and antioxidants in yogurt

Probiotic bacteria grow slowly in milk due to their low proteolytic activity (Bari et al, 2009; Güler-Akın & Akın, 2007) and, moreover, their survival in yogurt is hindered by low pH, high oxygen tension and nutrition depletion (Vasiljevic et al., 2007). These adverse effects can be minimized by using selected probiotic strains (such as low-acid producers or more acid resistant) and supplementing yogurt mix with peptides and amino acids, prebiotics or antioxidants (Güler-Akın & Akın, 2007; Vasiljevic et al, 2007).

Bifidobacterium strains have the ability to utilize prebiotics (complex carbohydrates) such as inulin; hence, inulin can enhance the metabolic activity of *Bifidobacterium* (Vasiljevic et al, 2007). Vasiljevic et al. (2007) studied the effect of prebiotics inulin, oat β-glucan or barley β-glucan on the viability of *S. thermophilus, L. bulgaricus* and *B. animalis* ssp. *lactis* in yogurt during 4 weeks of storage at 4°C. They reported no significant difference in the counts of *S.*

thermophilus and *L. bulgaricus* during storage, but at the end of storage the counts of *B. animalis* ssp. *lactis* were 0.7, 1.0 and 1.1 log cfu mL^{-1} greater for yogurts containing barley β-glucan, inulin and oat β-glucan, respectively when compared with the non-supplemented yogurt (7.4 log cfu mL^{-1}). Greater *L. acidophilus* counts in fat-free plain yogurt supplemented with1.5% inulin of short (6.94 log cfu g^{-1}), medium (7.01 log cfu g^{-1}) or long (7.01 log cfu g^{-1}) chain compared with non-supplemented yogurt (6.85 log cfu g^{-1}) have been reported by Aryana et al. (2007). Akalin et al. (2007) reported greater *S. thermophilus* (8.96 log cfu g^{-1}), *L. bulgaricus* (5.51 log cfu g^{-1}) and *B. animalis* (8.80 log cfu g^{-1}) counts in reduced-fat yogurt supplemented with 1.5% FOS compared with the counts (8.67, 5.07 and 8.65 log cfu g^{-1}, respectively) in non-supplemented yogurt at the end of storage (28 days).

Dave and Shah (1998) studied the effect of cysteine.HCl supplementation (50, 250 or 500 mg L^{-1}) of fermented milk manufactured with *S. thermophilus, L. acidophilus* and bifidobacteria during 35 days of storage. They reported that cysteine.HCl supplementation at 50, 250 or 500 mg L^{-1} decreased the milk Eh to -130, -180 or -217 mV, respectively, compared with the non-supplemented milk (-70 mV); and on day 35, the Eh of all supplemented fermented milks was < 25 mV compared with 160 mV for the non-supplemented fermented milk. They reported that on day 35, *S. thermophilus* counts in fermented milks supplemented with 250 or 500 mg L^{-1} cysteine.HCl (8.11 or 6.59 log cfu g^{-1}, respectively) were less than the counts in non-supplemented fermented milk (8.69 log cfu g^{-1}); whereas supplementation at 50 mg L^{-1} yielded greater counts (9.03 log cfu g^{-1}). They also reported that *L. acidophilus* counts (≥ 6.11 log cfu g^{-1}) in all supplemented fermented milks were greater than the non-supplemented fermented milk (5.71 log cfu g^{-1}) and bifidobacteria counts in all supplemented fermented milks remained ≥ 4.76 log cfu g^{-1} compared with 2.30 log cfu g^{-1} for the non-supplemented fermented milk. Dave and Shah (1998) concluded that lower Eh improved the viability of *L. acidophilus* and bifidobacteria but decreased the viability of *S. thermophilus* due to adverse effects of suppressed Eh on the cell wall and cell membrane of *S. thermophilus*.

In a different study, Dave and Shah (1997b) also reported that on day 0, yogurts manufactured with *L. acidophilus* and bifidobacteria, and supplemented with 50, 250 or 500 mg L^{-1} cysteine.HCl had Eh ranges of -10 to -30, -25 to -80 mV, or -30 to -100 mV, respectively compared to an Eh range of 50 to 100 mV for the non-supplemented yogurts; and the Eh increased in all yogurts during storage, and on day 35, the Eh of supplemented yogurts ranged

from 10 to 120 mV compared with 120 to 150 mV for non-supplemented yogurts. They reported that on day 35, *S. thermophilus* counts in yogurts supplemented with 250 or 500 mg L^{-1} cysteine.HCl (7.82 to 8.03 log cfu g^{-1}) were less than non-supplemented and 50 mg L^{-1} cysteine.HCl yogurts (8.31 to 8.39 log cfu g^{-1}); whereas *L. bulgaricus* counts in all supplemented yogurts were > 6 log cfu g^{-1} compared with < 5 log cfu g^{-1} for the non-supplemented yogurts; *L. acidophilus* counts for 250 or 500 mg L^{-1} cysteine.HCl (≥ 5.48 log cfu g^{-1}) supplemented yogurts were greater compared with the non-supplemented (≤ 4.92 log cfu g^{-1}); and bifidobacteria counts (7.02 to 7.34 log cfu g^{-1}) were not effected by 50 mg L^{-1} cysteine.HCl supplementation but the counts were less in 250 or 500 mg L^{-1} cysteine.HCl supplemented yogurts. Bari et al. (2009) reported similar results for yogurts manufactured with 0, 0.25, 0.50 or 0.75% L-cysteine.HCl with the exception that L-cysteine.HCl had no significant effect on the *L. acidophilus* viability.

2.11 Sodium acetate

Sodium acetate ($C_2H_3NaO_2$), the sodium salt of acetic acid, is a FDA approved buffering and flavoring agent (Lindsay, 2007; Manju et al., 2007). Sodium acetate has been reported to possess antioxidant and antimicrobial activities (Sallam, 2007; Serdengect, Yildirim & Gokoglu, 2006), and is used in the meat industry to control unwanted natural microflora.

No research has been reported in the literature on the effect of sodium acetate on the viability of starter and probiotic bacteria in yogurt; however, two studies reported that the growth yield and acid production of some lactic acid bacteria (LAB) were affected if growth media was supplemented with sodium acetate. Lino et al. (2001) reported that out of 49 strains of LAB (23 strains of *Lactobacillus* spp., 5 strains of *Leuconostoc* spp., 3 strains of *Weissella* spp., 7 strains of *Pediococcus* spp., 3 strains of *Enterococcus* spp., 2 strains of *Lactococcus* spp., 4 strains of *Streptococcus* spp., *Sporolactobacillus inulinus* and *Bacillus coagulans*) grown individually in GYP broth supplemented with 50 mM sodium acetate for 2 days, 32 strains produced 1.2 × more lactic acid compared with the non-supplemented broth, while the remaining strains produced similar levels of lactic acid. They further reported better growth (absorbance of GYP broth at 660 nm) of *L. sakei* NRIC 1077, *L. coryniformis* ssp. *coryniformis* NRIC 1638 and *L. plantarum* NRIC 1067 in 10, 20, 50 or 100 mM sodium acetate supplemented GYP broth compared with the growth in non-supplemented broth after 2 or 3 days of fermentation. Activation of L-lactate dehydrogenase and/or strengthening of the glycolytic pathway or pentose cycle were given as

possible explanations for the increased lactic acid production in sodium acetate supplemented broth (Lino et al., 2001). Lino, Uchimura and Komagata (2002) reported that growth yield (g dry bacteria per mol glucose) of *L. sakei* NRIC 1071^T and *L. plantarum* NRIC 1067^T grown in GYP broth supplemented with 50 mM sodium acetate for 24 h was 21.3 g and 19.9 g, respectively compared with *L. sakei* NRIC 1071^T (13.6 g) and *L. plantarum* NRIC 1067^T (16.0 g) grown in non-supplemented GYP broth. They further reported that *L. sakei* NRIC 1071^T and *L. plantarum* NRIC 1067^T produced ~ 2 to 2.5× more lactic acid in sodium acetate supplemented GYP broth after 24 h fermentation compared with the lactic acid produced by *L. sakei* NRIC 1071^T (10 mM) and *L. plantarum* NRIC 1067^T (20 mM) in non-supplemented GYP broth. The pH in sodium acetate supplemented GYP broth after 24 h decreased from 6.8 to ~ 4.0 compared with 6.8 to ~ 3.6 in non-supplemented GYP broth in the study by Lino et al. (2002) suggested that sodium acetate supplementation provided buffering ability to GYP broth.

2.12 Plant extract

According to Cognis Nutrition & Health (Monheim, Germany), Cegemett® Fresh is an extract prepared from an oleoresin mixture (based on olive, garlic-onion, and citrus extracts, glycerol E422 and ascorbic acid E300) and possesses antimicrobial and antioxidant properties. Plant extract contains sodium acetate as a carrier and constitutes ~ 50% of the plant extract. This product is being used in Europe in food products such as sausages and bread, and surface treatment of poultry and carcasses in a concentration ranging from 0.3% to 3% for reducing the bacterial load and inhibiting mold.

CHAPTER 3 - Research Question

Can plant extract (Cegemett® Fresh) supplementation enhance the viability of yogurt starter (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) and probiotic (*Bifidobacterium animalis* ssp. *animalis* ATCC 25527 and *Lactobacillus acidophilus* ATCC 4356) bacteria in nonfat yogurt during refrigerated storage?

CHAPTER 4 - Impact of a plant extract on the viability of yogurt starter cultures in nonfat yogurt (experiment-I)

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4.1 Introduction

Yogurt, a nutrient-dense food, is one of the most popular fermented milk products worldwide (McKinley, 2005). In some cases, the beneficial health effects of yogurt are attributed to the starter cultures (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*); however, disagreements exist whether yogurt starter cultures are considered as probiotics or not (Lomax & Calder, 2009). The yogurt bacteria fulfill all criteria to be considered as probiotics (Guarner et al., 2005; Salminen, Lahtinen & Gueimonde, 2005) and have been reported to confer health benefits within the yogurt system (Dzida, 2009a; Dzida, 2009b; McKinley, 2005; Sarkar, 2008; Strnad & Babus, 1997). To confer these health benefits, probiotic bacteria should be viable at the time of consumption at a recommended concentration of 6 to 8 log cfu g^{-1} (Ross, Desmond, Fitzgerald & Stanton, 2005; Vasiljevic & Shah, 2008). In the U.S., refrigerated yogurts displaying the "Live & Active Cultures" seal on the containers should have $\geq$ 8 log cfu g^{-1} yogurt cultures at the time of manufacture (NYA, 2009).

In most yogurt products, viability of yogurt bacteria typically declines below the suggested concentration during refrigerated storage, significantly limiting the time retailers have to sell "active-culture" products (Dave & Shah, 1998; Dave & Shah, 1997b; Dave & Shah, 1997c; Vasiljevic & Shah, 2008). This decrease in the viability has been attributed to the low pH, the high oxygen tension and/or the accumulation of starter culture metabolites such as hydrogen peroxide and lactic acid formed during fermentation and storage (Dave & Shah, 1997c; Tamime & Robinson, 1999b; Vasiljevic, Kealy & Mishra, 2007). Researchers have attempted to extend yogurt bacteria viability during storage by making the yogurt environment more conducive to the yogurt bacteria. Supplementation of yogurt mix with antioxidants such as cysteine or ascorbic acid has been reported to reduce the redox potential (Eh) of the yogurt mix (Dave & Shah, 1997a; Dave & Shah, 1997b) and improve the viability of *L. bulgaricus* (an anaerobe/aerotolerant) and *S. thermophilus* (a facultative anaerobe), which prefer to grow at a reduced Eh of -250 to +100 and -100 to +300 mV, respectively (Ray, 2004). Dave and Shah

(1997b) reported greater *L. bulgaricus* counts (> 7 log cfu g^{-1}) on day 25 in yogurt supplemented with cysteine at 50, 250 or 500 mg L^{-1} compared with non-supplemented yogurt (< 5 log cfu g^{-1}); *S. thermophilus* counts were not affected during the storage if cysteine was added at 50 mg L^{-1} but the counts were adversely affected if cysteine was added at 250 or 500 mg L^{-1} compared with non-supplemented yogurt. They attributed this decrease in *S. thermophilus* counts at the greater cysteine concentrations to the suppressed Eh which in turn adversely affected the *S. thermophilus* cell wall and cell membrane. Bari et al. (2009) reported similar results for yogurt supplemented with cysteine at 0.25, 0.50 or 0.75%. Dave and Shah (1997a) reported that *L. bulgaricus* counts were > 7 log cfu g^{-1} on day 20 if yogurt was supplemented with ascorbic acid at 50, 150 or 250 mg kg^{-1} but < 5 log cfu g^{-1} in the non-supplemented yogurt; *S. thermophilus* counts were not affected if yogurt was supplemented with ascorbic acid at 50 mg kg^{-1}, but the counts in yogurt supplemented with ascorbic acid at 150 or 250 mg kg^{-1} on day 35 were less than those in non-supplemented yogurts.

Cegemett® Fresh (Cognis, Nutrition & Health, Monheim, Germany) is a plant extract (PE) prepared from an oleoresin mixture (based on olive, garlic, onion, citrus extract and uses sodium acetate as a carrier) and possesses antioxidant properties. Plant extract is a less costly antioxidant compared to cysteine or ascorbic acid; therefore supplementing yogurt with PE could be a more economical option for reducing the yogurt Eh and hence improving yogurt bacteria viability.

I hypothesized that supplementing yogurt mix with PE could reduce the Eh of the yogurt and thus improve the longevity of yogurt bacteria in the product, especially anaerobic *L. bulgaricus*. Therefore, the objective of this study was to investigate the effect of PE supplementation on the Eh and viability of *L. bulgaricus* and *S. thermophilus* in nonfat yogurt stored for 50 days at 5 ºC, while monitoring selected physicochemical parameters. Cysteine supplementation, which has been reported to effectively reduce the Eh of yogurt mix (Dave & Shah, 1998; Dave & Shah, 1997b), was included as a comparison treatment.

4.2 Materials and methods

4.2.1 Experimental design

Five yogurt samples (treatments) with various levels of supplements [non-supplemented (NS), supplemented with 0.5% or 1.0% (w/v) plant extract (Cegemett® Fresh; $PE_{0.5}$ and PE_1) or

supplemented with 0.014% or 0.028% (w/w) L-cysteine.HCl (Fisher Biotech, Fisher Scientific, Fair Lawn, NJ, USA; $Cys_{0.014}$ and $Cys_{0.028}$)] were prepared. For the fermentation study, NS and supplemented yogurt mixes were fermented in a bioreactor (Bioflo 3000, New Brunswick Scientific Co. Inc., Edison, NJ, USA) to determine the fermentation time (to pH 4.50) of each yogurt formulation. Titratable acidity (TA), pH, Eh and microbial analyses were done at 1 h intervals during fermentation to track these changes. Two replications were conducted with each test done in duplicate, and the average was used for analysis. The five treatments were done in a randomized order before starting the second replication. A one-way analysis of variance was done using Statistical Analysis System (SAS®) version 9.1 (SAS Institute Inc., Cary, NC, USA), and differences in means were determined using LSD at $\alpha = 0.05$.

For the shelf life study, NS and supplemented yogurts were prepared and stored at 5 ºC for 50 days. Yogurts were analyzed on the day after fermentation (day 1) and weekly thereafter. Firmness and total solids were determined only on day 1. Three replications were conducted with each test done in duplicate, and the average was used for analysis. This study was designed as a split plot [supplement type and concentration (treatment) as the whole plot and time as the split plot]. Data were analyzed using SAS® version 9.1 (SAS Institute Inc.) and Tukey's test at $\alpha = 0.05$ was used to differentiate among the significant means of the main effects and of interactions.

4.2.2 Yogurt starter cultures propagation

Nonfat dry milk (NFDM; low heat, spray processed, Grade A, Dairy America™, Fresno, CA, USA) was rehydrated at 140 g L^{-1} in distilled-deionized water, sterilized at 121 ºC and 15 psi for 15 min, and cooled to 35 ºC. Sterilized, rehydrated NFDM (Dairy America™) was inoculated with 1% (w/v) freeze-dried yogurt cultures (Yo-Mix™ Yogurt Cultures, Yo-Mix 161 LYO 375 DCU, Danisco, New Century, KS, USA), incubated (Isotemp Incubator, Fisher Scientific) at 35 ºC for 18 h, and then maintained at 5 ºC (Equatherm® Incubator, Lab-Line Instruments, Inc, Melrose Park, IL, USA) until it was used as the mother culture for yogurt (within 48 h). During the preliminary study, no significant differences were found in *L. bulgaricus* and *S. thermophilus* ratio of the freeze-dried cultures vs. the prepared mother culture.

4.2.3 Yogurt preparation

Yogurt mix was prepared by dissolving 140 g NFDM (Dairy America™) and 40 g sugar (Pure Cane Sugar, Domino Foods, Inc., Yonkers, NY, USA) per liter of distilled-deionized water. The mix was pasteurized at 90 ºC for 10 min, cooled to 40 to 43 ºC and inoculated with 3% (w/w) mother culture. Plant extract supplementation was done during the mixing along with the NFDM (Dairy America™) and sugar, prior to pasteurization; whereas Cys supplementation was done at the point of culture addition (Greene & Jezeski, 1957). For the fermentation study, inoculated yogurt mix was transferred to the bioreactor (Bioflo 3000) and fermentation was done at 43 ºC until pH 4.50. Whereas, for the shelf life study, inoculated yogurt mix was transferred to sterilized plastic (polypropylene) cups (Fisherbrand 118 mL, Fisher Scientific, Pittsburg, PA, USA) and 50 mL centrifuge tubes (Oak Ridge Centrifuge Tubes, Nalge Nunc International, Rochester, NY, USA), and incubated (Isotemp Incubator, Fisher Scientific) at 43 ºC until pH 4.50 ± 0.05. Yogurt samples were then stored at 5 ºC (Equatherm® Incubator) until testing.

4.2.4 pH

pH was measured with a pH meter (Thermo Scientific Orion 2 Star Benchtop, Thermo Fisher Scientific Inc., Beverly, MA, USA) that was calibrated with standardized pH buffer solutions 4.0 and 7.0 (Fisher Scientific) prior to the analysis.

4.2.5 Titratable acidity (TA)

Titratable acidity expressed as % lactic acid was measured as described by Chandan and O'Rell (2006c). Nine milliliters of sample were pipetted into a 100 mL titration flask, and the pipette was rinsed with approximately 18 mL distilled-deionized water. Titration against 0.1 N sodium hydroxide (Fisher Scientific) was done using 0.5 mL phenolphthalein (Fisher Scientific) as an indicator. Titratable acidity was calculated using the following formula:

$$TA\ (\%\ lactic\ acid) = mL\ of\ 0.1\ N\ NaOH\ used \times 0.1$$

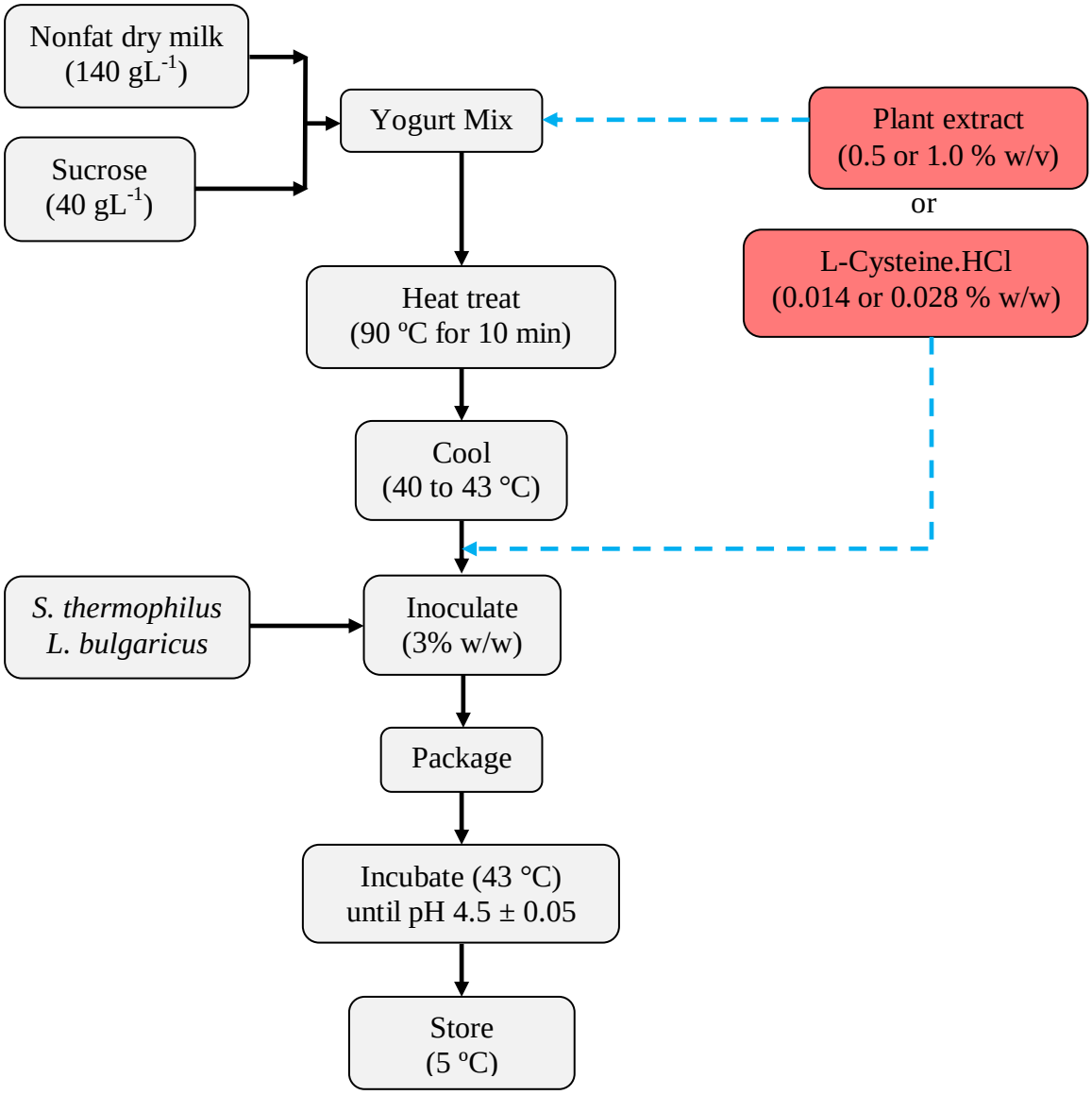

Figure 4.1 Schematic of yogurt manufacture

4.2.6 Redox potential (Eh)

Redox potential was measured at 25 ± 2°C with a platinum electrode (Platinum Combination Electrode, Fisher Scientific) with an internal Ag/AgCl reference electrode (Fisher Scientific) filled with 4 M KCl solution and connected to a pH meter (Accumet® Portable, AP63 pH/mV/ion meter, Fisher Scientific) following the method described by Bolduc, Bazinet, Lessard, Chapuzet, and Vuillemard (2006). Zobell's solution (Ricca Chemical Company, Arlington, TX, USA), with a standard Eh of 228 mV (at 25 ºC) against platinum electrode (filled with 4M KCl with AgCl), was used to verify the electrode potential prior to each measurement.

The measured Eh values were converted to terms of standard hydrogen electrode by adding 200 mV to the observed values (Nordstrom & Wilde, 2005).

4.2.7 Syneresis

Syneresis was measured as described by Amatayakul, Sherkat, and Shah (2006). A cup of yogurt was weighed and maintained at an angle of 45 º for 2 h at 5 ºC. The whey was removed from the surface with a syringe, and the yogurt cup was re-weighed. Syneresis was reported in terms of the percentage of whey lost. Syneresis was calculated using the following formula:

$$Syneresis\ (\%) = (Whey\ Lost / Sample\ Weight) \times 100$$

4.2.8 Water holding capacity (WHC)

Water holding capacity was measured as described by Parnell-Clunies, Kakuda, Mullen, Arnott, and deMan (1986). Yogurt directly fermented in a 50 mL centrifuge tube (Oak Ridge Centrifuge Tubes) was weighed and centrifuged (Marathon 21000R, Fisher Scientific) at 13,500× g and 10 ºC for 30 min. Separated supernatant was drained, and the pellet was weighed. Water holding capacity was reported as the percentage of pellet weight. Water holding capacity was calculated using the following formula:

$$WHC\ (\%) = (Pellet\ Weight / Sample\ Weight) \times 100$$

4.2.9 Firmness

Yogurt firmness was measured as described by Salvador and Fiszman (2004) with some modifications. Firmness was measured at 5 ºC with a TA.XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK) at 2 mm s^{-1} speed and 10 mm penetration with a 2.54 cm diameter probe. Firmness was measured in g force as the force at breaking (i.e. the first significant discontinuity in the curve obtained from the texture analyzer).

4.2.10 Total solids

Total solids were determined as described by Hooi et al. (2004) with some modifications. Approximately 3 g of yogurt sample were placed in the pre-weighed, pre-dried aluminum pan (Fisher Scientific), and transferred to an atmospheric oven (Isotemp Oven, Fisher Scientific) at 100 ºC for 5 h. Samples were cooled in a desiccator before final weights were recorded.

$$Total\ Solids\ (\%) = (Sample\ weight\ after\ drying / Sample\ weight\ before\ drying) \times 100$$

4.2.11 Peptone solution (0.1%) preparation

One g of peptone (Bacto, Becton Dickinson and Company, Sparks, MD, USA) was dissolved per liter of distilled-deionized water, 9 mL transferred to test tube, sterilized at 121 ºC and 15 psi for 15 min, and used for making serial dilutions for microbial plating.

4.2.12 Streptococcus thermophilus counts

S. thermophilus counts were determined as described by Dave and Shah (1996) with some modifications. Yogurt samples were serially diluted using sterilized 0.1% peptone (Bacto) water, pour plated using *S. thermophilus* isolation agar (Fluka, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and incubated (Blue M, Dry Type Bacteriological Incubator, Blue Island, IL, USA) aerobically at 37 ºC for 48 h. *S. thermophilus* colonies were confirmed using Gram staining and Rapid ID 32 STREP system (bioMérieux, Inc., Durham, NC, USA).

4.2.13 Lactobacillus delbrueckii ssp. bulgaricus counts

L. bulgaricus counts were determined as described by Duncan, Yaun, Sumner, and Bruhn (2004) with some modifications. Yogurt samples were serially diluted using sterilized 0.1% peptone (Bacto) water, pour plated using MRS (de Man, Rogosa and Sharpe) agar (Oxoid, Basingstoke, Hampshire, England) at pH 5.4 ± 0.1 and incubated anaerobically with anaerobe gas packs (Mitsubishi Pack-Anaero, Fisher Scientific) at 37 ºC for 72 h. *L. bulgaricus* colonies were confirmed using Gram staining, and API® 50 CH system (kit of 50 biochemical tests for the identification of *Lactobacillus* and related genera) and API® CHL medium (bioMérieux, Inc.).

4.3. Results and discussion

4.3.1 Fermentation study

As no information was available in regards to the use of PE supplementation in yogurt, a fermentation study was done to determine the effect of PE on the yogurt fermentation process. Supplementing yogurt mixes with PE or Cys did not affect the initial pH and TA, as the pH and TA of yogurt mixes ranged from 6.45 to 6.50 and from 0.20 to 0.22%, respectively (Fig. 4.2A). However, the initial Eh of all supplemented yogurt mixes (ranged from 129 to 227 mV) was significantly less than that of the NS yogurt (258 mV; Fig. 4.2B). During preliminary work, the cysteine concentrations of 0.014 and 0.028% (w/w) provided a similar Eh reduction as 0.5 and

1% PE, respectively and these concentrations did not exceed the amounts that had a negative effect on viability of the starter bacteria (Dave & Shah, 1998; Dave & Shah, 1997b). However, in this study, supplementing cysteine at 0.014% decreased the yogurt mix Eh to a greater extent (~ 20%) compared with the 0.5% PE supplementation, resulting in a significantly lower Eh in $Cys_{0.014}$ yogurt mix (183 mV) compared with the $PE_{0.5}$ yogurt mix (227 mV; Fig. 4.2B).

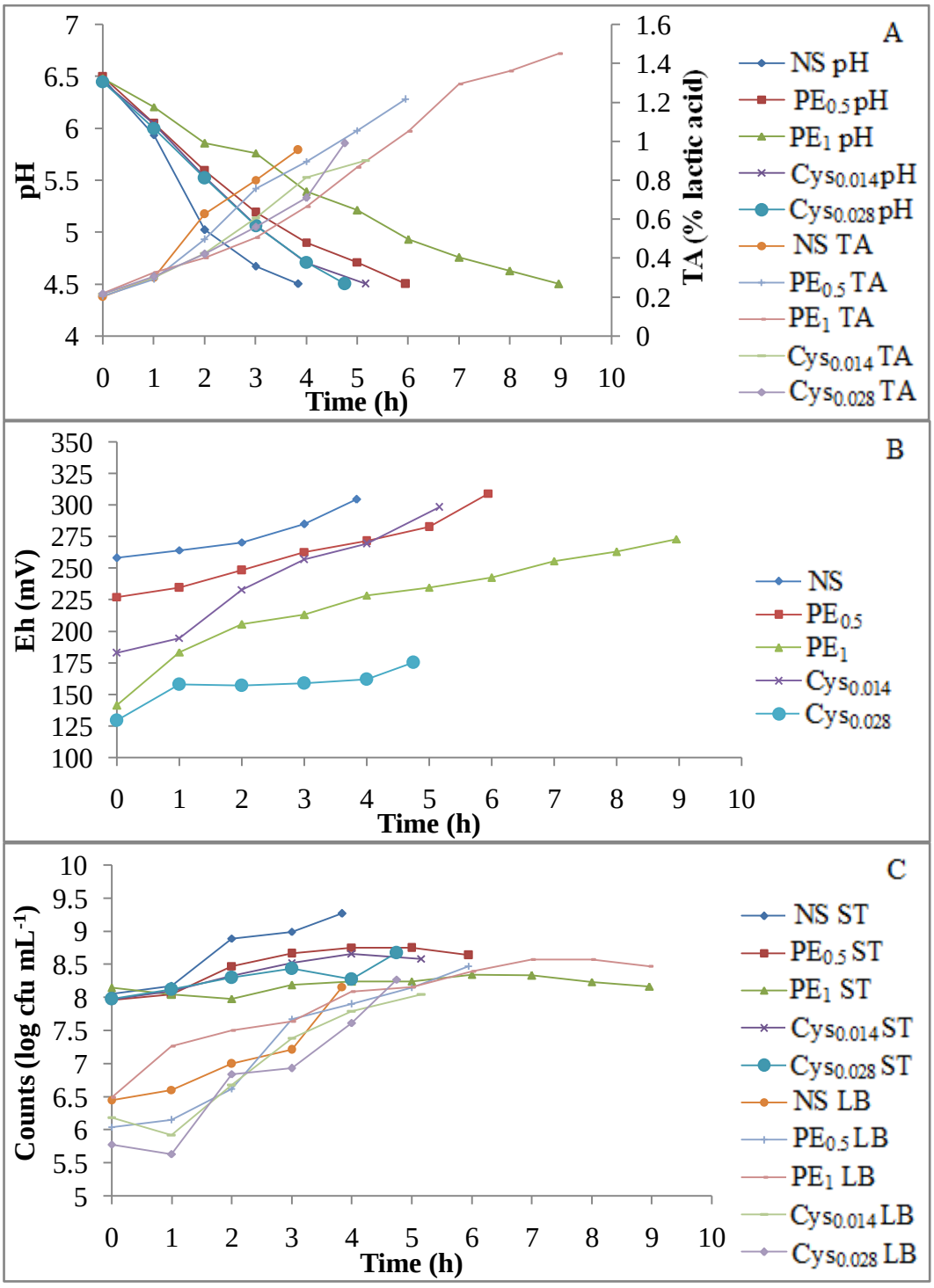

Figure 4.2 Change in characteristics of yogurt mixes during fermentation: (A) mean pH (n = 2) and mean titratable acidity (TA; n = 2); (B): mean redox potential (Eh; n = 2); (C): mean *L. bulgaricus* counts (LB; n = 2) and *S. thermophilus* counts (ST; n = 2). NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with

1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl.

Fermentation time (to pH 4.5) increased significantly to 5.95 h, 8.97 h, 5.17 h and 4.75 for $PE_{0.5}$, PE_1, $Cys_{0.014}$ and $Cys_{0.028}$ yogurts, respectively, compared with 3.83 h for NS yogurt. Substantial pH decrease and TA increase occurred during the first 2 h of fermentation for the NS yogurt compared with supplemented yogurts, which had substantial pH and TA change during the subsequent hours of fermentation (Fig. 4.2A). At the end of fermentation, the TA of PE-supplemented yogurts (1.22 and 1.45% for $PE_{0.5}$ and PE_1 yogurts, respectively) was significantly greater than NS (0.96%) and Cys-supplemented yogurts (0.90 and 0.99% for $Cys_{0.014}$ and $Cys_{0.028}$, respectively; Fig. 4.2A). Greater TA increase over the same pH change in the PE-supplemented yogurts (> 1.0%) compared with the NS and Cys-supplemented yogurts (< 0.8%) at the end of fermentation (Table 4.1) suggests that PE-supplemented yogurts had greater buffering capacities; possibly a function of the sodium acetate carrier, a known buffering agent (Lindsay, 2007), in the PE. Therefore, the longer fermentation times of PE-supplemented yogurts could be attributed to the increased buffering capacities, which in turn resisted the pH change as acid accumulated.

At the end of fermentation, the Eh of the NS, $Cys_{0.014}$ and $PE_{0.5}$ yogurts were similar (~ 304 mV) but greater than the PE_1 yogurt (273.2 mV) and $Cys_{0.028}$ yogurt (175.3 mV; Fig. 4.2B). Redox potential of all yogurts significantly increased during fermentation, but the increase was greatest for PE_1 yogurt (131.8 mV) and least for NS and $Cys_{0.028}$ yogurts (~ 46 mV; Table 4.1). The Eh of a food system depends upon the pH, microbial activity, packaging material, partial pressure of oxygen in the storage environment, and food composition (IFT, 2003). As redox potential is inversely related to pH (Morris, 2000), the increase in yogurt Eh during fermentation may be attributed to the decreased pH. Dave and Shah (1998) reported that milk mixes supplemented with 0, 50, 250, and 500 mg L^{-1} cysteine had initial Eh values of -70, -130, -180 and -217 mV, respectively. The lower Eh values reported by Dave and Shah (1998) compared to our Eh values might be attributed to differences in total solids (> 15.4% vs. 14.6%) of the yogurt mixes or heat treatments (85 ºC for 30 min vs. 90 ºC for 10 min). Redox potential decreases during milk pasteurization as results of whey proteins denaturation (which expose sulfhydryl groups) and dissolved oxygen expulsion (Chandan & O'Rell, 2006a; Dave & Shah, 1998;

Morris, 2000; Tamime & Robinson, 1999a; Walstra & Jenness, 1984). Therefore, the greater total milk solids in yogurt mix and longer heat exposure in the Dave & Shah (1998) study could have contributed to greater numbers of exposed sulfhydryl groups, less dissolved oxygen and the lower Eh values.

L. bulgaricus and *S. thermophilus* counts during fermentation are presented in Fig. 4.2C. *L. bulgaricus* counts in all yogurts increased from 1.71 to 2.49 log cfu mL^{-1} during fermentation (Table 4.1), but no significant differences were observed in *L. bulgaricus* counts between NS and supplemented yogurts at the end of fermentation. At the end of fermentation, the increase in *S. thermophilus* counts was 1.21 log cfu mL^{-1} in NS yogurt but < 0.7 log cfu mL^{-1} in supplemented yogurts (Table 4.1).

Table 4.1 Change in characteristics of yogurts[x] from the start to the end of fermentation

	NS	$PE_{0.5}$	PE_1	$Cys_{0.014}$	$Cys_{0.028}$
pH	1.98 ± 0.01^{a}	2.00 ± 0.00^{a}	1.98 ± 0.00^{a}	1.95 ± 0.02^{a}	1.95 ± 0.03^{a}
Eh[e] (mV)	46.4 ± 0.2^{d}	81.8 ± 1.0^{c}	131.8 ± 3.6^{a}	115.4 ± 1.4^{b}	45.8 ± 3.0^{d}
TA[f] (% lactic acid)	0.76 ± 0.02^{c}	1.01 ± 0.00^{b}	1.23 ± 0.00^{a}	0.68 ± 0.02^{d}	0.78 ± 0.00^{c}
L. bulgaricus (log cfu mL^{-1})	1.71 ± 0.23^{a}	2.44 ± 0.60^{a}	1.98 ± 0.26^{a}	1.86 ± 0.04^{a}	2.49 ± 0.30^{a}
S. thermophilus (log cfu mL^{-1})	1.21 ± 0.02^{a}	0.68 ± 0.24^{ab}	0.02 ± 0.01^{b}	0.62 ± 0.31^{ab}	0.70 ± 0.18^{a}

[a-d] Means (n = 2) ± SE with different superscripts for individual characteristic differ ($P \leq 0.05$)

[x] NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl

[e] Redox potential

[f] Titratable acidity

4.3.2 Shelf life study

4.3.2.1 Yogurt on day 1

Yogurt formulations were modified with supplements so as to create a more conducive environment to support the bacteria in yogurt; however, it was critical to ascertain that neither supplement changed the physicochemical characteristics of the yogurt. Thus, yogurts were analyzed on day 1 to determine effects of supplementation. To confirm consistency in yogurt making, total solids were analyzed. The total solids ranged from 14.61 to 14.74%, and no significant differences were observed (Table 4.2). On day 1, yogurt pH and TA were affected by supplementation ($P = 0.016$ for pH and $P < 0.0001$ for TA; Table 4.2). Yogurt pH ranged from 4.41 to 4.51. The pH of PE-supplemented yogurts (~ 4.50) was significantly greater than that of NS and Cys-supplemented yogurts (~ 4.42). Cys-supplemented yogurts had similar TA as the NS yogurt (1.04%), but TA of PE-supplemented yogurts was greater compared with NS yogurt and significantly increased as the concentration increased (1.27 and 1.59% for $PE_{0.5}$ and PE_1 yogurts, respectively).

Yogurt firmness on day 1 was measured to verify that a gel structure occurred in the supplemented yogurts. Yogurt firmness on day 1 was significantly affected by supplementation ($P < 0.0001$; Table 4.2). The $PE_{0.5}$ yogurt firmness was similar to the NS yogurt (147.2 g). The firmness of Cys-supplemented yogurts was significantly greater than that of NS yogurt and significantly increased as concentration increased (~ 20 and ~ 38% for $Cys_{0.014}$ and $Cys_{0.028}$, respectively), whereas the firmness of PE_1 yogurt was significantly less (~ 32%) than NS yogurt. The greater firmness of Cys-supplemented yogurts is probably a function of the greater number of disulfide linkages in the protein network that forms the gel (Damodaran, 2007). The lower firmness of PE_1 yogurt might be due to the greater proteolysis in PE_1 yogurt as a result of the longer fermentation time compared with that of other yogurts (Sodini, Montella & Tong, 2005).

On day 1, yogurt Eh ranged from 307.3 to 346.8 mV. The Eh of all yogurts was similar except the Eh of $Cys_{0.028}$ yogurt was less than NS, $Cys_{0.014}$ and $PE_{0.5}$ yogurts (Table 4.2). On day 1, *L. bulgaricus* counts in all yogurts were > 8 log cfu mL^{-1} and the counts in the PE_1 yogurt were greater than in the NS, $PE_{0.5}$ and $Cys_{0.014}$ yogurts (Table 4.2). *S. thermophilus* counts in PE_1, $Cys_{0.014}$ and $Cys_{0.028}$ yogurts were less compared with NS yogurt (9.27 log cfu mL^{-1}) but $PE_{0.5}$ yogurt had counts similar to the other yogurts (Table 4.2). Syneresis on day 1 was greater in PE_1

yogurt (8.61%) than in the other yogurts (≤ 6.11%). The greater syneresis of PE_1 yogurt may be explained by two factors. First, the greater TA of PE_1 yogurt may have induced greater gel contraction that expelled a greater amount of whey and hence the greater syneresis (Aryana et al., 2007; Rašić & Kurmann, 1978). Second, the longer fermentation time for the PE_1 yogurt could have resulted in greater proteolysis compared with the other yogurts and might have also contributed to the greater syneresis in PE_1 yogurt (Gassem & Frank, 1991). No differences were observed in WHC of various yogurts as a function of supplementation, as all yogurts had WHC ~ 20.35% (Table 4.2).

Table 4.2 Characteristics of yogurts[x] on day 1

Characteristics	NS	$PE_{0.5}$	PE_1	$Cys_{0.014}$	$Cys_{0.028}$
Total solids (%w/w)	14.64 ± 0.24^{a}	14.72 ± 0.26^{a}	14.66 ± 0.42^{a}	14.61 ± 0.21^{a}	14.74 ± 0.26^{a}
pH	4.43 ± 0.01^{b}	4.50 ± 0.02^{a}	4.51 ± 0.02^{a}	4.41 ± 0.01^{b}	4.42 ± 0.02^{b}
TA[e] (% lactic acid)	1.04 ± 0.03^{c}	1.27 ± 0.08^{b}	1.59 ± 0.06^{a}	1.01 ± 0.02^{c}	1.00 ± 0.03^{c}
Eh[f] (mV)	346.0 ± 5.2^{a}	346.8 ± 2.5^{a}	335.3 ± 6.2^{ab}	343.8 ± 13.9^{a}	307.3 ± 14.4^{b}
Firmness (g)	147.2 ± 3.6^{c}	137.2 ± 3.2^{c}	99.7 ± 1.9^{d}	175.9 ± 6.4^{b}	202.6 ± 9.8^{a}
Syneresis (%)	4.00 ± 0.51^{b}	5.34 ± 0.39^{b}	8.61 ± 0.99^{a}	5.44 ± 0.67^{b}	6.11 ± 0.78^{b}
WHC[g] (%)	19.58 ± 1.25^{a}	20.18 ± 0.38^{a}	20.09 ± 1.39^{a}	21.26 ± 0.84^{a}	20.64 ± 2.05^{a}
L. bulgaricus (log cfu mL^{-1})	8.31 ± 0.08^{b}	8.22 ± 0.13^{b}	8.64 ± 0.09^{a}	8.18 ± 0.09^{b}	8.48 ± 0.10^{ab}
S. thermophilus (log cfu mL^{-1})	9.27 ± 0.04^{a}	8.97 ± 0.13^{ab}	8.78 ± 0.18^{b}	8.91 ± 0.08^{b}	8.88 ± 0.09^{ab}

[a-d] Means (n = 3) ± SE with different superscripts within a row differ ($P \leq 0.05$)

[x] NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl

[e] Titratable acidity

[f] Redox potential

[g] Water holding capacity

4.3.2.2 Yogurts during storage

4.3.2.2.1 pH and titratable acidity

During storage, yogurt pH and TA were significantly affected by supplementation ($P < 0.0001$ for pH and TA) and storage ($P = 0.0002$ for pH and $P < 0.0001$ for TA), but the interactions between supplementation and storage were not significant. The pH of PE-supplemented yogurts (4.49) was significantly greater than NS and Cys-supplemented yogurts (~ 4.32; Fig. 4.3A). Overall, yogurt pH decreased from day 1 (4.46) to 8 (4.40) and then remained constant through day 50 (Fig. 4.3B). On day 50, the pH of NS and Cys-supplemented yogurts was ~ 4.30, whereas the pH of PE-supplemented yogurts was ~ 4.48. Titratable acidity of Cys-supplemented yogurts was similar to NS yogurt (1.11%), but TA of PE-supplemented yogurts was significantly greater and significantly increased as concentration increased (~ 18 and ~ 47% for $PE_{0.5}$ and PE_1 yogurts, respectively; Fig. 4.4A). Overall, the TA of yogurts increased significantly from day 1 (1.18%) to 8 (1.24%) and then remained constant until day 50 (Fig. 4.4B). On day 50, TA of NS and Cys-supplemented yogurts was ~ 1.10%, whereas the TA of $PE_{0.5}$ and PE_1 yogurts was 1.35 and 1.65%, respectively. The greater pH and TA of PE-supplemented yogurts were possibly due to the presence of sodium acetate in the PE.

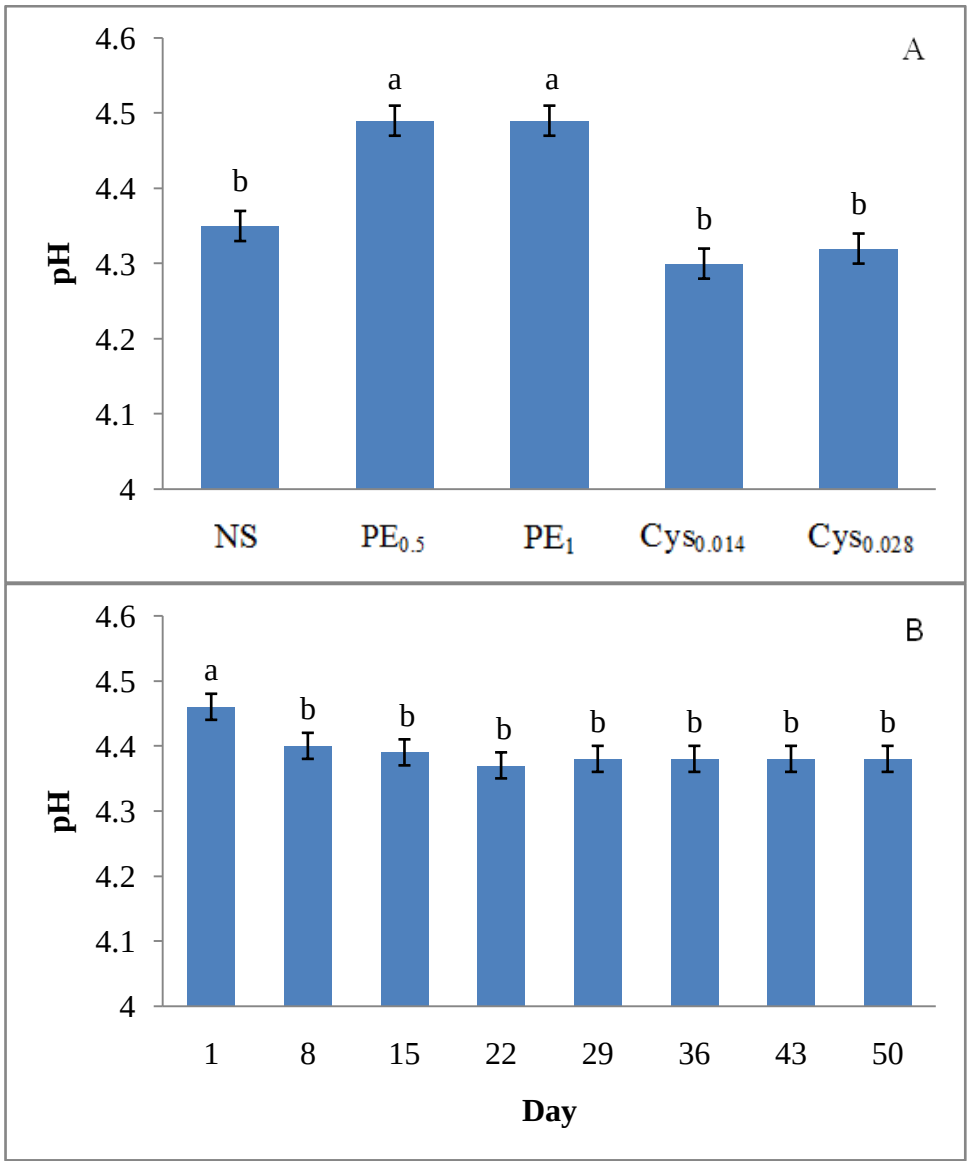

Figure 4.3 pH of yogurts as a function of supplementation (A) or storage (B): (A) means (n = 24) averaged for days with pooled SE (0.02); (B) means (n = 15) averaged for all yogurts with pooled SE (0.02). $^{a-b}$ Bars with different letters differ ($P \leq 0.05$). NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl.

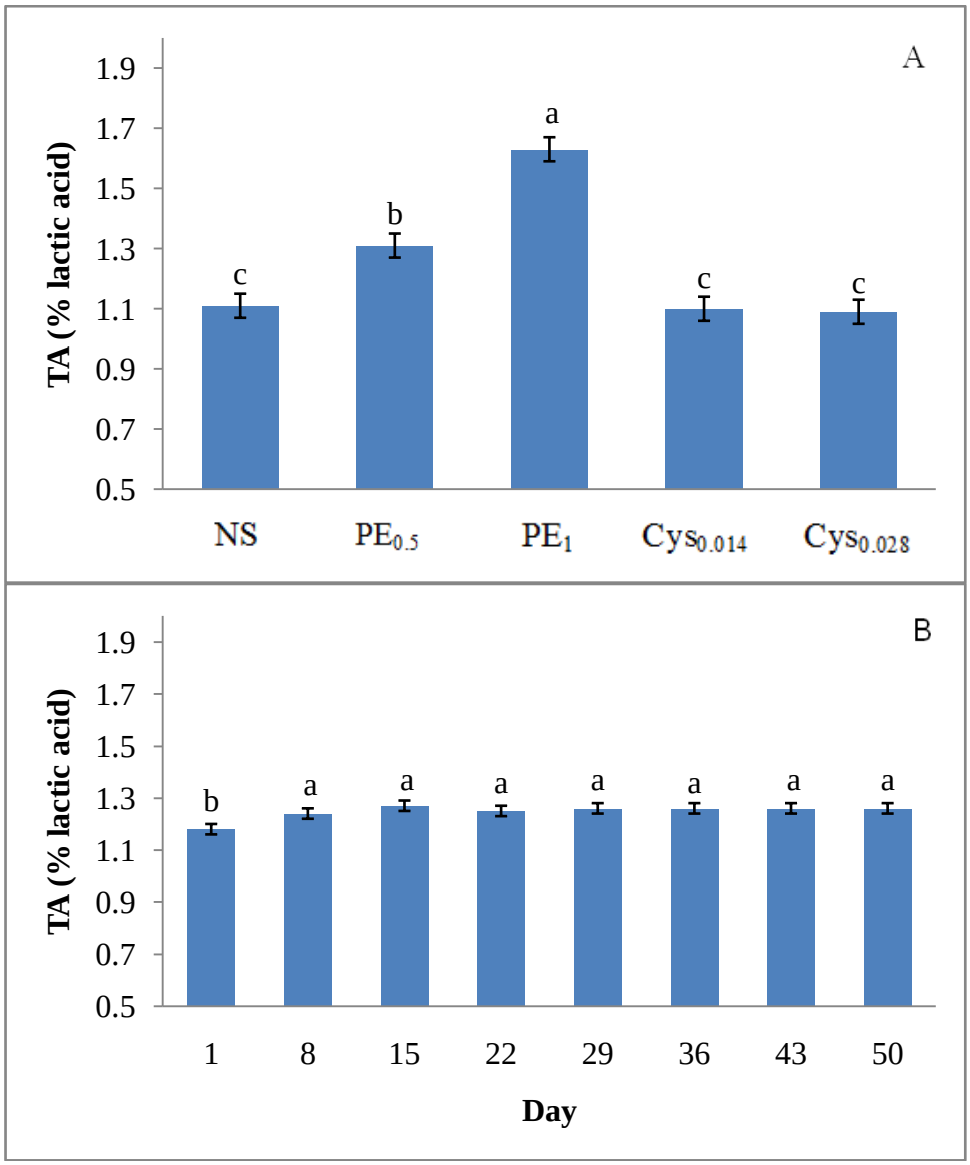

Figure 4.4 Titratable acidity (TA) of yogurts as a function of supplementation (A) or storage (B): (A) means (n = 24) averaged for days with pooled SE (0.04); (B) means (n = 15) averaged for all yogurts with pooled SE (0.02). $^{a-c}$ **Bars with different letters differ (*P* ≤ 0.05). NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl.**

4.3.2.2.2 Redox potential

Yogurt Eh was significantly affected by supplementation ($P = 0.0481$) and storage ($P < 0.0001$). Overall, $Cys_{0.014}$ and PE-supplemented yogurts had Eh similar to NS yogurt (~ 375 mV), whereas the Eh of $Cys_{0.028}$ yogurt was significantly less (~ 13%; Fig. 4.5A). The Eh of all yogurts increased significantly from day 1 (336 mV) to 8 (362 mV), from day 8 to 22 (375 mV) and then decreased from day 22 to 43 (360 mV; Fig. 4.5B). On day 50, the Eh of NS, $PE_{0.5}$ and PE_1 yogurts was ~ 372 mV; and the Eh for $Cys_{0.014}$ and $Cys_{0.028}$ yogurts was ~ 359 and 333 mV, respectively.

The Eh of fermented milks stored in plastic containers has been reported to be directly related to the increase in oxygen tension in yogurt due to oxygen permeability through the plastic containers during storage (Dave & Shah, 1998; Dave & Shah, 1997a; Dave & Shah, 1997c). Dave and Shah (1997c) reported that dissolved oxygen content of fermented milk stored in plastic containers was greater than fermented milk stored in glass containers during 35 days of storage. Redox potential of a growth medium has an inverse relationship with pH (Morris, 2000). Therefore, this increase in yogurt Eh from day 1 to 8 could be attributed to the decrease in pH over the same storage period and/or increase in oxygen tension due to air permeability through the plastic containers during storage.

Redox potential of raw milk has been reported to range between 200 and 300mV, and that of pasteurized milk is ~ 180 mV (Bolduc et al., 2006). Dave and Shah (1997b) reported that on day 0, the Eh of fermented milk supplemented with cysteine at 0, 50, 250 or 500 mg L^{-1} and fermented with *L. acidophilus*, bifidobacteria and yogurt bacteria ranged from 50 to 100 mV, -10 to -30 mV, -25 to -80 and -30 to -100 mV, respectively, and after 35 days of storage the Eh increased to 150 to 160, 110 to 120, 40 to 50 and 10 to 40 mV, respectively. Dave and Shah (1997a) reported similar results for fermented milk supplemented with 0, 50, 150 or 250 mg kg^{-1} ascorbic acid and fermented with *L. acidophilus*, bifidobacteria and yogurt bacteria during 35 days of storage. Dave and Shah (1997a, 1997b) reported that the Eh increased throughout the 35 days of storage; however, in our study no significant increase in Eh was observed after day 22 but rather Eh decreased from day 22 to 43. Redox potential of fermented milk during storage has been reported to be affected by the strains of bacteria used during fermentation (Dave & Shah, 1997a; Dave & Shah, 1997b); therefore, these differences in Eh trends during storage may be

attributed to the differences in the starter bacteria strains used and presence of probiotics in Dave and Shah (1997a, 1997b) studies, while in our study only yogurt starter bacteria was used.

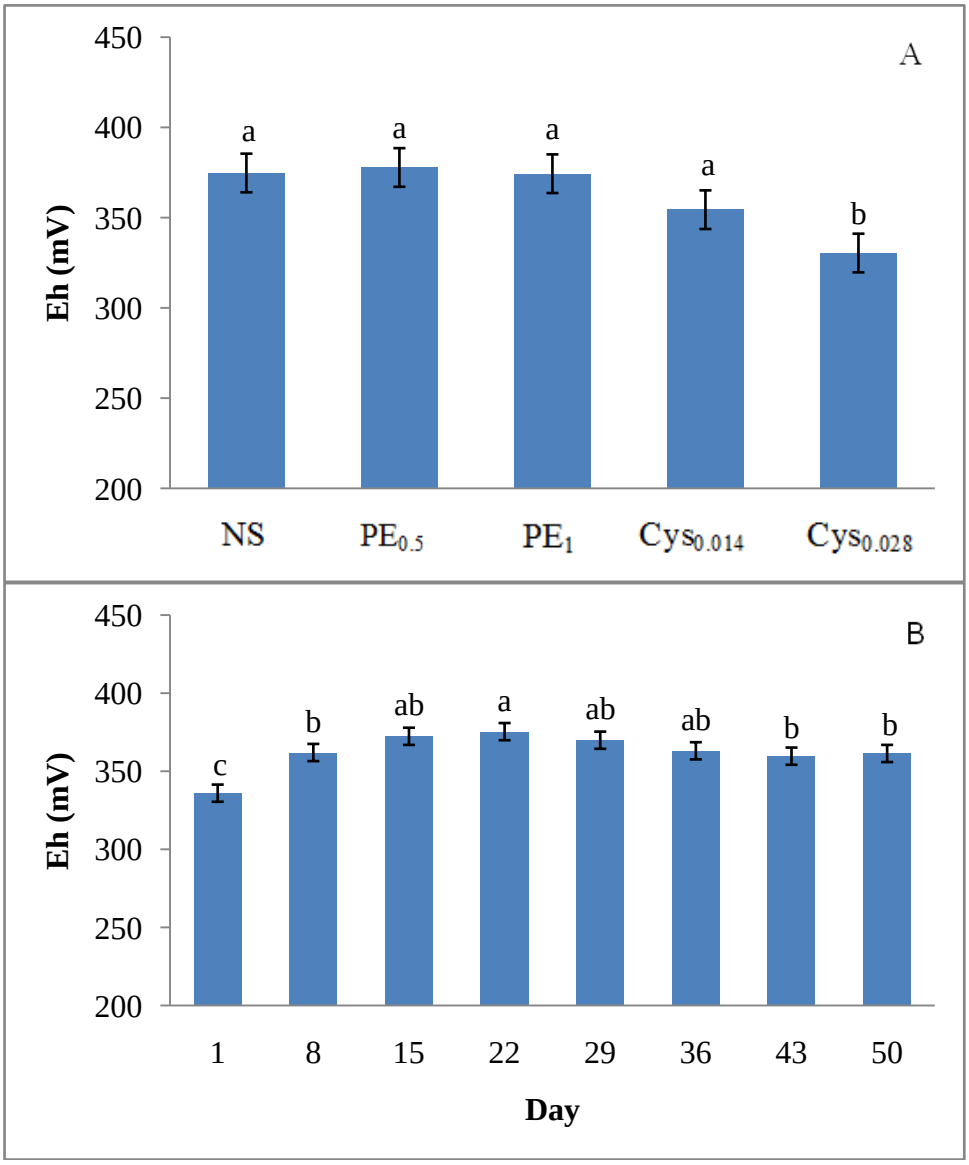

Figure 4.5 Redox potential (Eh) of yogurts as a function of supplementation (A) or storage (B): (A) means (n = 24) averaged for days with pooled SE (10.7); (B) means (n = 15) averaged for all yogurts with pooled SE (5.5). $^{a-c}$ Bars with different letters differ ($P \leq 0.05$). NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl.

4.3.2.2.3 Microbial counts

L. bulgaricus counts during storage are presented in Table 4.3. *L. bulgaricus* counts were affected by supplementation ($P = 0.0021$), storage ($P < 0.0001$) and the interaction between supplementation and storage ($P < 0.0001$). *L. bulgaricus* counts in all yogurts decreased significantly during storage. On day 36, *L. bulgaricus* counts in NS yogurt were < 3 log cfu mL^{-1}, whereas counts were > 3 log cfu mL^{-1} in all supplemented yogurts until day 43 (Table 4.3). *L. bulgaricus* counts in NS yogurt decreased below the recommended concentration of 6 log cfu mL^{-1} on day 15, whereas the counts in Cys-supplemented, PE_1 and $PE_{0.5}$ yogurts were > 6 log cfu mL^{-1} until day 15, 22 and 29, respectively. Therefore, supplementing yogurt with PE and Cys maintained *L. bulgaricus* counts > 6 log cfu mL^{-1} for a longer time compared with the NS yogurt. Dave and Shah (1997b) reported that the reduction in *L. bulgaricus* counts was less if fermented milk was supplemented with 50, 250 or 500 mg cysteine L^{-1} compared with the NS fermented milk during 35 days of storage. Bari et al. (2009) reported similar results for yogurt supplemented with 0.25, 0.5 or 0.75% cysteine and stored for 15 days. The improvement in the longevity of *L. bulgaricus* in PE-supplemented yogurts in our study cannot be attributed to the Eh alone because the NS and PE-supplemented yogurts had similar Eh throughout storage. Presence of some prebiotics or sodium acetate in PE could account for the improved *L. bulgaricus* viability in PE-supplemented yogurts.

S. thermophilus counts during 50 days of storage are presented in Table 4.3. A significant interaction existed between supplementation and storage ($P = 0.0009$). *S. thermophilus* counts decreased significantly in PE_1 yogurt by day 29 (~ 1 log cfu mL^{-1}); however, the counts in NS, $PE_{0.5}$ and Cys-supplemented yogurts on day 50 were similar (> 8 log cfu mL^{-1}), showing that addition of Cys (at 0.014 or 0.028%) or PE (at 0.5%) did not adversely affect the viability of *S. thermophilus*. As *S. thermophilus* is more sensitive to lactic acid accumulation during fermentation, the lesser *S. thermophilus* counts in PE_1 yogurt could be attributed to the greater TA of PE_1 yogurts (Chandan & O'Rell, 2006a; Lourens-Hattingh & Viljoen, 2001). *S. thermophilus* counts in all yogurts remained > 6 log cfu mL^{-1} for 50 days of storage. Dave and Shah (1997b, 1998) reported that addition of 50 mg L^{-1} Cys in fermented milk had no adverse affect on *S. thermophilus* counts but supplementing fermented milk with 250 mg Cys L^{-1} (0.025% w/v) or more decreased *S. thermophilus* counts. However, in our study Cys

supplementation at 0.028% did not affect *S. thermophilus* counts, probably due to the greater Eh of $Cys_{0.028}$ yogurt compared with < 50 mV in Dave and Shah (1997b, 1998) studies.

Table 4.3 *L. bulgaricus* and *S. thermophilus* counts of yogurts[x] during storage

Counts	Day of Storage	NS	$PE_{0.5}$	PE_1	$Cys_{0.014}$	$Cys_{0.028}$
L. bulgaricus	1	8.31^{abc}	8.22^{abc}	8.64^{a}	8.18^{abc}	8.48^{ab}
(log cfu mL^{-1})	8	7.19^{abcde}	8.00^{abcd}	8.31^{abc}	7.18^{abcdef}	7.80^{abcd}
	15	5.50^{fghijk}	7.72^{abcde}	8.16^{abcd}	$6.18^{defghij}$	7.44^{abcde}
	22	4.16^{jkl}	$6.66^{abcdefg}$	$6.53^{bcdefgh}$	5.14^{ghijkl}	$5.82^{efghijk}$
	29	3.11^{lm}	$6.30^{cdefghi}$	5.27^{fghijk}	4.30^{ijkl}	$5.16^{fghijkl}$
	36	$< 3.00^{m}$	4.92^{hijkl}	4.38^{ijkl}	3.64^{kl}	4.53^{hijkl}
	43	$< 3.00^{m}$	4.14^{jkl}	3.72^{kl}	3.93^{kl}	3.88^{klm}
	50	$< 3.00^{m}$	$< 3.00^{m}$	$< 3.00^{m}$	$< 3.00^{m}$	$< 3.00^{m}$
S. thermophilus	1	9.27^{a}	8.97^{ab}	8.78^{ab}	8.91^{ab}	8.88^{ab}
(log cfu mL^{-1})	8	9.16^{a}	8.90^{ab}	8.51^{abc}	9.02^{ab}	8.97^{ab}
	15	9.06^{ab}	8.91^{ab}	8.49^{abc}	9.07^{ab}	8.56^{abc}
	22	9.15^{a}	8.80^{ab}	8.16^{bcd}	8.93^{ab}	8.80^{ab}
	29	9.15^{a}	8.84^{ab}	7.73^{cde}	8.92^{ab}	8.86^{ab}
	36	9.09^{ab}	8.76^{ab}	7.37^{de}	8.55^{abc}	8.45^{abc}
	43	8.78^{ab}	8.59^{abc}	7.23^{e}	8.85^{ab}	8.61^{abc}
	50	8.64^{abc}	8.44^{abc}	7.23^{e}	8.56^{abc}	8.60^{abc}

[a-m] Means (n = 3) ± SE (0.37 for *L. bulgaricus* and 0.16 for *S. thermophilus*) with different superscripts for individual bacteria differ ($P \leq 0.05$)

[x] NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl

4.3.2.2.4 Syneresis

Syneresis of yogurts was significantly affected by supplementation ($P = 0.0005$) and storage ($P = 0.0045$). Syneresis in PE_1 yogurt (7.31%) was significantly greater than the other yogurts, in which syneresis ranged from 4.10 to 5.54% (Fig. 4.6A). The greater syneresis of PE_1 yogurt may be explained by the greater TA of PE_1 yogurt (Aryana et al., 2007; Rašić & Kurmann, 1978) and/or the greater proteolysis in PE_1 yogurt (Gassem & Frank, 1991) due to the longer fermentation time compared with the other yogurts. Overall, syneresis in all yogurts was significantly less on day 50 (4.93%) compared with day 1 (5.91%); however, syneresis fluctuated within the range of 4.67 to 5.34% from day 8 to 43 (Fig. 4.6B). Conflicting results have been reported in regards to yogurt syneresis during storage. Gassem and Frank (1991) reported that syneresis in yogurt manufactured from 9% NFDM during 15 days of storage increased from day 1 (~ 35%) to 8 (~ 50%) and then decreased to the original value on day 15. On the other hand, Salvador and Fiszman (2004) reported increased syneresis in skim milk yogurt from day 0 (~ 0.5%) to 49 (~ 1.8%). These differences in syneresis values in Gassem and Frank (1991) and Salvador and Fiszman (2004) compared with our values could be due to differences in the method used for the determination of syneresis and/or differences in total solids of the yogurts.

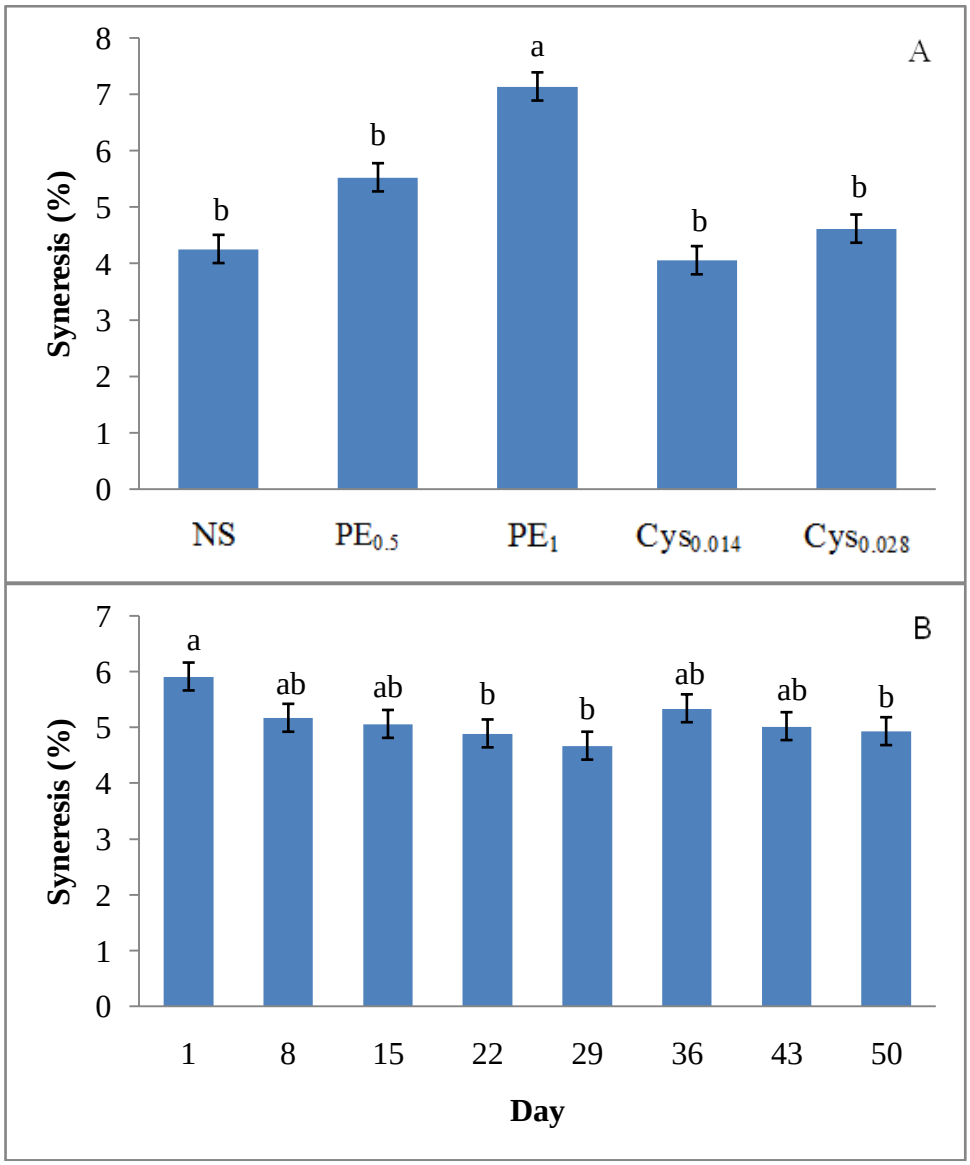

Figure 4.6 Syneresis of yogurts as a function of supplementation (A) or storage (B). (A) means (n = 24) averaged for days with pooled SE (0.34); (B) means (n = 15) averaged for all yogurts with pooled SE (0.25). $^{a-b}$ **Bars with different letters differ ($P \leq 0.05$). NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl.**

4.3.2.2.5 Water holding capacity

Water holding capacity was affected by supplementation ($P = 0.0323$) and storage ($P < 0.0001$). Water holding capacity of $Cys_{0.028}$ yogurt (23.31%) was greater than $PE_{0.5}$ yogurt (21.12%); however, all supplemented yogurts had similar WHC compared with NS yogurt (21.75%; Fig. 4.7A). Overall, WHC increased significantly from day 8 (20.66%) to15 (23.25%) and remained constant thereafter, but WHC on days 22, 43 and 50 was similar to WHC on day 1 and 8 (Fig. 4.7B). Parnell-Clunies et al. (1986) reported that WHC of yogurt prepared from pasteurized milk (85 ºC for 10 min) decreased on day 42 (30.88%) compared with day 1 (27.51%). In contrast, Gassem and Frank (1991) reported that WHC of yogurt manufactured with 9% NFDM decreased from day 1 (~ 14%) to 8 (~ 13%) and then increased to the initial value on day 15. Perhaps the differences in WHC patterns in these studies are a function of the differences in the total solids contents, especially the protein contents.

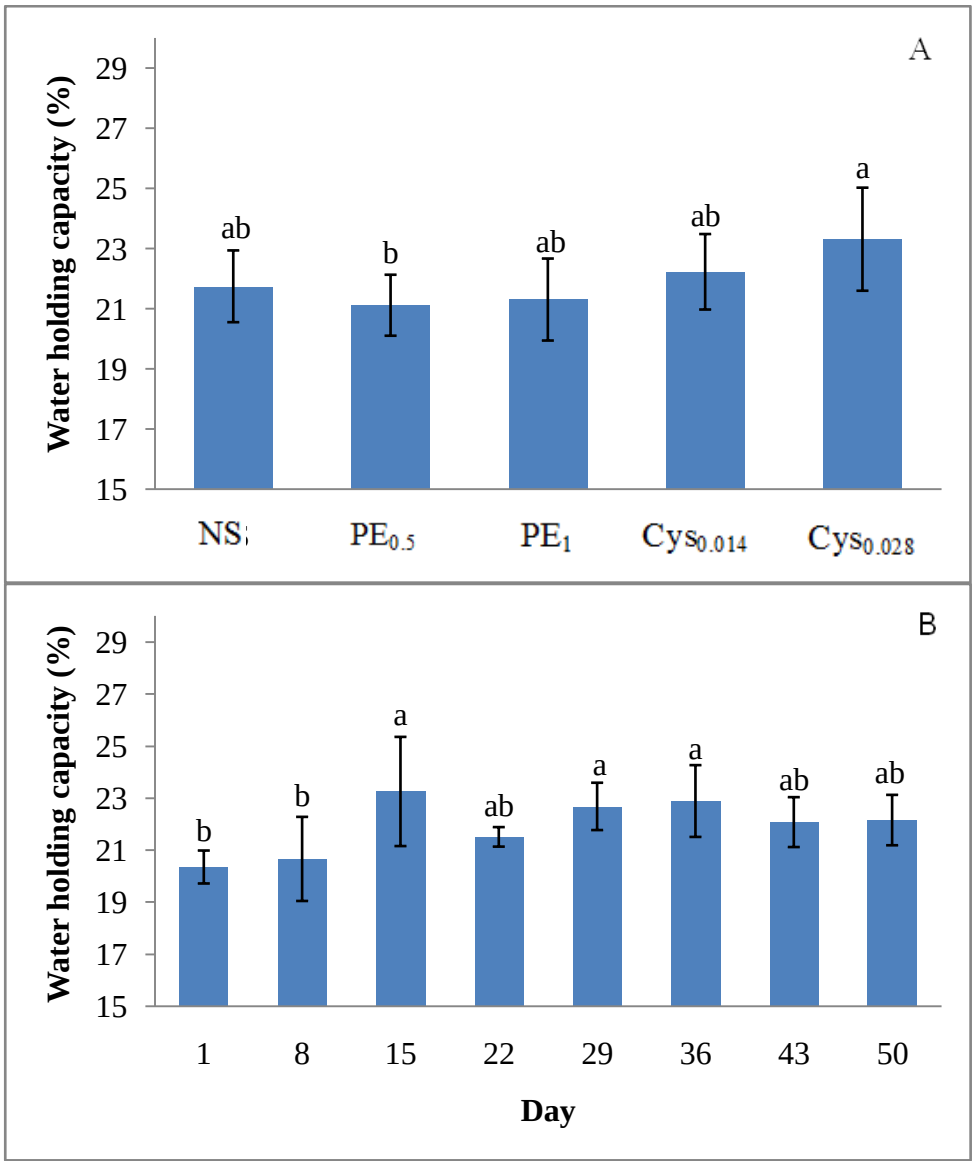

Figure 4.7 Water holding capacity (WHC) of yogurts as a function of supplementation (A) or storage (B): (A) means (n = 24) averaged for days with pooled SE (0.43); (B) means (n = 15) averaged for all yogurts with pooled SE (0.46). $^{a-b}$ **Bars with different letters differ (*P* ≤ 0.05). NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl.**

4.4 Conclusions

Supplementing yogurt with 0.5% PE, 1% PE or Cys maintained the viability of *L. bulgaricus* at > 6 log cfu mL^{-1} for an additional 21, 14 and 7 days, respectively compared to non-supplemented yogurt during storage at 5 °C. Supplementing yogurt with 1% PE significantly increased TA and syneresis compared with the NS yogurt, whereas yogurt supplemented with 0.5% PE had greater TA but similar physicochemical properties and *S. thermophilus* counts compared with NS yogurt. Although Cys-supplemented yogurts had similar pH, TA and *S. thermophilus* counts compared with the NS yogurt, the firmness of Cys-supplemented yogurts was significantly greater and the Eh of $Cys_{0.028}$ yogurt was significantly less compared with the NS yogurt. Because of its lower price, PE supplementation at 0.5% could to be a more economical method of supplementing yogurt to improve the viability of *L. bulgaricus* while having a minimal effect on other physicochemical yogurt properties.

The NS and PE-supplemented yogurts demonstrated similar Eh during storage; therefore the improvement in the longevity of *L. bulgaricus* cannot be attributed to Eh alone. Further research should be conducted to study the exact reason behind the ability of PE to improve the longevity of *L. bulgaricus* (e.g., sodium acetate or possible presence of prebiotics such as inulin or fructooligosaccharides). The effect of this plant extract on the viability of yogurt bacteria along with probiotics in yogurt should also be studied.

4.5 Acknowledgement

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4.6 Summary

The effect of a plant extract (prepared from olive, garlic, onion, citrus and uses sodium acetate as a carrier) on the viability of yogurt starter cultures was studied. Nonfat yogurt was prepared with various levels of supplements: plant extract (0, 0.5 or 1.0% w/v) or L-cysteine.HCl (0.014 or 0.028% w/w). Microbial and physicochemical analyses were done weekly for 50 days. Fermentation time increased for supplemented yogurts compared with the non-supplemented

yogurt. *Lactobacillus bulgaricus* counts in supplemented yogurts were > 6 log cfu mL^{-1} for a longer time (7 to 21 days) compared with the non-supplemented yogurt. *Streptococcus thermophilus* counts in all yogurts were > 6 log cfu mL^{-1} throughout the storage. Overall, redox potential and titratable acidity increased during storage, but pH and syneresis decreased. Plant extract at 0.5% enhanced *L. bulgaricus* viability in non-fat yogurt while least affecting physicochemical characteristics.

4.7 Determination of presence of inulin/fructooligosaccharides in the plant extract

4.7.1 Introduction

It was concluded from experiment-I that Eh was not the factor responsible for the observed improvement in the longevity of *Lactobacillus bulgaricus* during 50 days of storage at 5 °C. According to Cognis (Nutrition & Health, Monheim, Germany) plant extract (PE) is extracted from garlic and onion, and contains ~ 50% sodium acetate as a carrier; therefore presence of some prebiotics and/or sodium acetate in the PE was thought to be the factor responsible for the prolonged viability of *L. bulgaricus*.

Onion and garlic are natural sources of prebiotics such as inulin and fructooligosaccharides, FOS (Chow, 2002; Frank & De Leenheer, 2002; Niness; 1999). Recently, a number of studies have been done to improve the viability of yogurt starter and probiotic bacteria by supplementing yogurt mixes with inulin or FOS. Inulin and FOS are used as fat replacers in reduced calorie food products (Akalin et al., 2007; Niness, 1999) and provide health benefits associated with dietary fibers (Niness, 1999). Akalin et al. (2007) reported greater *S. thermophilus* (8.47 log cfu g^{-1}) and *L. bulgaricus* (5.39 log cfu g^{-1}) counts in 1.5% FOS supplemented reduced fat yogurt on day 28 compared with the counts (8.08 and 4.65 log cfu g^{-1}, respectively) in the non-supplemented yogurt; however, *B. animalis* counts were similar in 1.5% supplemented (8.70 log cfu g^{-1}) and non-supplemented (8.62 log cfu g^{-1}) yogurts. Oliveira (2009) reported significantly greater *L. bulgaricus* counts (~ 8.25 log cfu mL^{-1}) in yogurt supplemented with 0.04 g g^{-1} inulin on day 1 compared with the counts (~ 7.80 log cfu mL^{-1}) in non-supplemented yogurt, but no significant differences were observed in *S. thermophilus* counts (~ 9.1 log cfu mL^{-1}). They further reported that at the end of storage (day 7), *L. bulgaricus* counts (~

0.25 log cfu mL^{-1}) were greater in supplemented yogurt compared with the counts (8.00 log cfu mL^{-1}) in non-supplemented yogurt, but this difference was not significant.

In this experiment, PE was analyzed for the presence of inulin and FOS. It was hypothesized that if inulin and/or FOS are present in the plant extract at significant levels, the improvement of *L. bulgaricus* viability in experiment -I could be attributed to the presence of inulin and/or FOS in the PE and results from this experiment could also be used in designing the experimental design for experiment-II.

4.7.2 Materials and methods

Inulin and FOS content in the PE was determined by the enzymatic, spectrophotometric method as described by Steegmans et al. (2004). The principle of this method was that inulin/FOS were dissolved and extracted with boiling water. One part of this solution was hydrolyzed with sucrase (Megazyme, Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland) and the other part was hydrolyzed with fructanase (Megazyme). These solutions were then analyzed to determine fructose using an enzymatic, spectrophotometer kit (ENZYTEC™ fluid D-Glucose/D-Fructose). Fructose (F_{if}) produced from inulin/FOS was obtained by subtracting fructose (F_i) present initially in the sample and produced from sucrose (F_s), from the total fructose (F_t).

$$F_{if} = F_t - F_i - F_s$$

Inulin/FOS content in the original sample is calculated by the following formula:

Inulin/fructooligosaccharides (g /g of sample) $= F_{if} \times 0.995$

4.7.2.1 Sample preparation and extraction

Plant extract (2 g) or inulin standard (0.5 g; Orafit® HP, Beneo-Orafti Inc., Morris Plains, NJ, USA) was weighed in 100 mL beaker; 40 mL boiling distilled-deionized water was added and mixed well with stirring rod. pH was adjusted to 6.5 to 8.0 if necessary (using 0.05N KOH (FisherChemical, Fisher Scientific) or 0.05N HCl (FisherChemical, Fisher Scientific)). The solution was transferred to 100 mL volumetric flask by the rinsing beaker with boiling distilled-deionized water and incubated in a water bath (Isotemp 220, Fisher Scientific) at 85 ºC for 15 min. It was cooled to room temperature (25 ºC) and diluted with distilled-deionized water up to the mark. This solution was filtered through 0.2 µm membrane filter (Fisherbrand®) before using.

4.7.2.2 Sucrose hydrolysis with sucrase (Assay A_1)

Inulin standard or PE solution (250µL) was pipetted into a cuvette; 1.4 mL phosphate buffer and 50µL sucrase (Megazyme) solution (100 units mL^{-1}) were added. The cuvette was covered with seal and parafilm, and homogenized gently using a vortex (Vortex Genie 2™, Fisherbrand®). The cuvette was incubated in a water bath (Isotemp, Fisher Scientific) at 40 ºC for 30 min with mild agitation and cooled to 25 ºC.

4.7.2.3 Inulin/FOS hydrolysis with fructanase (Assay A_2)

The working inulin standard or PE solution (0.5 mL) was pipetted into a cuvette; 1.4 mL acetate buffer and 0.1 mL fructanase (Megazyme) solution (2000 units mL^{-1}) was added. The cuvette was covered with seal and parafilm and homogenized gently using a vortex (Vortex Genie 2™). The cuvette was incubated in a water bath (Isotemp 202, Fisher Scientific) at 60 ºC for 60 min with mild agitation and cooled to 25 ºC.

7.4.2.4 Enzymatic, spectrophotometric measurement using ENZYTEC™ fluid D-glucose/D-fructose kit

Following the procedure described by Steegmans et al. (2004) and the ENZYTEC™ fluid D-glucose/D-fructose kit absorbance of Assay A_1 and A_2 was measured at 340 nm, and inulin and FOS concentration in PE was calculated.

4.7.3 Results and discussion

The detection limit for the method used for the quantitative analysis of inulin and FOS was 1% in any food matrix (Steegmans et al., 2004). The determined inulin/ FOS content of the PE was much lower than the detection limit (0.042 ± 0.026%). The method was also verified using a standard inulin (Orafti® HP). Inulin content of the standard was determined as 93.82 ± 2.4%.

Such low levels of inulin and FOS in the PE eliminates the possibility of an inulin or FOS role in the improvement of the viability of *L. bulgaricus* in PE supplemented yogurts in experiment-I. Since PE supplemented yogurt mixes in experiment-I contained 0.5 and 1.0%, the concentration of these low levels of inulin/FOS would have been further diluted and had no practical impact on the viability of yogurt starter cultures. Inulin/FOS supplementation of 2 to

5% has been reported to have any nutritional or functional benefits in the yogurt (Ramchandran & Shah, 2008).

4.7.4 Conclusions

Inulin/FOS concentrations were below the detection limits of the method used; therefore improvement in *L. bulgaricus* could not be attributed to the inulin or FOS in the PE. Sodium acetate has been reported to increase the growth yield of some probiotic bacteria, and because PE contains ~ 50% of sodium acetate, the possibility of sodium acetate in the PE being responsible for the improved viability of *L. bulgaricus* in PE supplemented yogurts in experiment-I should be studied.

CHAPTER 5 - Impact of a plant extract on the viability of starter and probiotic cultures in nonfat yogurt (experiment-II)

5.1 Introduction

According to the FAO/WHO, probiotics are defined as live microorganisms which when administered in adequate amounts confer health benefits on the host (Vasiljevic & Shah, 2008; Moriya, Fachin, Gândara & Viotto, 2006). The market for probiotic foods is growing rapidly and yogurt is one of the most popular media/vectors for delivering probiotics (Food Ingredient First, 2010; Stanton et al., 2001). Several health benefits are associated with the consumption of yogurt containing active starter and/or probiotic bacteria (McKinley, 2005; Sarkar, 2008; Strnad & Babus, 1997). As an example, regular consumption of yogurt has been reported to reduce *Helicobacter pylori* colonization and infection in humans (Sheu et al., 2006; Wang et al., 2004). Keiling, Schneider and Jahreis (2002) reported that regular consumption of probiotic yogurt containing *Lactobacillus acidophilus* 145 and *Bifidobacterium longum* 913 increased the HDL cholesterol concentration and decreased the LDL/HDL cholesterol ratio in humans. In other studies, regular consumption of probiotic yogurt containing *L. acidophilus* and *B. lactis* (Ataie-Jafari, Larijani, Majd & Tahbaz, 2009), and fermented milk containing *Streptococcus thermophilus* MUH34 and *L. acidophilus* L1 (Anderson & Gilliland, 1999) have been reported to lower serum cholesterol levels in humans. Larsson, Andersson, Johansson and Wolk (2008) reported that regular consumption of yogurt and sour cream lowered the risk of bladder cancer.

Although no general agreement has been made on the minimum concentration of live probiotic bacteria that should be present in a food product at the time of consumption (Donkor, Henriksson, Vasiljevic & Shah, 2006), the recommended concentration ranges from 6 to 8 log cfu g^{-1} (Ross, Desmond, Fitzgerald & Stanton, 2005; Vasiljevic & Shah, 2008). The viability of probiotic bacteria in yogurt often decreases below the recommended concentration during storage and this has been attributed to the low pH, high oxygen tension, increased redox potential (Eh) and/or increased hydrogen peroxide concentration (Dave & Shah, 1997a; Dave & Shah, 1997c; Donkor et al., 2006; Lourens-Hattingh & Viljoen, 2001; Sarkar, 2008; Vasiljevic, Kealy, & Mishra, 2007). Shah, Lankaputhra, Britz and Kyle (1995) reported that out of 5 commercial probiotic yogurt brands obtained directly from the manufacturers, *B. bifidum* counts in 3 yogurt brands were < 6 log cfu g^{-1} on day 0 and the counts in the other 2 yogurt brands decreased to < 6

log cfu g^{-1} by days 3 and 12. In the same probiotic yogurt brands *L. acidophilus* counts in 2 yogurt brands were < 6 log cfu g^{-1} on day 0 and the counts in the other 3 yogurt brands decreased to < 6 log cfu g^{-1} by day 33. Supplementing yogurt with antioxidants such as cysteine or ascorbic acid (Bari, Ashrafi, Alizade & Rofehgarineghad, 2009; Dave & Shah, 1998; Dave & Shah, 1997a; Dave & Shah, 1997b) and prebiotics such as inulin, fructooligosaccharides (FOS) or β-glucan (Akalin, Gönç, Ünal, & Fenderya, 2007; Aryana, Plauche & Nia, 2007; Vasiljevic et al., 2007) have been reported to improve the viability of starter and probiotic bacteria during storage.

Some probiotic strains, especially those of *Bifidobacterium* spp., are sensitive to low pH and their viability in yogurt decreases rapidly during storage (Lourens-Hattingh & Viljoen, 2001). Greater buffering ability in yogurt could reduce the lethal effect of the acidic environment on the starter and probiotic bacteria (Ainaz & Ehsani, 2008). Sodium acetate ($C_2H_3NaO_2$), the sodium salt of acetic acid, is a FDA approved buffering and flavoring agent in foods (Lindsay, 2007; Manju, Jose, Gopal, Ravishankar & Lalitha, 2007).

Although no study has been reported addressing the effect of sodium acetate on the viability of starter and probiotic bacteria in yogurt, researchers have reported that the growth yield and acid production ability of some lactic acid bacteria is enhanced if grown in media supplemented with sodium acetate. Lino, Manome, Okada, Uchimura and Komagata (2001) reported that out of 49 strains of lactic acid bacteria (23 strains of *Lactobacillus* spp., 5 strains of *Leuconostoc* spp., 3 strains of *Weissella* spp., 7 strains of *Pediococcus* spp., 3 strains of *Enterococcus* spp., 2 strains of *Lactococcus* spp., 4 strains of *Streptococcus* spp., *Sporolactobacillus inulinus* and *Bacillus coagulans*) grown individually in glucose yeast extract peptone (GYP) broth supplemented with 50 mM sodium acetate for 2 days, 32 strains produced 1.2 × more lactic acid compared with the non-supplemented broth, while the remaining 17 strains produced similar amounts of lactic acid. They further reported greater growth (measured as absorbance of GYP broth at 660 nm) of *L. sakei* NRIC 1077, *L. coryniformis* ssp. *coryniformis* NRIC 1638 and *L. plantarum* NRIC 1067 in 10, 20, 50 or 100 mM sodium acetate supplemented GYP broth compared with non-supplemented broth after 2 or 3 days of fermentation. These researchers proposed that the activation of L-lactate dehydrogenase and/or the strengthening of the glycolytic pathway or pentose cycle contributed to the greater lactic acid production in sodium acetate supplemented broth. Lino, Uchimura and Komagata (2002) reported that the growth yield (g dry bacteria per mol glucose) of *L. sakei* NRIC 1071^T and *L. plantarum* NRIC

1067^T grown in GYP broth supplemented with 50 mM sodium acetate for 24 h was 21.3 g and 19.9 g, respectively, compared with *L. sakei* NRIC 1071^T (13.6 g) and *L. plantarum* NRIC 1067^T (16.0 g) grown in the non-supplemented GYP broth. They further reported that *L. sakei* NRIC 1071^T and *L. plantarum* NRIC 1067^T produced ~ 2 to 2.5 × more lactic acid in sodium acetate supplemented GYP broth after 24 h fermentation than in the non-supplemented broth. The pH decrease in sodium acetate supplemented GYP broth after 24 h was from 6.8 to ~ 4.0 compared with the pH decrease from 6.8 to ~ 3.6 in the non-supplemented broth suggested that sodium acetate supplementation provided a good buffering ability to GYP broth (Lino et al., 2002).

Cegemett® Fresh (Cognis, Nutrition & Health, Monheim, Germany) is a plant extract (PE) prepared from an oleoresin mixture [olive, garlic, onion and citrus extract, with sodium acetate (~ 50%) as a carrier], and possesses antioxidant properties. Plant extract (~ \$10 kg^{-1}) is a less expensive supplement compared to cysteine, ascorbic acid or inulin (ranging from ~ \$90 to over \$1000 kg^{-1}; alfa.com, 2010; ajiaminoacids.com, 2010); therefore supplementing yogurt with PE may be a more economical option for improving the viability of starter and probiotic bacteria. Michael, Phebus and Schmidt (2010) reported that the Eh did not differ in PE-supplemented and non-supplemented yogurts but *L. bulgaricus* counts in yogurts supplemented with 0.5 and 1.0% PE were > 6 log cfu mL^{-1} for an additional 14 and 21 days, respectively, compared with the non-supplemented yogurt. Perhaps the enhanced buffering ability (instead of reduced Eh) of PE-supplemented yogurts was responsible for the improved *L. bulgaricus* viability. Therefore, the objective of this study was to investigate the effect of PE supplementation on the buffering capacity of yogurt mix, and on the viability of yogurt starter (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) and probiotic (*Bifidobacterium animalis* ssp. *animalis* and *Lactobacillus acidophilus*) bacteria in nonfat yogurt stored for 50 days at 5 ºC.

5.2 Materials and methods

5.2.1 Experimental design

Yogurt mixes were formulated with 0.5 % (w/v) plant extract (PE; Cegemett® Fresh) supplementation, 0.25 % (w/v) sodium acetate (SA; Fisher Biotech, Fisher Scientific, Fair Lawn, NJ, USA) supplementation or without supplementation. Because PE contains 50 % sodium acetate, SA supplementation (0.25 %) was used as a comparison treatment. Each yogurt mix formulation was fermented with starter cultures and *B. animalis* (BA), *L. acidophilus* (LA) or

both probiotics (*B. animalis* and *L. acidophilus*; P). Abbreviations used for various yogurts in the study are described in Table 5.1.

Table 5.1 Abbreviations used for various yogurts

	Formula		
Culture	Non -supplemented (NS)	Plant extract (PE) supplemented	Sodium acetate (SA) supplemented
B. animalis (BA)	NS-BA	PE-BA	SA-BA
L. acidophilus (LA)	NS-LA	PE-LA	SA-LA
B. animalis and *L. acidophilus* (P)	NS-P	PE-P	SA-P

For the fermentation study, yogurts were fermented in a bioreactor (Bioflo 3000, New Brunswick Scientific Co. Inc., Edison, NJ, USA) to determine the fermentation time as well as to track the changes in microbial counts, Eh, pH and titratable acidity (TA) during fermentation. These analyses were done at 1 h intervals during the fermentation period. Prior to the fermentation, buffering curves for NS, PE and SA yogurt mixes were generated. Two replications were conducted with each test done in duplicate and the average was used for statistical analysis. For the shelf life study, yogurts were manufactured and stored for 50 days at 5 ºC. Yogurts were analyzed on the day after fermentation (day 1), and weekly thereafter. Firmness and total solids were determined only on day 1. Three replications were conducted with each test done in duplicate and the average was used for statistical analysis.

A 3 (formula) × 3 (culture) factorial, randomized complete block design (RCBD) with fixed blocks (replications) was used for statistical analysis of the fermentation study and day 1 yogurts. Whereas, a repeated measure analysis in a 3×3 factorial, RCBD with fixed blocks (replications) was used for statistical analysis of the shelf life study. Analysis of variance (ANOVA) and least-square means at $\alpha = 0.05$ were used to differentiate the means of the significant main effects and interactions. All analyses were performed using the procedures for "PROC MIXED" of Statistical Analysis System (SAS®) version 9.1 (SAS® Institute Inc, Cary, NC, USA).

5.2.2 Yogurt starter and probiotic cultures propagation

Nonfat dry milk (NFDM; low heat, spray processed, Grade A, Dairy America™, Fresno, CA, USA) was rehydrated at 140 g L^{-1} in distilled-deionized water, sterilized at 121 ºC and 105 kPa for 15 min, and cooled to 37 ºC. Sterilized, reconstituted NFDM was inoculated with 1% (w/w) freeze-dried yogurt cultures (Yo-Mix™ Yogurt Cultures, Yo-Mix 161 LYO 375 DCU, Danisco, New Century, KS, USA), incubated (Isotemp Incubator, Fisher Scientific) at 37 ºC for 18 h, and maintained at 5 ºC (Equatherm® Incubator, Lab-Line Instruments, Inc, Melrose Park, IL, USA) until it was used to culture the yogurt (within 48 h). *S. thermophilus* was confirmed using Gram staining and Rapid ID 32 STREP system (bioMérieux, Inc., Durham, NC, USA); and *L. bulgaricus* was confirmed using Gram staining, API® 50 CH system (bioMérieux, Inc.) and API® CHL medium (bioMérieux, Inc.).

Bifidobacterium animalis ssp. *animalis* ATCC 25527 culture (American Type Culture Collection, Manassas, VA, USA) was propagated initially according to supplier's instructions. Nonfat dry milk was rehydrated at 140 g L^{-1} in distilled-deionized water, supplemented with 10 g L^{-1} glucose (Fisher Scientific) and 10 g L^{-1} yeast extract (Acros Organic, Fisher Scientific), sterilized at 121 ºC and 105 kPa for 15 min, and cooled to 37 ºC. Sterilized, reconstituted NFDM (90 mL) was supplemented with 10 mL 0.5% L-cysteine.HCl (Fisher Biotech, Fisher Scientific) solution, inoculated with 3% (w/w) *B. animalis* culture, incubated at 37 ºC for 18 h, and maintained at 5 ºC until it was used to culture the yogurt (within 48 h). *B. animalis* was confirmed using Gram staining and API® 20 A system (bioMérieux, Inc.)

Lactobacillus acidophilus ATCC 4356 culture (Microbiologics®, St. Cloud, MN, USA) was propagated initially according to supplier's instructions. Nonfat dry milk was rehydrated at 140 g L^{-1} in distilled-deionized water, supplemented with 10 g L^{-1} glucose and 10 g L^{-1} yeast extract, sterilized at 121 ºC and 105 kPa for 15 min, and cooled to 37 ºC. Sterilized, reconstituted NFDM was inoculated with 3% (w/w) *L. acidophilus* culture, incubated at 37 ºC for 18 h, and maintained at 5 ºC until it was used to culture the yogurt (within 48 h). *L. acidophilus* was confirmed using Gram staining and API® 20 A system.

5.2.3 Yogurt preparation

Yogurt mix was prepared by dissolving 140 g NFDM and 40 g sucrose (Pure Cane Sugar, Domino Foods, Inc., Yonkers, NY, USA) per liter of distilled-deionized water, supplemented

with PE or SA (Fig. 5.1). The mix was pasteurized at 90 ºC for 10 min, cooled to 37 ºC, inoculated with 3% (w/w) respective cultures (Fig. 5.1), transferred to sterile plastic (polypropylene) cups (Fisherbrand 118 mL, Fisher Scientific, Pittsburg, PA, USA), and incubated at 37 ºC until pH 4.50. Yogurt samples were then stored at 5 ºC until testing.

5.2.4 pH

pH was measured with a pH meter (Thermo Scientific Orion 2 Star Benchtop, Thermo Fisher Scientific Inc., Beverly, MA, USA) that was calibrated with standardized pH buffer solutions 4.0 and 7.0 (Fisher Scientific) prior to the analysis.

5.2.5 Titratable acidity (TA)

Titratable acidity (expressed as % lactic acid) was measured as described by Chandan and O'Rell (2006c). Sample (9 mL) was pipetted into a 100 mL titration flask and the pipette was rinsed using ~ 18 mL distilled-deionized water, and titrated against 0.1 N sodium hydroxide (NaOH; Fisher Scientific) using 0.5 mL phenolphthalein (Fisher Scientific) as an indicator. Titratable acidity was calculated using the following formula:

$$TA\ (\%\ lactic\ acid) = mL\ of\ 0.1\ N\ NaOH\ used \times 0.1$$

5.2.6 Redox potential (Eh)

Redox potential was measured at 25 ± 2^{o}C with a platinum electrode (Platinum Combination Electrode, Fisher Scientific) with an internal Ag/AgCl reference electrode (Fisher Scientific) filled with 4 M KCl solution and connected to a pH meter (Accumet® Portable, AP63 pH/mV/ion meter, Fisher Scientific) following the method described by Bolduc, Bazinet, Lessard, Chapuzet and Vuillemard (2006). Zobell's solution (Ricca Chemical Company, Arlington, TX, USA), with a standard Eh of 228 mV (at 25 ºC) against platinum electrode (filled with 4M KCl with AgCl), was used to verify the electrode potential prior to each measurement. The measured Eh values were converted to terms of standard hydrogen electrode by adding 200 mV to the observed values (Nordstrom & Wilde, 2005).

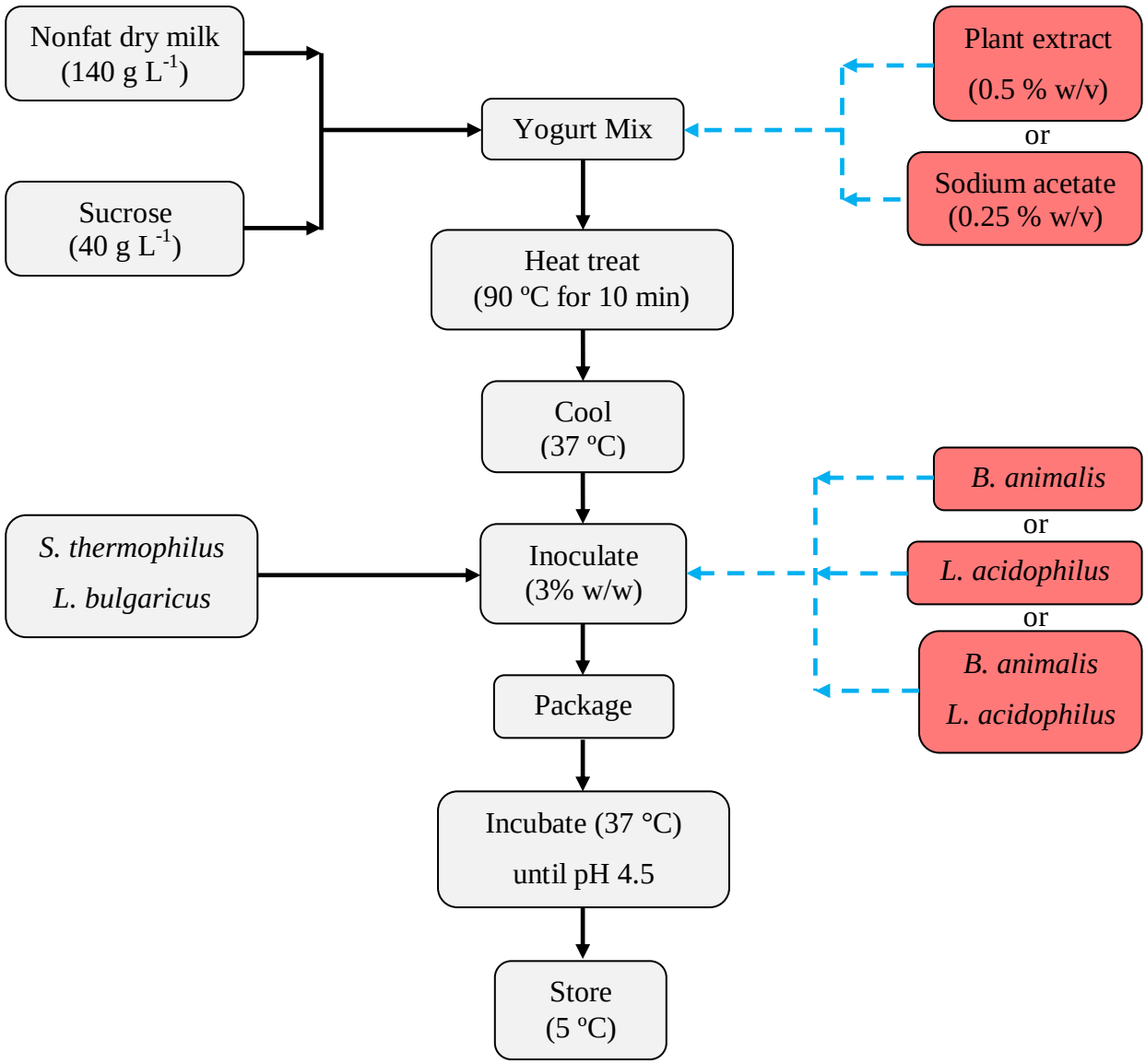

Figure 5.1 Schematic of yogurt manufacture

5.2.7 Syneresis

Syneresis was measured as described by Amatayakul, Sherkat and Shah (2006). A cup of yogurt was weighed and maintained at an angle of 45 ^{o} for 2 h at 5 ºC. The whey was removed from the surface using a syringe and the yogurt cup was re-weighed. Syneresis was reported in terms of percent of whey lost. Syneresis was calculated using the following formula:

$$Syneresis\ (\%) = (Whey\ Lost\ /\ Sample\ Weight) \times 100$$

5.2.8 Buffering capacity/curves

Buffering capacity was measured at 25 ºC as described by Salaün, Mietton and Gaucheron (2007) with some modifications. Acid titration was performed on 10 mL yogurt mix from initial pH to 4.00 using 1N hydrochloric acid (HCl; Fisher Scientific) added in 0.05 mL increments at 30 sec intervals. Buffering capacities were calculated using the formula described by Van Slyke (1922), and plotted against the corresponding pH values to generate buffering curves. The buffering curves of NS, PE or SA yogurt mixes from all replications were plotted, and the curves best presenting the average of all replications for NS, PE or SA yogurt mix were selected and used for interpretation. The following formula was used for calculating buffering capacity:

$$\textit{Buffering capacity } (\beta) = |dB / dpH|$$

where, dB = mL of acid added / mL of sample

dpH = pH after adding acid - pH before adding acid

5.2.9 Firmness

Yogurt firmness was measured as described by Salvador and Fiszman (2004) with some modifications. Firmness was measured at 5 ºC with a TA.XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK) at 2 mm s^{-1} speed and 10 mm penetration with a 2.54 cm diameter probe. Firmness was measured in g force as the force at breaking (i.e. the first significant discontinuity in the curve obtained from the texture analyzer).

5.2.10 Total solids

Total solids were determined as described by Hooi et al. (2004) with some modifications. Approximately 3 g of yogurt sample was weighed in the pre-weighed, pre-dried aluminum pan (Fisher Scientific), and placed in an atmospheric oven (Isotemp Oven, Fisher Scientific) at 100 ºC for 5 h. Samples were cooled in a desiccator before final weights were recorded. The following formula was used for calculating total solids:

$$\textit{Total Solids (\%)} = (\textit{Sample weight after drying} / \textit{Sample weight before drying}) \times 100$$

5.2.11 Streptococcus thermophilus counts

S. thermophilus counts were determined as described by Dave and Shah (1996) with some modifications. Yogurt samples were serially diluted using sterilized 0.1% peptone (Bacto,

Becton Dickinson and Company, Sparks, MD, USA) water (9 mL), pour plated using *S. thermophilus* isolation agar (Fluka, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) prepared according to manufacturer's directions and incubated (Blue M, Dry Type Bacteriological Incubator, Blue Island, IL, USA) aerobically at 37 ºC for 48 h. *S. thermophilus* colonies were confirmed using Gram staining and Rapid ID 32 STREP system.

5.2.12 Lactobacillus delbrueckii ssp. *bulgaricus* counts

L. bulgaricus counts were enumerated as described by Duncan, Yaun, Summer and Bruhn (2004) with some modifications. Yogurt samples were serially diluted using sterilized 0.1 % peptone water, pour plated using MRS (de Man, Rogosa and Sharpe) agar (Oxoid, Basingstoke, Hampshire, England) prepared according to manufacturer's directions and adjusted to pH 5.4 ± 0.1, and incubated anaerobically using anaerobe gas packs at 37 °C for 72 hours. *L. bulgaricus* colonies were confirmed using Gram staining, and API® 50 CH system and API® CHL medium.

5.2.13 Bifidobacterium animalis ssp. *animalis* counts

B. animalis counts were enumerated as described by Moriya, Fachin, Gândara and Viotto (2006) with some modifications. MRS agar was prepared and tempered to 45 °C in a water bath. A supplement solution consisting of L-cysteine.HCl (0.5 g), nalidixic acid (15 mg), neomycin sulfate (100 mg), lithium chloride (3 g) and paromomycin sulphate (200 mg) dissolved in 40 mL distilled-deionized water was prepared (all chemicals obtained from Fisher Scientific). The solution was filter-sterilized through a 0.42 µ pore membrane (Fisher Scientific) and 4 mL of solution was mixed with 96 mL of the tempered agar just before plating. Yogurt samples were serially diluted using sterilized 0.1 % peptone water, pour plated using supplemented MRS agar and incubated anaerobically using anaerobe gas packs at 37 ºC for 72 hours. *B. animalis* colonies were confirmed using Gram staining and API® 20 A system.

5.2.14 Lactobacillus acidophilus counts

L. acidophilus counts were enumerated as described by Dave and Shah (1996) with some modifications. Sterilized and tempered MRS agar (90 mL) was supplemented with filter-sterilized D-sorbitol solution (10 mL), prepared by dissolving 10 g D-sorbitol (Fisher Scientific) in 100 mL of distilled-deionized water, just before plating. Yogurt samples were serially diluted

using sterilized 0.1 % peptone water, pour plated using supplemented MRS agar and incubated anaerobically using anaerobe gas packs at 37 ºC for 72 hours. *L. acidophilus* colonies were confirmed using Gram staining and API® 20 A system.

5.3 Results and discussion

5.3.1 Buffering capacity/curves

Buffering curves for NS and supplemented yogurt mixes are presented in Fig. 5.2. Overall, buffering capacities of the PE and SA yogurt mixes were greater compared with the NS yogurt mix at pH < 6; however, buffering capacity of the PE yogurt mix was greater than the SA yogurt mix. Non-supplemented and SA yogurt mixes had maximum buffering capacity (exhibited as peaks; 0.050 and 0.071, respectively) at pH 4.83 and 4.73, respectively. Buffering compounds exhibit maximum buffering capacity at the pH equal to their pKa (Van Slyke, 1922) and the pKa value for sodium acetate is 4.76 (Ruzin, 1999); therefore buffering action of SA yogurt mixes could be attributed to the presence of sodium acetate. Plant extract supplemented yogurt had two buffering capacity peaks (0.083); one at pH 4.83 and the other at pH 4.61. The maximum buffering capacity of PE yogurt mix was also greater than SA yogurt mix. These results suggest the presence of additional component(s) other than sodium acetate in the plant extract that resulted in the increased buffering capacity. Maximum buffering capacity of raw milk during acid titration has been reported to occur at ~ pH 5.1 (Lucey, Hauth, Gorry & Fox, 1993b); whereas milk that has been heat-treated at 90 ºC for 10 min had maximum buffering capacity at ~ pH 5.0 (Lucey, Gorry & Fox, 1993a). This difference between the pH for maximum buffering capacity in the Lucey et al. (1993a) study and NS yogurt mix in this study could be attributed to differences in the total solids. Gastaldi et al. (1997) reported an increase in buffering capacity in reconstituted skim milk when total solids increased from 10% (~ 0.038) to 15% (~ 0.062) or 20% (~ 0.085). They also reported the shift of pH for maximum buffering capacity from ~ pH 5.0 for 10% total solids reconstituted skim milk to ~ pH 4.8 for 15 or 20% total solids.

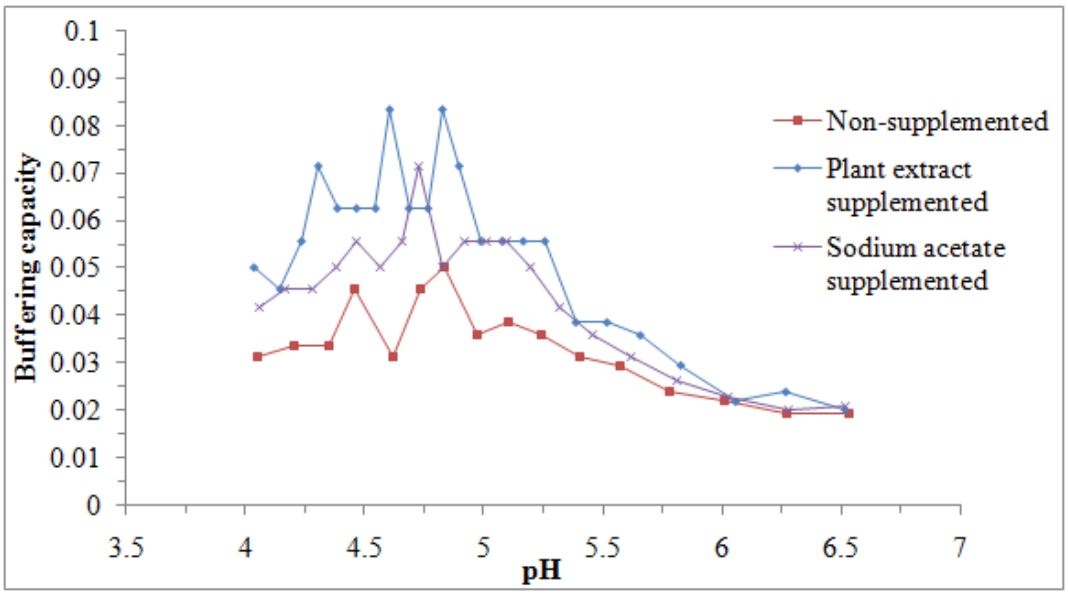

Figure 5.2 Buffering curves of yogurt mixes.

5.3.2 Fermentation study

Supplementing yogurt mixes with PE or SA did not affect the initial yogurt pH and TA, as yogurt mixes prior to the fermentation had similar pH [~ 6.49; Appendix D (Fig. D.1A)] and TA [0.20%; Appendix D (Fig. D.1B)]. However, the Eh of supplemented yogurt mixes (~ 230 mV) was less compared with NS yogurt mixes [~ 278 mV; Appendix D (Fig. D.1C)].

Yogurt fermentation time (to pH 4.50) was significantly affected by formula and culture (Table 5.2). Plant extract yogurts had the longest average fermentation time (8.17 h) followed by SA yogurts (6.90 h) and then NS yogurts (5.80 h; Table 5.3). Longer fermentation times of supplemented yogurts compared with NS yogurts could be attributed to the greater buffering capacities of supplemented yogurt mixes which would have resisted the pH decrease as acid was produced during fermentation. Yogurts fermented with LA-culture had the longest average fermentation time (7.49 h) followed by yogurts fermented with P-culture (7.04 h), whereas yogurts fermented with BA-culture had the shortest average fermentation time (6.35 h; Table 5.3).

The increase in TA of yogurts during fermentation was significantly affected by formula and culture (Table 5.2). The increase in TA was greater in PE (1.10 %) and SA (0.96 %) yogurts compared with the increase in NS yogurts (0.78 %; Table 5.3). The greater acid production in PE yogurts compared with that in SA yogurts over the same pH change confirms that the plant

extract had greater buffering capacity than sodium acetate; therefore, other component(s) besides sodium acetate in the plant extract contributed in greater buffering capacity of PE yogurt mixes. Yogurts fermented with BA- or P-culture had greater increase in TA (0.95 and 0.97 %, respectively) compared with the increase in yogurts fermented with LA-culture (0.91 %; Table 5.3). The pH decrease during fermentation was slower in supplemented yogurt mixes compared with NS yogurts (Appendix D; Fig. D.1A), whereas the TA increase was similar in all yogurt mixes except in PE-LA yogurt mix which had a slower increase in TA (Appendix D; Fig. D.1B). Titratable acidity of supplemented yogurts at the end of fermentation ranged from 1.09 to 1.34% compared with ~ 0.98% for NS yogurts (Appendix D; Fig. D.1B).

The increase in Eh of yogurts during fermentation was significantly affected by formula (Table 5.2). The increase in Eh in supplemented yogurts (~ 89 mV) was greater compared with the increase in NS yogurts (37.77 mV; Table 5.3). The increase in Eh of supplemented yogurt mixes was rapid during the first hour but attained a steady increase thereafter, and at the end of fermentation all yogurts attained similar Eh [~ 318 mV; Appendix D (Fig. D.1C)].

All yogurt mixes were inoculated with similar concentrations (~ 7 log cfu mL^{-1}) of respective bacteria at the start of fermentation. Increases in *S. thermophilus* and *L. bulgaricus* counts during fermentation were significantly affected by formula but not by culture (Table 5.2). During fermentation, *S. thermophilus* and *L. bulgaricus* counts increased > 1 log cfu mL^{-1} in all yogurts, and the final counts ranged from 8.2 and 9.0 log cfu mL^{-1} (Appendix D; Fig. D.2A, D.2B). Increases in *S. thermophilus* and *L. bulgaricus* counts were greater in PE and SA yogurts (ranged from 1.49 and 1.63 log cfu mL^{-1}) compared with NS yogurts (~ 1.2 log cfu mL^{-1}; Table 5.3).

Table 5.2 *P*-values (α = 0.05) of main effects (formula and culture) and interaction for fermentation time, and change in titratable acidity (TA), redox potential (Eh) and microbial counts of yogurts from the start to the end of fermentation

Effect	Fermentation time	TA	Eh	*S. thermophilus*	*L. bulgaricus*	*B. animalis*	*L. acidophilus*
Formula	< 0.0001	< 0.0001	< 0.0001	0.0038	0.0499	0.0664	0.5244
Culture	< 0.0001	0.0114	0.7740	0.1451	0.1477	0.2454	0.3682
Formula × Culture	0.1832	0.1362	0.9745	0.2020	0.1680	0.1002	0.1882

Table 5.3 Fermentation time, and change in titratable acidity (TA), redox potential (Eh) *S. thermophilus* and *L. bulgaricus* counts of yogurts[x] from the start to the end of fermentation as a function of formula or culture

	Formula			Culture			
Characteristics	NS	PE	SA	BA	LA	P	Pooled SE
Fermentation Time (h)	5.80[c]	8.17[a]	6.90[b]	6.35[C]	7.49[A]	7.04[B]	0.11
TA (% lactic acid)	0.78[c]	1.10[a]	0.96[b]	0.97[A]	0.91[B]	0.95[A]	0.02
Eh (mV)	37.77[b]	89.93[a]	88.18[a]				5.70
S. thermophilus (log cfu mL^{-1})	1.24[b]	1.58[a]	1.63[a]				0.09
L. bulgaricus (log cfu mL^{-1})	1.21[b]	1.51[a]	1.49[a]				0.11

[a-c] Means (n = 6, averaged for formula) with different lower case superscripts within a row (formula) differ ($P \leq 0.05$)

[A-C] Means (n = 6, averaged for culture) with different upper case superscripts within a row (culture) differ ($P \leq 0.05$)

[x] NS, non-supplemented yogurts; PE, plant extract supplemented yogurts; SA, sodium acetate supplemented yogurts; BA, yogurts fermented with *B. animalis*; LA, yogurts fermented *L. acidophilus*; P, yogurts fermented with both probiotics (*B. animalis* and *L. acidophilus*)

Increases in *B. animalis* and *L. acidophilus* counts during fermentation were neither affected by formula nor by culture (Table 5.2). *B. animalis* had less growth and the increase in *B. animalis* counts in yogurts was < 1 log cfu mL^{-1} with the final counts ~ 7.5 log cfu mL^{-1} (Appendix D; Fig. D.3A). Less growth of *B. animalis* in yogurts fermented with BA-culture allowed yogurt starter bacteria to grow fast, hence resulting in shorter fermentation times. Final *L. acidophilus* counts in yogurts were ~ 8.5 log cfu mL^{-1} and the counts increased > 1 log cfu mL^{-1} during fermentation (Appendix D; Fig. D.3B). *L. acidophilus* grew well in yogurts, and greater fermentation times for yogurts fermented with LA-culture could be attributed to the increased competition for nutrients.

5.3.3 Yogurts on day 1

Statistical analysis indicated that the total solids and firmness of the yogurts did not differ by formula or culture on day 1 (Table 5.4). It was expected that total solids of supplemented yogurts would be greater than that of NS yogurts but supplementation levels did not affect the yogurt total solids, as total solids of all yogurts were similar and ranged from 14.43 to 14.73 % w/w (Appendix C; Table C.23). Firmness on day 1 was measured to assure gel formation. All yogurts had similar firmness, ranging from 123.3 to 145.4 g (Appendix C; Table C.24); indicating that neither formula nor culture affected the initial gel structure.

On day 1, yogurt pH was significantly affected by formula × culture (Table 5.4). The pH of yogurts fermented with BA-culture was greater in PE and SA yogurts (4.45) compared with that of NS yogurt (4.35), and the pH of yogurt fermented with LA-culture was greater in PE yogurt (4.49) compared with that of NS yogurt (4.39); however, no significant differences in pH were observed in the yogurts fermented with P-culture (Table 5.5). Yogurt pH was not affected by the culture in NS and SA yogurts, but the pH of PE yogurt fermented with LA-culture (4.49) was greater than that of PE yogurt fermented with P-culture (4.42; Table 5.5). Titratable acidity on day 1 was significantly affected by formula and culture (Table 5.4). Titratable acidity of PE and SA yogurts (1.43 and 1.32 %, respectively) was greater compared with that of NS yogurts (1.16 %); whereas yogurts fermented with P-culture had the greatest TA (1.34 %) and yogurts fermented with LA-culture had the least TA (1.27 %; Table 5.6). Redox potential of all yogurts on day 1 was similar [~ 330 mV (Appendix C; Table C.17)] and did not differ by formula or culture (Table 5.4).

On day 1, yogurt syneresis was significantly affected by formula × culture (Table 5.4). As presented in Table 5.5, syneresis of yogurts fermented with BA- or P-culture did not differ; however, yogurt fermented with LA-culture had greater syneresis in PE yogurt (3.68 %) compared with that in NS and SA yogurts (2.23 and 2.56 %, respectively). Syneresis in NS yogurts was similar (ranged from 2.23 to 2.88 %); however, PE yogurt fermented with LA-culture had greater syneresis (3.68 %) than PE yogurt fermented with P-culture (2.80 %), but SA yogurt fermented with LA-culture had less syneresis (2.56 %) than SA yogurt fermented with P-culture (3.39 %; Table 5.5).

On day 1, only *L. bulgaricus* counts were significantly affected by formula (Table 5.4). *L. bulgaricus* counts in PE and SA yogurts (8.65 and 8.72 log cfu mL^{-1}, respectively) were greater compared with NS yogurts (8.37 log cfu mL^{-1}; Table 5.6). *S. thermophilus* and *L. acidophilus* counts in yogurts ranged from 8.3 to 8.8 log cfu mL^{-1} (Appendix C; Table C.19, C.22), and *B. animalis* counts in yogurts ranged from 6.3 to 7.3 log cfu mL^{-1} (Appendix C; Table C.21).

Table 5.4 *P*-values (α = 0.05) of main effects (formula and culture) and interaction for total solids, firmness, pH, titratable acidity (TA), redox potential (Eh), syneresis and microbial counts of yogurts on day 1

Effect	Total solids	Firmness	pH	TA	Eh	Syneresis	*S. thermophilus*	*L. bulgaricus*	*B. animalis*	*L. acidophilus*
Formula	0.1350	0.1008	0.0006	< 0.0001	0.0955	0.0245	0.2895	0.0337	0.1652	0.1016
Culture	0.0523	0.3234	0.6495	0.0381	0.5203	0.3210	0.1322	0.3431	0.3830	0.6681
Formula × Culture	0.2061	0.3252	0.0322	0.5463	0.1666	0.0371	0.8542	0.5877	0.0522	0.8521

Table 5.5 pH and syneresis of yogurts[x] on day 1 as a function of formula × culture

	pH			Syneresis (%)		
	Formula			Formula		
Culture	NS	PE	SA	NS	PE	SA
BA	4.35^{e}	4.45^{abc}	4.45^{abc}	2.44^{cd}	3.03^{abc}	2.61^{bcd}
LA	4.39^{de}	4.49^{a}	4.40^{cde}	2.23^{d}	3.68^{a}	2.56^{cd}
P	4.40^{cde}	4.42^{bcd}	4.42^{bcd}	2.88^{bcd}	2.80^{bcd}	3.39^{ab}

[a-e] Means (n = 3, averaged for formula and culture; with pooled SE of 0.02 for pH and 0.37 for syneresis) with different lower case superscripts within each interaction differ ($P \leq 0.05$)

[x] NS, non-supplemented yogurts; PE, plant extract supplemented yogurts; SA, sodium acetate supplemented yogurts; BA, yogurts fermented with *B. animalis*; LA, yogurts fermented with *L. acidophilus*; P, yogurts fermented with both probiotics (*B. animalis* and *L. acidophilus*)

Table 5.6 Titratable acidity (TA) and *L. bulgaricus* counts of yogurts[x] on day 1 as a function of formula or culture

	Formula			Culture			
Characteristics	NS	PE	SA	BA	LA	P	Pooled SE
TA (% lactic acid)	1.16^{c}	1.43^{a}	1.32^{b}	1.30^{AB}	1.27^{B}	1.34^{A}	0.03
L. bulgaricus (log cfu mL^{-1})	8.37^{b}	8.65^{a}	8.72^{a}				0.13

[a-c] Means (n = 9, averaged for formula) with different lower case superscripts within a row (formula) differ ($P \leq 0.05$)

[A-C] Means (n = 9, averaged for culture) with different upper case superscript within a row (culture) differ ($P \leq 0.05$)

[x] NS, non-supplemented yogurts; PE, plant extract supplemented yogurts; SA, sodium acetate supplemented yogurts; BA, yogurts fermented with *B. animalis*; LA, yogurts fermented with *L. acidophilus*; P, yogurts fermented with both probiotics (*B. animalis* and *L. acidophilus*)

5.3.3 Shelf life study

5.3.3.1 pH and titratable acidity

Yogurt pH and TA were significantly affected by formula and storage (Table 5.7). The pH of PE and SA yogurts (4.36 and 4.33, respectively) was similar but greater than that of NS yogurts (4.25; Table 5.8). Overall, yogurt pH decreased from day 1 (4.42) to 15 (4.30) and then remained constant until day 50 (Table 5.9). Yogurts supplemented with plant extract had the greatest TA (1.50 %) followed by SA yogurts (1.38 %) and then NS yogurts (1.24 %; Table 5.8). Overall, TA increased from day 1 (1.30%) to 15 (1.37%) and remained constant; however, TA on day 15 was less than that on day 43 and 50 (Table 5.9). These results agree with Gassem and Frank (1991), who reported a decrease in pH and an increase in TA in nonfat yogurt during 15 days of storage. Akalin et al. (2007) also reported that the pH of reduced-fat and full fat yogurt fermented with *B. animalis* decreased from ~ 4.50 to ~ 4.30 and from ~ 4.49 to ~ 4.39, respectively, during 28 days of storage.

5.3.3.2 Redox potential

Yogurt Eh was significantly affected by storage (Table 5.7), as yogurt Eh increased from day 1 (328.8 mV) to 29 (381.4 mV) and remained constant until day 50 (Table 5.9). This increase in yogurt Eh could be related to the decrease in pH (from 4.42 to 4.30) during day 1 to 15 and/or the increase in oxygen tension due to air permeability through plastic storage containers (Dave & Shah, 1998; Dave & Shah, 1997a; Dave & Shah, 1997c; Morris, 2000). Dave and Shah (1997a) reported that the Eh of non-supplemented yogurt manufactured with *L. acidophilus* and bifidobacteria on day 0 was 129.8 mV and increased to 180 mV on day 35. In a different study, Dave and Shah (1997b) reported that the yogurt Eh during refrigerated (4 °C) storage was a function of the fermentation culture (different strains of yogurt starter and same strains of *L. acidophilus* and bifidobacteria). However, in this study no differences were noted among the yogurts fermented with the 3 different culture combinations.

Table 5.7 *P*-values (α = 0.05) of main effects (formula, culture and storage) and interactions for pH, titratable acidity (TA), redox potential (Eh), syneresis and microbial counts of yogurts

Effect	pH	TA	Eh	Syneresis	*S. thermophilus*	*L. bulgaricus*	*B. animalis*	*L. acidophilus*
Formula	< 0.0001	< 0.0001	0.2023	0.0003	0.1107	< 0.0001	0.0881	< 0.0001
Culture	0.8503	0.1824	0.1863	0.1089	0.7952	0.0002	0.7491	0.0074
Storage	< 0.0001	< 0.0001	< 0.0001	0.1747	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Formula × Culture	0.1365	0.5244	0.0927	0.1430	0.0734	0.0056	0.0580	0.1564
Formula × Storage	0.4418	0.9316	0.9012	0.7654	0.0537	< 0.0001	0.0400	0.0009
Culture × Storage	0.9459	0.3722	0.9419	0.6218	0.9593	< 0.0001	0.5048	0.0098
Formula × Culture × Storage	0.7814	0.6755	0.9874	0.2449	0.8426	0.4821	0.7275	0.0020

Table 5.8 pH, titratable acidity (TA) and syneresis of yogurts[x] as a function of formula

	Formula			
Characteristics	NS	PE	SA	Pooled SE
pH	4.25[b]	4.36[a]	4.33[a]	0.01
TA (% lactic acid)	1.24[c]	1.50[a]	1.38[b]	0.02
Syneresis (%)	2.60[b]	3.50[a]	3.17[a]	0.13

[a-c] Means (n = 72; averaged for culture and storage days) with different lower case superscripts within a row (formula) differ ($P \leq 0.05$)

[x] NS, non-supplemented yogurts; PE, plant extract supplemented yogurts; SA, sodium acetate supplemented yogurts

Table 5.9 pH, titratable acidity (TA), redox potential (Eh) and *S. thermophilus* counts of yogurts as a function of storage

	Storage day								
Characteristics	1	8	15	22	29	36	43	50	Pooled SE
pH	4.42[a]	4.34[b]	4.30[c]	4.29[c]	4.29[c]	4.30[c]	4.29[c]	4.29[c]	0.01
TA (% lactic acid)	1.30[d]	1.34[c]	1.37[b]	1.39[ab]	1.39[ab]	1.39[ab]	1.41[a]	1.41[a]	0.01
Eh (mV)	328.8[e]	342.9[d]	361.7[c]	373.9[b]	381.4[a]	382.3[a]	386.9[a]	381.5[a]	2.2
S. thermophilus (log cfu mL^{-1})	8.54[a]	7.81[b]	7.53[bc]	7.68[b]	7.76[b]	7.70[b]	7.86[b]	7.20[c]	0.12

[a-e] Means (n = 27; averaged for formula and culture) with different lower case superscripts within a row (storage day) differ ($P \leq 0.05$)

5.3.3.3 Syneresis

Syneresis was significantly affected by yogurt formula (Table 5.7). Syneresis in PE and SA yogurts (3.50 and 3.17 %, respectively) was greater compared with that in NS yogurts (2.60 %; Table 5.8). Greater syneresis of supplemented yogurt could be attributed to their greater TA and/or greater proteolysis due to longer fermentation times (Gassem & Frank, 1991).

5.3.3.4 Microbial counts

S. thermophilus counts in yogurt were significantly affected by storage but not by formula or culture (Table 5.7). Overall, *S. thermophilus* counts in all yogurts decreased from day 1 (8.54 log cfu mL^{-1}) to 8 (7.81 log cfu mL^{-1}) and then remained constant until day 43. These counts decreased further on day 50 (7.20 log cfu mL^{-1}); however, on day 15 and 50 the counts were similar (Table 5.9). The *S. thermophilus* counts in all yogurts remained above the minimum recommended concentration (6 log cfu mL^{-1}; Ross et al., 2005; Vasiljevic & Shah, 2008) throughout storage.

L. bulgaricus counts were significantly affected by formula × culture, formula × storage, and culture × storage (Table 5.7). *L. bulgaricus* counts in yogurts fermented with BA- or LA-culture were greatest in PE yogurts (7.38 and 6.63 log cfu mL^{-1}, respectively) followed by SA yogurts (6.08 and 6.00 log cfu mL^{-1}, respectively) and NS yogurts (5.18 and 4.86 log cfu mL^{-1}, respectively). *L. bulgaricus* counts in yogurts fermented with P-culture were similar in PE and SA yogurts (6.99 and 6.60 log cfu mL^{-1}, respectively), but the counts in PE yogurts were greater than NS yogurts (6.38 log cfu mL^{-1}; Table 5.10). For NS yogurts, *L. bulgaricus* counts were greater in yogurt fermented with P-culture (6.38 log cfu mL^{-1}) than yogurts fermented with BA- or LA-culture (5.18 and 4.86 log cfu mL^{-1}, respectively); however, no trend was observed in PE or SA yogurts (Table 5.10). During storage, *L. bulgaricus* counts were greater in PE yogurts compared with NS yogurts from day 15 to 50; whereas *L. bulgaricus* counts were greater in PE yogurts compared with SA yogurts from day 22 to 50, except the counts were similar on day 36 (Table 5.11). *L. bulgaricus* counts were > 6 log cfu mL^{-1} until day 22 in NS yogurts, day 29 in SA yogurts and day 36 in PE yogurts. Overall, *L. bulgaricus* counts in yogurts fermented with probiotic cultures were similar until day 22; but from day 36, the counts were greater in yogurts fermented with P-culture (Table 5.11). Greater *L. bulgaricus* counts in yogurts fermented with P-culture could be attributed to the synergetic effect of probiotic bacteria with *L. bulgaricus* and

improved proteolytic activity that could have produced more amino acids required for sustaining the viability of *L. bulgaricus* (Donkor et al., 2006; Mortazavian et al., 2006a; Shihata & Shah, 2000). Greater *L. bulgaricus* counts in supplemented yogurts might be a function of greater buffering capacity.

Table 5.10 *L. bulgaricus* counts (log cfu mL^{-1}) of yogurts[x] as a function of formula × culture

	Formula		
Culture	NS	PE	SA
BA	5.18^{e}	7.38^{a}	6.08^{cd}
LA	4.86^{e}	6.63^{bc}	6.00^{d}
P	6.38^{cd}	6.99^{ab}	6.60^{bc}

[a-e] Means (n = 24; averaged for storage days; with pooled SE of 0.19) with different lower case superscripts differ ($P \leq 0.05$)

[x] NS, non-supplemented yogurts; PE, plant extract supplemented yogurts; SA, sodium acetate supplemented yogurts; BA, yogurts fermented with *B. animalis*; LA, yogurts fermented with *L. acidophilus*; P, yogurts fermented with both probiotics (*B. animalis* and *L. acidophilus*)

Table 5.11 *L. bulgaricus* counts of yogurts[x] as a function of formula × storage and culture × storage

Storage	Formula			Culture		
day	NS	PE	SA	BA	LA	P
1	8.37^{abc}	8.65^{ab}	8.72^{a}	8.49^{AB}	8.57^{A}	6.86^{A}
8	7.46^{def}	8.06^{bcd}	7.80^{cde}	7.62^{CD}	7.91^{BC}	7.80^{CD}
15	6.55^{ghi}	7.47^{def}	7.07^{fg}	6.68^{EFG}	7.19^{DE}	7.22^{DE}
22	6.12^{ijk}	7.35^{ef}	6.49^{hij}	6.54^{FG}	6.60^{EFG}	6.81^{EF}
29	4.81^{mn}	6.89^{fgh}	6.10^{ijk}	6.15^{G}	5.39^{H}	6.25^{FG}
36	4.33^{n}	6.41^{hijk}	5.87^{kl}	5.49^{H}	4.88^{HI}	6.24^{FG}
43	3.47^{o}	5.89^{jkl}	4.36^{n}	4.64^{IJ}	3.76^{K}	5.31^{H}
50	2.67^{p}	5.30^{lm}	3.41^{o}	4.12^{JK}	2.35^{L}	4.92^{HI}

[a-p] Means (n = 9; averaged for formula; with pooled SE of 0.35) with different lower case superscripts within formula differ ($P \leq 0.05$)

[A-L] Means (n = 9; averaged for culture; with pooled SE of 0.35) with different upper case superscripts within culture differ ($P \leq 0.05$)

[x] NS, non-supplemented yogurts; PE, plant extract supplemented yogurts; SA, sodium acetate supplemented yogurts; BA, yogurts fermented with *B. animalis*; LA, yogurts fermented with *L. acidophilus*; P, yogurts fermented with both probiotics (*B. animalis* and *L. acidophilus*)

B. animalis counts were significantly affected by formula × storage (Table 5.7). Overall, *B. animalis* counts in yogurts with different formulas were similar during storage, except the counts in SA yogurts were greater than NS and PE yogurts on day 15, and the counts in PE yogurts were less than SA yogurts on day 29 and 36 (Table 5.12). *B. animalis* counts decreased to < 6 log cfu mL^{-1} on day 8 in NS and PE yogurts, and day 22 in SA yogurts. Better viability of *B. animalis* in SA yogurts compared with PE yogurts could be attributed to the lower TA of SA yogurts than PE yogurts. Overall, increased TA and Eh of yogurts during storage could have contributed to the rapid decrease in *B. animalis* counts, as *Bifidobacterium* spp. is less acid tolerant and more oxygen sensitive in yogurt than *Lactobacillus* spp. (Lourens-Hattingh & Viljoen, 2001). Dave and Shah (1997c) and Talwalkar et al. (2004) have reported that dissolved oxygen increased in yogurt stored in plastic cups during storage. Dave & Shah (1997c) reported

that at the end of storage (35 days), yogurt stored in glass bottles had ~ 1.5 ppm less dissolved oxygen compared with yogurt stored in plastic cups (~ 9 ppm) and the survival rate of bifidobacteria during storage was 30 to 70% more in yogurt stored in glass bottles compared with plastic cups. Thus, they concluded that oxygen permeability was a critical factor in determining the viability of bifidobacteria. Although Talwalkar et al. (2004) reported less dissolved oxygen (< 4.29 ppm) in yogurt stored in Nupak™ (polyester-based gas barrier) containers, compared with yogurt stored in high-impact polystyrene containers (~ 58 ppm) on day 42, the packaging material did not affect *Bifidobacterium* spp. counts (7.85 log cfu mL^{-1}). They concluded that oxygen might be the significant factor for *Bifidobacterium* spp. viability in yogurt during storage but the viability could also be strain specific.

Table 5.12 *B. animalis* counts of yogurts[x] as a function of formula × storage

Storage	Formula		
day	NS	PE	SA
1	6.98^{a}	6.60^{ab}	6.98^{a}
8	5.99^{bcde}	5.64^{cdef}	6.09^{bcd}
15	5.18^{fgh}	5.18^{fghi}	6.33^{abc}
22	5.45^{defg}	4.60^{ghijkl}	5.39^{defg}
29	4.76^{ghij}	4.66^{hijk}	5.46^{defg}
36	4.54^{hijkl}	4.16^{ijklm}	5.27^{efgh}
43	3.66^{mn}	4.11^{jklm}	4.07^{jklm}
50	3.90^{klmn}	3.89^{lmn}	3.22^{n}

[a-n] Means (n = 9; averaged for culture; with pooled SE of 0.29) with different lower case superscripts differ ($P \leq 0.05$)

[x] NS, non-supplemented yogurts; PE, plant extract supplemented yogurts; SA, sodium acetate supplemented yogurts

L. acidophilus counts were significantly affected by formula × culture × storage (Table 5.7), which are presented in Table 5.13. Overall, *L. acidophilus* counts were > 6 log cfu mL^{-1} for a longer time in supplemented yogurts compared with NS yogurts and in yogurts fermented with P-culture compared with yogurts fermented with LA-culture. *L. acidophilus* counts in NS

yogurts were > 6 log cfu mL^{-1} until day 22 and 15 in NS-P and NS-LA, respectively; whereas in PE and SA yogurts the counts were > 6 log cfu mL^{-1} until day 50 and 36 in PE-P and PE-LA, respectively, and day 29 and 22 in SA-P and SA-LA, respectively (Table 5.13). Longer fermentation time and improved proteolytic activity in yogurts fermented with P-culture could have produced more amino acids which sustained the viability of *L. acidophilus* (Donkor et al., 2006; Mortazavian et al., 2006a; Shihata & Shah, 2000), and the better viability of *L. acidophilus* in supplemented yogurts could also be attributed to the greater buffering ability (Ainaz & Ehsani, 2008).

Table 5.13 *L. acidophilus* counts of yogurts[x] as a function of formula × culture × storage

	Formula					
	NS		PE		SA	
Day of	Culture		Culture		Culture	
storage	LA	P	LA	P	LA	P
1	8.54^{ab}	8.44^{abc}	8.65^{a}	8.67^{a}	8.76^{a}	8.73^{a}
8	$7.70^{abcdefgh}$	7.93^{abcdef}	7.99^{abcde}	$7.84^{abcdefg}$	8.14^{abcd}	$7.50^{abcdefgh}$
15	$7.22^{abcdefghi}$	$6.98^{abcdefghij}$	$7.24^{abcdefghi}$	$7.57^{abcdefgh}$	$7.05^{abcdefghij}$	$7.06^{abcdefghij}$
22	$5.99^{efghijkl}$	$6.87^{abcdefghij}$	$7.38^{abcdefghi}$	$7.04^{abcdefghij}$	$6.50^{bcdefghijk}$	$6.47^{cdefghijk}$
29	4.32^{lmnopq}	$5.40^{ijklmnop}$	$6.20^{defghijkl}$	$6.68^{abcdefghij}$	$5.74^{hijklmno}$	$6.15^{defghijkl}$
36	2.97^{q}	$5.84^{ghijklmn}$	$6.08^{efghijkl}$	$6.80^{abcdefghij}$	$5.83^{ghijklmn}$	$5.69^{hijklmno}$
43	3.74^{opq}	$4.58^{klmnopq}$	$5.71^{hijklmno}$	$5.94^{fghijklm}$	4.31^{lmnopq}	$5.12^{jklmnop}$
50	3.73^{opq}	3.61^{pq}	3.84^{nopq}	$6.19^{defghijkl}$	3.86^{nopq}	3.91^{mnopq}

[a-q] Means (n = 3; with pooled SE of 0.35) with different lower case superscripts differ ($P \leq 0.05$)

[x] NS, non-supplemented yogurts; PE, plant extract supplemented yogurts; SA, sodium acetate supplemented yogurts; BA, yogurts fermented with *B. animalis*; LA, yogurts fermented with *L. acidophilus*; P, yogurts fermented with both probiotics (*B. animalis* and *L. acidophilus*)

5.4 Conclusions

Greater buffering capacity of PE yogurts compared with SA yogurts suggested the presence of additional component(s) in PE other than sodium acetate that resulted in greater buffering capacity. Because of its lower cost, PE supplementation could be a more economical

method to produce probiotic yogurt with enhanced viability of *L. bulgaricus* and *L. acidophilus* without affecting the viability of *S. thermophilus* and *Bifidobacterium animalis* ssp. *animalis*. In the future, qualitative and quantitative analyses of the commercial plant extract could elucidate the active component(s) in PE responsible for greater buffering capacity and improved viability of *L. bulgaricus* and *L. acidophilus* in yogurt. The effect of PE supplementation in yogurt on different starter and probiotic bacteria strains should also be studied.

5.5 Summary

The effect of a plant extract (prepared from olive, garlic, onion and citrus, and uses ~ 50% sodium acetate as a carrier) on the viability of yogurt starter and probiotic bacteria was studied. Yogurt samples were prepared with 3 different formulas (0.5% plant extract, 0.25% sodium acetate or no supplement) and fermented with 3 different cultures (yogurt starter and *B. animalis*, *L. acidophilus* or both probiotics). Microbial and chemical analyses were done weekly during 50 days of storage at 5 ºC. The plant extract and sodium acetate supplemented yogurt mixes had greater buffering capacities compared with non-supplemented yogurt mixes. *L. bulgaricus* and *L. acidophilus* counts in supplemented yogurts were > 6 log cfu mL^{-1} for an additional 7 to 35 days compared with non-supplemented yogurts. *S. thermophilus* and *B. animalis* counts were not affected by supplementation. These results suggested that the greater buffering ability could enhance the longevity of *L. bulgaricus* and *L. acidophilus* in yogurt during storage.

CHAPTER 6 - Research Summary

6.1 Summary

6.1.1 Experiment-I

Yogurt starter bacteria, *S. thermophilus* and *L. bulgaricus* are facultative anaerobe and anaerobe/aerotolerant, respectively; therefore I hypothesized that supplementing yogurt mix with plant extract could reduce the yogurt redox potential (Eh) and thus improve the longevity of starter bacteria in yogurt. The objective of this study was to investigate the effect of PE supplementation on the Eh and on the viability of *L. bulgaricus* and *S. thermophilus* in nonfat yogurt stored for 50 days at 5 ºC, while monitoring selected physicochemical parameters. Cysteine supplementation, which has been reported to effectively reduce the Eh of yogurt mix, was included as a comparison treatment.

Yogurts supplemented with 0.5% plant extract, 1% plant extract or cysteine (0.014 or 0.028 %) maintained *L. bulgaricus* counts > 6 log cfu mL^{-1} for an additional 21, 14 and 7 days, respectively, compared with non-supplemented yogurt during 50 days of storage at 5 °C. Whereas, *S. thermophilus* counts were > 6 log cfu mL^{-1} in all yogurts. On day 50, *S. thermophilus* count in 1% plant extract supplemented yogurt (7.23 log cfu mL^{-1}) was less than non-supplemented yogurt (8.64 log cfu mL^{-1}). Although cysteine supplemented yogurts had similar chemical properties compared with non-supplemented yogurt, the firmness of cysteine supplemented yogurts was greater than non-supplemented yogurt. Yogurt supplemented with 1% plant extract had greater titratable acidity (TA), pH and syneresis compared with non-supplemented yogurt; whereas yogurt supplemented with 0.5% plant extract had greater TA and pH compared with non-supplemented yogurt but had similar physical properties. The enhanced longevity of *L. bulgaricus* could not be attributed to the Eh of yogurt as plant extract supplemented and non-supplemented yogurts had similar Eh during storage. These results suggested that 0.5% plant extract supplementation could enhance the longevity of *L. bulgaricus* in yogurt without affecting its physical properties.

6.1.2 Experiment-II

Although *L. bulgaricus* counts in Experiment-I were greater in plant extract supplemented yogurts for a longer time compared with non-supplemented yogurt, no differences were observed in the Eh of non-supplemented and plant extract supplemented yogurts. Therefore, I hypothesized that the greater buffering capacity of plant extract supplemented yogurt, due presence of sodium acetate in the plant extract, might have enhanced the longevity of *L. bulgaricus*. Therefore, the objective of this study was to investigate the effect of plant extract (0.5%) supplementation on the buffering capacity of yogurt mix, and on the viability of yogurt starter and probiotic (*Bifidobacterium animalis* ssp. *animalis* and *Lactobacillus acidophilus*) bacteria in nonfat yogurt stored for 50 days at 5 ºC. Because plant extract contains 50% sodium acetate, sodium acetate supplementation at 0.25% was used as a comparison treatment.

Yogurt mixes supplemented with 0.5% plant extract or 0.25% sodium acetate had greater buffering capacity compared with non-supplemented yogurt mix. Probiotic yogurts supplemented with 0.5% plant extract and 0.25% sodium acetate maintained *L. bulgaricus* and *L. acidophilus* counts > 6 log cfu mL^{-1} for an additional 14 to 35 and 7 to 14 days, respectively, compared with non-supplemented probiotic yogurts during 50 days of storage at 5 °C; and the yogurts fermented with *B. animalis* and *L. acidophilus* had *L. bulgaricus* and *L. acidophilus* counts > 6 log cfu mL^{-1} for additional 7 to 14 days compared with yogurts fermented with *B. animalis* or *L. acidophilus*. *S. thermophilus* and *B. animalis* counts were not affected by supplementation or fermentation culture during storage. *S. thermophilus* counts in all yogurts were > 6 log cfu mL^{-1} throughout the storage; however, *B. animalis* counts decreased to < 6 log cfu mL^{-1} by day 8 in 0.5% plant extract supplemented and non-supplemented probiotic yogurts, and by day 15 in 0.25% sodium acetate supplemented probiotic yogurts. Supplemented probiotic yogurts had greater pH, TA and syneresis compared with non-supplemented probiotic yogurts but had similar Eh. The greater buffering capacity of plant extract supplemented yogurt mix compared with sodium acetate supplemented yogurt mix suggested the presence of other component(s) than in the plant extract that contributed in the buffering capacity. These results suggested that greater buffering capacity in yogurt mix could enhance the longevity of *L. bulgaricus* and *L. acidophilus* in probiotic yogurt.

6.2 Conclusions

Currently, cysteine, ascorbic acid and inulin are supplements, which can be added to yogurt mix to enhance the longevity of starter and/or probiotic bacteria in yogurt during storage. Plant extract is a cheaper supplement; therefore supplementing yogurt mix with 0.5% plant extract may be more economical to extend the shelf life of regular and probiotic yogurt. The greater syneresis in plant extract supplemented yogurt may be addressed by using stabilizer(s) in the yogurt mix that could restrict the whey loss. In the future, quantitative and qualitative analyses of commercial plant extract preparation could identify the factors responsible for its buffering action. The effects of plant extract supplementation on different strains of starter and probiotic bacteria should also be studied in future.

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Appendix A - SAS codes used for statistical analyses in experiment-I

A.1 Fermentation study and day 1

The following SAS code was used for the analyses of the fermentation data and day 1 data of shelf life study of pH, titratable acidity, redox potential, syneresis, water holding capacity, *S. thermophilus* counts and *L. bulgaricus* counts. The term "Parameter" used in the code referred to the above mentioned individual parameters used during analyses.

```
filename Parameter 'Path for datafile to be imported';

proc import
datafile = Parameter    out = Parameter1
replace dbms = excel;
range = 'Sheet1$B6:E16';
run;

data Parameter2;
set Parameter1;
if (Compound = "Control" and Percent = "zero") then trt = 1;
if (Compound = "PE" and Percent = "low") then trt = 2;
if (Compound = "PE" and Percent = "high") then trt = 3;
if (Compound = "CYS" and Percent = "low") then trt = 4;
if (Compound = "CYS" and Percent = "high") then trt = 5;
run;

proc print data = Parameter2;
run;

proc glm data = Parameter2;
class trt;
model ParameterStats = trt;
means trt;
means trt/lsd;

run;

quit;
```

A.2 Shelf life study

The following SAS code was used for the analyses of the shelf life study data of pH, titratable acidity, redox potential, syneresis, water holding capacity, *S. thermophilus* counts and *L. bulgaricus* counts. The term “Parameter” used in the code referred to the above mentioned individual parameters used during analyses.

```
filename Parameter 'Path for datafile to be imported';

proc import
datafile = Parameter              out=Parameter1
replace dbms = excel;
range = 'Sheet3$A21:F141';
run;

data Parameter2;
set Parameter1;
if (Compound = "Control" and Percent = "zero") then trt = 1;
if (Compound = "PE" and Percent = "low") then trt = 2;
if (Compound = "PE" and Percent ="high") then trt = 3;
if (Compound = "CYS" and Percent = "low") then trt = 4;
if (Compound = "CYS" and Percent = "high") then trt = 5;
run;

proc mixed data = Parameter2;
class trt day Replication;
model Parameter = trt day day*trt/ddfm = satterth;
random Replication(trt);
lsmeans trt/pdiff adjust = tukey;
lsmeans day/pdiff adjust = tukey;
run;
quit;
```

Appendix B - SAS codes used for statistical analyses in experiment-II

B.1 Fermentation study and day 1

The following SAS code was used for the analyses of the fermentation data and day1 data of shelf life study of pH, titratable acidity, redox potential, buffering capacity *S. thermophilus* counts and *L. bulgaricus* counts. The term "Parameter" used in the code referred to the above mentioned individual parameters used during analyses.

```
filename Parameter 'Path for datafile to be imported';

proc import
datafile=Parameter      out=Parameter1
replace dbms=excel;
range='Sheet2$A2:K26';
run;

proc transpose data=Parameter1        out=Parameter2        name=trt        prefix=response;
var NS_P NS_BA NS_LA PE_P PE_BA PE_LA SA_P SA_BA SA_LA;
by Day Replication;
run;

data Parameter2;
set Parameter2;
drop _LABEL_;
rename response1=response;
run;

data Parameter3;
set Parameter2;
supp=substrn(trt,1,2);
bact=substrn(trt,4,2);
run;

proc mixed data=Parameter3 cl;
class supp bact Replication Day;
model response=supp bact supp*bact day day*supp day* bact day*supp*bact/ddfm=satterth;
repeated Day/type=cs subject=Replication(supp bact);
lsmeans supp bact supp*bact day day*supp day* bact day*supp*bact/pdiff;
run;
quit;
```

B.2 Shelf life study

The following SAS code was used for the analyses of the shelf life study data of pH, titratable acidity, redox potential, syneresis, *S. thermophilus* counts and *L. bulgaricus* counts. The term "Parameter" used in the code referred to the above mentioned individual parameters used during analyses.

```
filename Parameter 'Path for datafile to be imported';

proc import
datafile=Parameter     out=Parameter1
replace dbms=excel;
range='Sheet6$A2:K4';
run;

proc transpose data=Parameter1        out=Parameter2        name=trt        prefix=response;
var NS_P NS_BA NS_LA PE_P PE_BA PE_LA SA_P SA_BA SA_LA;
by Day Replication;
run;

data Parameter2;
set Parameter2;
drop _LABEL_;
rename response1=response;
run;

data Parameter3;
set Parameter2;
supp=substrn(trt,1,2);
bact=substrn(trt,4,2);
where Day=0;
run;

proc mixed data=Parameter3;
class Replication supp bact;
model response=Replication supp bact supp*bact;
lsmeans supp bact supp*bact/pdiff;
run;


run;
quit;
```

Appendix C - Raw data

C.1 Experiment-I: Shelf life study

Table C.1 pH of various yogurts[x] during storage

Treatment		Day											
		I.V.	0	1	8	15	22	29	36	43	50	57	64
NS	R1	6.51	4.52	4.43	4.39	4.36	4.34	4.39	4.35	4.37	4.39	4.4	4.4
	R2	6.49	4.54	4.42	4.27	4.29	4.29	4.31	4.32	4.28	4.33	4.33	4.3
	R3	6.52	4.55	4.44	4.29	4.37	4.32	4.3	4.32	4.35	4.38	4.4	4.36
$PE_{0.5}$	R1	6.48	4.54	4.51	4.53	4.57	4.53	4.57	4.54	4.53	4.55	4.55	4.56
	R2	6.48	4.54	4.53	4.5	4.48	4.54	4.42	4.58	4.41	4.44	4.46	4.48
	R3	6.49	4.52	4.47	4.4	4.38	4.49	4.5	4.48	4.46	4.42	4.46	4.48
$Cys_{0.014}$	R1	6.48	4.48	4.42	4.39	4.23	4.23	4.23	4.25	4.23	4.2	4.24	4.24
	R2	6.46	4.52	4.4	4.37	4.26	4.27	4.28	4.28	4.34	4.29	4.23	4.26
	R3	6.46	4.48	4.42	4.28	4.28	4.29	4.32	4.31	4.37	4.32	4.33	4.32
PE_1	R1	6.49	4.57	4.54	4.5	4.56	4.52	4.5	4.48	4.46	4.5	4.5	4.5
	R2	6.48	4.5	4.49	4.5	4.43	4.45	4.46	4.45	4.52	4.44	4.44	4.51
	R3	6.48	4.5	4.49	4.5	4.46	4.5	4.51	4.5	4.5	4.52	4.52	4.5
$Cys_{0.028}$	R1	6.42	4.5	4.44	4.43	4.54	4.2	4.24	4.24	4.22	4.22	4.24	4.21
	R2	6.42	4.46	4.38	4.32	4.24	4.24	4.22	4.2	4.28	4.23	4.25	4.28
	R3	6.41	4.4	4.44	4.28	4.38	4.38	4.37	4.32	4.35	4.4	4.4	4.36

[x] NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl

I.V. Initial values before fermentation

R1, R2 & R3 Replication 1, 2 & 3

Table C.2 Titratable acidity, TA (% lactic acid) of various yogurts[x] during storage

Treatment		Day											
		I.V.	0	1	8	15	22	29	36	43	50	57	64
NS	R1	0.2	0.9	1	1.03	1.04	1.04	1.03	1.04	1.04	1.02	1.02	1.02
	R2	0.21	0.95	1.09	1.17	1.16	1.17	1.16	1.15	1.15	1.13	1.13	1.14
	R3	0.21	0.96	1.02	1.11	1.16	1.17	1.19	1.16	1.15	1.13	1.12	1.13
$PE_{0.5}$	R1	0.2	1.13	1.16	1.17	1.23	1.18	1.19	1.22	1.22	1.21	1.22	1.23
	R2	0.21	1.19	1.24	1.31	1.45	1.3	1.43	1.26	1.42	1.42	1.41	1.4
	R3	0.22	1.23	1.4	1.42	1.43	1.35	1.35	1.36	1.38	1.41	1.4	1.41
$Cys_{0.014}$	R1	0.21	0.94	1.05	1.05	1.12	1.11	1.11	1.12	1.11	1.16	1.12	1.16
	R2	0.21	0.9	0.98	1.1	1.12	1.13	1.12	1.12	1.09	1.09	1.13	1.1
	R3	0.22	0.9	1.01	1.11	1.11	1.09	1.1	1.11	1.11	1.12	1.11	1.12
PE_1	R1	0.22	1.42	1.48	1.53	1.49	1.53	1.6	1.64	1.62	1.6	1.65	1.65
	R2	0.22	1.53	1.66	1.67	1.69	1.7	1.69	1.68	1.6	1.64	1.67	1.61
	R3	0.21	1.49	1.63	1.65	1.64	1.64	1.63	1.64	1.66	1.71	1.66	1.64
$Cys_{0.028}$	R1	0.22	0.93	0.98	1.1	1.19	1.12	1.13	1.14	1.13	1.11	1.17	1.2
	R2	0.24	0.91	1.05	1.09	1.13	1.12	1.13	1.12	1.12	1.08	1.07	1.12
	R3	0.24	0.92	0.96	1.09	1.05	1.04	1.04	1.07	1.06	1.06	1.05	1.06

[x] NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl

I.V. Initial values before fermentation

R1, R2 & R3 Replication 1, 2 & 3

Table C.3 Redox potential, Eh (mV) of various yogurts[x] during storage

Treatment		Day											
		I.V.	0	1	8	15	22	29	36	43	50	57	64
NS	R1	234.8	305.5	354.8	415.7	424.8	420.1	415.5	368.8	376	380.2	388.3	382
	R2	251.3	330.5	346.5	362.4	396.1	383.5	368.5	371.1	363.6	369.2	364.8	369.7
	R3	276	330	336.8	342.4	356.4	377.5	370.3	368.7	360.1	364.9	369.1	370.6
$PE_{0.5}$	R1	218.9	296.4	351.7	409.2	418.5	419.3	418.7	394.3	386.7	388.4	391.8	387.6
	R2	230.1	305.2	343.6	391.2	398.7	395.4	372.6	368.4	370.3	360.3	361.1	382
	R3	224.5	308.6	345.1	361.7	364.5	361.2	363.9	361.4	360.1	362.1	361.4	362.2
$Cys_{0.014}$	R1	200.5	307	369.5	370.6	377.8	374.6	373.2	372.8	360	367.6	391.4	339.9
	R2	190.4	301.7	340	342.2	343.8	364.6	360.9	334.4	355.4	353.8	351.3	353.6
	R3	185.9	294.1	321.8	349	348.3	348	337.1	341.1	344.6	354.9	350.3	348.9
PE_1	R1	127.4	279.7	344.3	360.6	405.2	405.1	406.2	407.4	380.5	385.9	408.9	392.1
	R2	135.4	263.3	323.3	384	384.6	396.1	398.5	379.4	369.9	368.4	372.6	369.3
	R3	140.2	270.1	338.4	366.3	367.2	363.4	360.1	361.9	363.1	364.6	363.7	366.8
$Cys_{0.028}$	R1	117.3	284.1	333.2	351.7	365.4	369.6	367.4	366.2	361.1	353.2	362.8	360.6
	R2	127.9	277.4	305.1	311.7	335.2	333.3	331.7	322.5	324.9	324.6	321.5	327.1
	R3	127	254.6	283.6	309.5	297.2	317.2	301.9	326.1	316.9	320.8	320.9	324.7

[x] NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl

I.V. Initial values before fermentation

R1, R2 & R3 Replication 1, 2 & 3

Table C.4 Syneresis (%) of various yogurts[x] during storage

Treatment		Day									
		1	8	15	22	29	36	43	50	57	64
NS	R1	3.01	4.18	3.61	4.17	3.67	4.71	5.33	5.27	6.11	5.12
	R2	4.72	3.68	3.94	3.12	5.08	3.94	4.24	5.22	4.81	3.34
	R3	4.26	4.01	4.54	4.56	4.36	4.2	4.58	3.93	3.56	3.06
$PE_{0.5}$	R1	4.67	5.57	4.09	5.47	5.69	5.26	5.88	6.06	7.13	6.43
	R2	6	6	5.58	6.41	5.82	7.87	6.61	5.37	5.34	4.88
	R3	5.58	5.78	6.12	5.48	3.97	4.33	4.45	4.68	4.42	4.38
$Cys_{0.014}$	R1	4.54	3.37	2.98	3.63	3.77	4.97	4.08	4.29	3.71	3.5
	R2	6.74	4.27	5.96	3.1	3.48	3.27	3.92	3.93	4.13	3.56
	R3	5.03	4.26	4.71	3.42	3.16	3.72	3.56	3.4	3.4	3.51
PE_1	R1	8.18	6.3	7.97	7.73	6.75	8.58	7.28	8.62	4.7	5.17
	R2	10.5	8.84	7.96	7.52	7.19	7.32	7.5	5.75	6.54	5.14
	R3	7.14	6.94	6.06	4.16	5.86	6.58	5.77	4.93	5.78	5.28
$Cys_{0.028}$	R1	6.97	5.17	4.41	5.11	3.49	5.72	4.54	5.32	3.46	3.94
	R2	6.8	4.77	4.4	4.85	4.72	4.54	3.94	3.51	3.78	3.12
	R3	4.56	4.37	3.6	4.59	3.08	5.12	3.68	3.62	3.48	3.08

[x] NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl R1, R2 & R3 Replication 1, 2 & 3

Table C.5 Water holding capacity, WHC (%) of various yogurts[x] during storage

Treatment		Day									
		1	8	15	22	29	36	43	50	57	64
NS	R1	80.49	81.11	77.43	74.57	76.45	78.87	79.51	76.09	73.21	77.32
	R2	79.14	81.28	77.68	79.73	78.44	79.08	78.54	78.56	79.48	79.95
	R3	81.64	74.01	74.81	79.96	78.2	79.22	76.69	76.6	78.09	78.06
$PE_{0.5}$	R1	80.25	80.6	78.64	76.19	80.09	80.13	80.87	76.59	73.47	78.69
	R2	79.72	81.3	78.33	79.88	78.14	79.09	78.68	79.14	80.27	80.57
	R3	79.5	80.4	79.02	79.48	78.02	74.48	77.49	77.16	76.62	77.2
$Cys_{0.014}$	R1	77.81	79.81	72.75	78.27	74.64	74.83	79.24	78.18	77.02	75.5
	R2	79.43	79.2	77.56	79.13	78.36	77.99	75.93	78.41	79.56	79.86
	R3	78.99	79.41	78.72	79.08	77.24	75.58	77.68	78.28	73.37	75.46
PE_1	R1	78.34	80.7	76.6	78.69	74.67	75.54	79.01	78.96	76.68	76.82
	R2	80.98	80.58	79.14	80.14	78.7	77.36	77.8	79.26	78.84	74.69
	R3	80.42	81.07	80.02	77.87	77.3	77.72	78.11	79.68	77.14	75.45
$Cys_{0.028}$	R1	77.15	78.41	72.31	78.47	75.45	75.54	80.32	78.3	75.95	76.15
	R2	79.74	79.12	75.68	78.22	77.34	76.54	74.38	78.22	78.39	72.96
	R3	81.19	73.14	72.6	77.75	76.84	74.82	74.68	74.32	75.54	76.78

[x] NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl R1, R2 & R3 Replication 1, 2 & 3

Table C.6 *S. thermophilus* counts (log cfu mL^{-1}) of various yogurts[x] during storage

Treatment		Day									
		1	8	15	22	29	36	43	50	57	64
NS	R1	9.3	9.2	9.15	9.04	9.04	9.18	8.93	8.49	7.81	8.58
	R2	9.32	9.1	9.05	9.21	9.16	8.77	8.25	8.17	8.99	9.11
	R3	9.19	9.17	8.97	9.19	9.25	9.32	9.16	9.26	9.16	8.91
$PE_{0.5}$	R1	9.08	8.92	8.73	8.65	8.76	8.81	8.66	8.67	7.45	8.56
	R2	9.11	9	9	8.82	8.88	8.56	8.59	8.86	8.96	8.59
	R3	8.71	8.78	9	8.94	8.88	8.92	8.52	7.78	8.13	8.25
$Cys_{0.014}$	R1	8.97	8.94	8.93	8.87	8.94	7.9	8.63	8.02	8.16	7.81
	R2	9	9.14	9.31	8.93	8.87	8.89	8.82	8.85	8.57	8.18
	R3	8.76	8.98	8.97	8.99	8.95	8.85	9.11	8.82	8.84	8.24
PE_1	R1	8.93	8.46	8.86	8.11	8.04	7.08	7.93	7.59	7.19	6.93
	R2	8.99	8.82	8.3	8.17	7.64	7.84	6.75	6.83	6.31	6.25
	R3	8.43	8.26	8.31	8.19	7.52	7.2	7	7.28	6.29	6.2
$Cys_{0.028}$	R1	8.99	8.96	8.23	8.85	8.86	7.96	8.78	8.56	8.29	7.97
	R2	8.7	8.99	8.84	8.85	8.78	8.68	8.46	8.62	8.46	8.57
	R3	8.94	8.95	8.62	8.69	8.94	8.71	8.58	8.62	8.82	8.77

[x] NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl R1, R2 & R3 Replication 1, 2 & 3

Table C.7 *L. bulgaricus* counts (log cfu mL^{-1}) of various yogurts[x] during storage

Treatment		Day									
		1	8	15	22	29	36	43	50	57	64
NS	R1	8.46	7.15	5.28	4.23	2.15	< 3	< 3	< 3	< 3	< 3
	R2	8.26	7.73	6.96	5.07	4.41	< 3	< 3	< 3	< 3	< 3
	R3	8.2	6.69	4.27	3.17	2.78	< 3	< 3	< 3	< 3	< 3
$PE_{0.5}$	R1	7.98	8	7.49	6.61	6.36	4.92	4.08	< 3	< 3	< 3
	R2	8.41	8.04	7.88	6.5	6.2	4.42	4	< 3	< 3	< 3
	R3	8.28	7.97	7.8	6.86	6.35	5.43	4.34	< 3	< 3	< 3
$Cys_{0.014}$	R1	8.18	7.15	6	4.61	3.43	< 3	3.84	< 3	< 3	< 3
	R2	8.34	7.71	7.54	6.83	5.54	4.01	4.5	< 3	< 3	< 3
	R3	8.02	6.67	5	3.97	3.92	3.9	3.44	< 3	< 3	< 3
PE_1	R1	8.76	8.32	8.71	6.98	5.65	5.15	3.64	< 3	< 3	< 3
	R2	8.7	8.73	7.94	6.91	4.93	3.54	3.3	< 3	< 3	< 3
	R3	8.47	7.89	7.84	5.71	5.23	4.45	4.23	< 3	< 3	< 3
$Cys_{0.028}$	R1	8.28	7.52	7.04	4.8	4.75	4.04	< 3	< 3	< 3	< 3
	R2	8.62	8.13	7.66	6.65	5.96	5.89	3.88	< 3	< 3	< 3
	R3	8.53	7.76	7.63	6.02	4.78	3.67	< 3	< 3	< 3	< 3

[x] NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl R1, R2 & R3 Replication 1, 2 & 3

Table C.8 Total solids (% w/w) of various yogurts[x] on day 1

Treatment	Total Solids		
	R1	R2	R3
Control	14.3	14.52	15.1
0.5CF	14.33	14.62	15.2
0.5CY	14.22	14.64	14.96
1CF	14.01	14.52	15.45
1CY	14.33	14.66	15.22

[x] NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl
R1, R2 & R3 Replication 1, 2 & 3

Table C.9 Firmness (g) of various yogurts[x] on day 1

Treatment	Firmness		
	R1	R2	R3
Control	150.27	151.4	139.93
0.5CF	142.13	138.46	131.14
0.5CY	181.14	163.26	183.34
1CF	96.57	103.23	99.26
1CY	186.43	220.36	201.1

[x] NS, non-supplemented yogurt; $PE_{0.5}$, yogurt supplemented with 0.5% (w/v) plant extract; PE_1, yogurt supplemented with 1.0% (w/v) plant extract; $Cys_{0.014}$, yogurt supplemented with 0.014% (w/w) L-cysteine.HCl; $Cys_{0.028}$, yogurt supplemented with 0.028% (w/w) L-cysteine.HCl
R1, R2 & R3 Replication 1, 2 & 3

C.2 Experiment-I: Fermentation study

Table C.10 Various parameters of non-supplemented (NS) yogurt during fermentation

Time (h:min)	pH		TA		Eh (mV)		*S. thermophilus* (log cfu mL^{-1})		*L. bulgaricus* (log cfu mL^{-1})	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
0:00	6.49	6.47	0.2	0.2	266.7	249.4	8.11	8	6.64	6.26
1:00	6.03	5.84	0.28	0.32	268.9	259.3	8.06	8.29	7.26	5.95
2:00	5.1	4.94	0.58	0.67	275.3	265.3	8.89	8.89	6.75	7.25
3:00	4.68	4.66	0.75	0.85	284.8	285.1	8.91	9.08	6.96	7.46
3:31		4.5		0.93		293.4		9.2		7.74
4:00	4.55		0.96		298.4		8.6		7.49	
4:09	4.5		0.98		315.5		9.34		8.58	

TA Titratable acidity (% lactic acid)
Eh Redox potential (mV)
R1 & R2 Replication 1 & 2

Table C.11 Various parameters of 0.5% plant extract supplemented ($PE_{0.5}$) yogurt during fermentation

Time (h:min)	pH		TA		Eh (mV)		*S. thermophilus* (log cfu mL^{-1})		*L. bulgaricus* (log cfu mL^{-1})	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
0:00	6.5	6.49	0.2	0.21	230.4	224.1	8.03	7.9	6.66	5.42
1:00	6.04	6.05	0.29	0.29	236.5	232.6	8.1	8	6.46	5.84
2:00	5.62	5.56	0.48	0.51	249.5	247	8.53	8.42	6.52	6.71
3:00	5.23	5.15	0.76	0.75	260.4	265.1	8.49	8.84	7.57	7.78
4:00	4.91	4.89	0.88	0.91	272.3	270.7	8.75	8.76	8.01	7.79
5:00	4.7	4.72	1.04	1.07	285.7	279.4	8.78	8.72	8.07	8.23
5:48	4.5		1.21		313.3		8.46		8.5	
6:06		4.5		1.22		304.9		8.82		8.45

TA Titratable acidity (% lactic acid)
Eh Redox potential (mV)
R1 & R2 Replication 1 & 2

Table C.12 Various parameters of 1% plant extract supplemented (PE_1) yogurt during fermentation

Time (h:min)	pH		TA		Eh (mV)		*S. thermophilus* (log cfu mL^{-1})		*L. bulgaricus* (log cfu mL^{-1})	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
0:00	6.48	6.48	0.23	0.21	142.3	140.5	8.16	8.13	6.76	6.22
1:00	6.23	6.18	0.3	0.35	184.5	182.6	8.07	8.02	7.4	7.12
2:00	5.88	5.83	0.36	0.44	205.1	206.1	8.08	7.88	7.81	7.2
3:00	5.74	5.78	0.52	0.49	214.4	212.1	8.18	8.2	8.04	7.23
4:00	5.31	5.47	0.7	0.63	228	229	8.33	8.16	8.18	7.99
5:00	5.18	5.24	0.91	0.82	235.4	234.2	8.27	8.22	8.25	8.06
6:00	4.87	4.99	1.09	1.01	244.5	240.7	8.31	8.38	8.51	8.28
7:00	4.71	4.8	1.34	1.25	252.1	259.4	8.37	8.3	8.72	8.42
8:00	4.6	4.65	1.39	1.33	261.5	264.6	8.23	8.24	8.71	8.43
8:45	4.5		1.46		270.5		8.2		8.49	
9:10		4.5		1.44		275.8		8.14		8.46

TA Titratable acidity (% lactic acid)
Eh Redox potential (mV)
R1 & R2 Replication 1 & 2

Table C.13 Various parameters of 0.014% L-cysteine.HCl supplemented ($Cys_{0.014}$) yogurt during fermentation

Time (h:min)	pH		TA		Eh (mV)		*S. thermophilus* (log cfu mL^{-1})		*L. bulgaricus* (log cfu mL^{-1})	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
0:00	6.47	6.43	0.22	0.21	185.7	180.1	8.07	7.87	6.26	6.1
1:00	6.09	5.99	0.28	0.32	195.1	194.2	8.03	8.17	6.14	5.7
2:00	5.64	5.42	0.38	0.47	226.1	239.9	8.19	8.46	6.63	6.72
3:00	5.17	4.96	0.53	0.68	255.6	258.1	8.4	8.65	7.23	7.53
4:00	4.76	4.65	0.78	0.85	262.8	275.8	8.7	8.62	7.72	7.87
5:00	4.6	4.5	0.8	0.92	276.4	294.1	8.43	8.75	8.16	8
5:20	4.5		0.88		302.4		8.42		8.09	

TA Titratable acidity (% lactic acid)
Eh Redox potential (mV)
R1 & R2 Replication 1 & 2

Table C.14 Various parameters of 0.028% L-cysteine.HCl supplemented ($Cys_{0.028}$) yogurt during fermentation

Time (h:min)	pH		TA		Eh (mV)		*S. thermophilus* (log cfu mL^{-1})		*L. bulgaricus* (log cfu mL^{-1})	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
0:00	6.42	6.48	0.21	0.22	130.6	128.5	7.88	8.08	5.46	6.1
1:00	6.01	5.99	0.3	0.31	163.2	152.8	8.04	8.2	5.73	5.54
2:00	5.55	5.49	0.4	0.44	155.7	158.8	8.15	8.45	7.02	6.66
3:00	5.03	5.09	0.56	0.56	160.8	157.1	8.52	8.36	6.63	7.23
4:00	4.7	4.72	0.71	0.71	164.1	160.1	8.4	8.15	7.84	7.39
4:40	4.5		0.98		179.4		8.75		8.25	
4:50		4.5		1		171.2		8.6		8.29

TA Titratable acidity (% lactic acid)
Eh Redox potential (mV)
R1 & R2 Replication 1 & 2

C.3 Experiment-II: Shelf life study

Table C.15 pH of various yogurts[y] during storage

Day	Replication	NS-P	PE-P	SA-P	NS-BA	PE-BA	SA-BA	NS-LA	PE-LA	SA-LA
I.V.	R1	6.52	6.52	6.53	6.52	6.52	6.53	6.5	6.52	6.52
	R2	6.51	6.53	6.52	6.52	6.54	6.51	6.52	6.52	6.53
	R3	6.53	6.54	6.54	6.53	6.5	6.53	6.52	6.5	6.5
0	R1	4.46	4.51	4.47	4.44	4.47	4.49	4.45	4.49	4.4
	R2	4.45	4.51	4.46	4.56	4.48	4.52	4.53	4.54	4.52
	R3	4.47	4.45	4.49	4.45	4.44	4.5	4.55	4.57	4.54
1	R1	4.4	4.38	4.46	4.35	4.45	4.44	4.34	4.48	4.38
	R2	4.43	4.44	4.46	4.36	4.45	4.46	4.44	4.5	4.4
	R3	4.37	4.44	4.35	4.35	4.46	4.45	4.4	4.48	4.43
8	R1	4.28	4.38	4.4	4.3	4.35	4.4	4.24	4.49	4.39
	R2	4.28	4.32	4.32	4.27	4.39	4.42	4.26	4.39	4.29
	R3	4.29	4.37	4.33	4.29	4.32	4.4	4.3	4.46	4.25
15	R1	4.26	4.38	4.33	4.21	4.38	4.31	4.23	4.44	4.27
	R2	4.16	4.28	4.22	4.19	4.28	4.36	4.18	4.39	4.31
	R3	4.26	4.38	4.33	4.24	4.46	4.36	4.3	4.41	4.32
22	R1	4.21	4.38	4.31	4.21	4.4	4.29	4.2	4.3	4.27
	R2	4.2	4.36	4.25	4.16	4.35	4.3	4.21	4.36	4.26
	R3	4.23	4.35	4.38	4.22	4.23	4.38	4.32	4.47	4.28
29	R1	4.14	4.34	4.29	4.16	4.41	4.26	4.26	4.38	4.3
	R2	4.16	4.3	4.3	4.23	4.33	4.2	4.2	4.33	4.32
	R3	4.31	4.38	4.32	4.32	4.2	4.43	4.3	4.46	4.29
36	R1	4.22	4.3	4.29	4.21	4.36	4.32	4.18	4.36	4.3
	R2	4.13	4.25	4.35	4.26	4.28	4.33	4.18	4.36	4.25
	R3	4.31	4.43	4.39	4.24	4.35	4.38	4.25	4.47	4.3
43	R1	4.14	4.36	4.34	4.24	4.32	4.38	4.21	4.32	4.31
	R2	4.18	4.3	4.28	4.18	4.19	4.36	4.16	4.41	4.3
	R3	4.31	4.41	4.4	4.3	4.23	4.28	4.25	4.35	4.23
50	R1	4.19	4.31	4.31	4.19	4.37	4.27	4.15	4.27	4.28
	R2	4.2	4.22	4.26	4.25	4.29	4.31	4.15	4.34	4.28
	R3	4.3	4.39	4.38	4.3	4.26	4.35	4.3	4.43	4.41

[y] NS-BA, non-supplemented yogurt inoculated with *B. animalis*; NS-LA, non-supplemented yogurt inoculated with *L. acidophilus*; NS-P, non-supplemented yogurt inoculated with both probiotics; PE-BA, 0.5% plant extract supplemented yogurt inoculated with *B. animalis*; PE-LA, 0.5% plant extract supplemented yogurt inoculated with *L. acidophilus*; PE-P, 0.5% plant extract supplemented yogurt inoculated with both probiotics; SA-BA, 0.25% sodium acetate supplemented yogurt inoculated with *B. animalis*; SA-LA, 0.5% sodium acetate supplemented yogurt inoculated with *L. acidophilus*; SA-P, 0.5% sodium acetate supplemented yogurt inoculated with both probiotics

I.V. Initial values before fermentation

Table C.16 Titratable acidity, TA (%lactic acid) of various yogurts[y] during storage

Day	Replication	NS-P	PE-P	SA-P	NS-BA	PE-BA	SA-BA	NS-LA	PE-LA	SA-LA
I.V.	R1	0.21	0.21	0.21	0.2	0.2	0.22	0.2	0.2	0.2
	R2	0.22	0.22	0.2	0.21	0.2	0.2	0.2	0.21	0.2
	R3	0.22	0.21	0.21	0.2	0.2	0.21	0.21	0.2	0.2
0	R1	1.08	1.29	1.26	1.06	1.32	1.21	1.03	1.27	1.41
	R2	1.04	1.3	1.21	1.03	1.33	1.27	1.02	1.26	1.2
	R3	0.97	1.32	1.15	1.01	1.29	1.26	0.97	1.25	1.16
1	R1	1.18	1.54	1.31	1.27	1.34	1.26	1.13	1.45	1.3
	R2	1.19	1.53	1.46	1.19	1.51	1.32	1.13	1.38	1.3
	R3	1.15	1.38	1.34	1.13	1.36	1.36	1.08	1.38	1.26
8	R1	1.25	1.48	1.38	1.21	1.57	1.35	1.26	1.38	1.33
	R2	1.23	1.55	1.38	1.24	1.51	1.3	1.19	1.44	1.37
	R3	1.2	1.42	1.33	1.18	1.52	1.33	1.11	1.37	1.37
15	R1	1.3	1.53	1.43	1.23	1.51	1.4	1.25	1.38	1.39
	R2	1.36	1.64	1.56	1.26	1.61	1.3	1.27	1.48	1.37
	R3	1.24	1.42	1.35	1.2	1.36	1.27	1.16	1.43	1.38
22	R1	1.35	1.5	1.43	1.28	1.46	1.42	1.23	1.54	1.46
	R2	1.32	1.51	1.46	1.32	1.57	1.38	1.29	1.43	1.43
	R3	1.3	1.44	1.3	1.17	1.67	1.28	1.13	1.33	1.42
29	R1	1.33	1.48	1.44	1.31	1.51	1.43	1.24	1.43	1.33
	R2	1.35	1.62	1.43	1.28	1.59	1.56	1.22	1.48	1.39
	R3	1.16	1.48	1.37	1.17	1.68	1.24	1.18	1.43	1.44
36	R1	1.33	1.63	1.46	1.24	1.5	1.37	1.25	1.5	1.41
	R2	1.35	1.68	1.42	1.28	1.66	1.44	1.26	1.51	1.4
	R3	1.16	1.42	1.32	1.16	1.43	1.27	1.19	1.4	1.38
43	R1	1.35	1.44	1.48	1.25	1.61	1.4	1.3	1.63	1.4
	R2	1.36	1.59	1.49	1.33	1.65	1.34	1.28	1.5	1.46
	R3	1.16	1.46	1.32	1.16	1.6	1.49	1.2	1.49	1.39
50	R1	1.37	1.61	1.42	1.34	1.52	1.49	1.28	1.63	1.4
	R2	1.36	1.62	1.47	1.27	1.6	1.34	1.28	1.47	1.46
	R3	1.17	1.48	1.33	1.2	1.6	1.33	1.2	1.39	1.35

[y] NS-BA, non-supplemented yogurt inoculated with *B. animalis*; NS-LA, non-supplemented yogurt inoculated with *L. acidophilus*; NS-P, non-supplemented yogurt inoculated with both probiotics; PE-BA, 0.5% plant extract supplemented yogurt inoculated with *B. animalis*; PE-LA, 0.5% plant extract supplemented yogurt inoculated with *L. acidophilus*; PE-P, 0.5% plant extract supplemented yogurt inoculated with both probiotics; SA-BA, 0.25% sodium acetate supplemented yogurt inoculated with *B. animalis*; SA-LA, 0.5% sodium acetate supplemented yogurt inoculated with *L. acidophilus*; SA-P, 0.5% sodium acetate supplemented yogurt inoculated with both probiotics

I.V. Initial values before fermentation

Table C.17 Redox potential, Eh (mV) of various yogurts[y] during storage

Day	Replication	NS-P	PE-P	SA-P	NS-BA	PE-BA	SA-BA	NS-LA	PE-LA	SA-LA
I.V.	R1	242.4	230	224.4	244.2	228.6	220.7	249.4	240.3	238.3
	R2	259.9	228.1	239.7	263.7	234.7	239.3	276.4	237.1	230.1
	R3	264.5	243.1	231.7	269.3	239.9	242.4	269.3	233.7	224.2
0	R1	298.6	297.1	304.4	306.4	318.2	295.2	322.4	308.4	310.4
	R2	322.7	305.2	313	292	310	299.7	291.2	293.3	323.4
	R3	302.5	289.1	299.7	294.5	304.1	306.3	326.9	318.2	291.7
1	R1	325.6	334.2	328.4	334.9	321.8	327.9	324.1	336.3	331.8
	R2	327.8	330.1	336.7	335.5	335.7	306.1	334.6	333.8	337
	R3	338.1	333.2	309.1	332.5	333.9	309.4	326.3	323.9	327.8
8	R1	347.5	341.4	366.6	335.2	332.1	330.1	346.8	330.9	345.9
	R2	334.8	338.8	331.3	335.8	325.3	332.8	333.1	338.3	336
	R3	349.1	359.1	340.4	361	343.1	339	362.5	356.2	364.8
15	R1	359.8	348	343.9	362.1	346.1	339.5	351	339.8	351.5
	R2	370.3	358.6	361.8	376.7	359.7	359.1	361	369.4	374
	R3	378.4	376.9	363.7	381.1	363.3	365.5	365.4	377.7	361.5
22	R1	364.3	362.8	376.7	366.8	360.4	366.1	369.4	357.3	370.3
	R2	375.1	380.8	379.5	388	369.1	374.3	368.7	380.1	383.1
	R3	374.6	375.4	365.8	375.7	380.1	381.5	383.1	377	389.7
29	R1	384.7	376.1	377.4	379.7	371.3	380.7	374.9	376.1	386.1
	R2	379.7	377.5	374.2	378.4	379.8	375.9	382.7	381.7	378.8
	R3	392.4	385.4	386.2	387.2	386.5	385.5	385.4	388.9	383.3
36	R1	380.9	376.2	379.5	382.2	381	375.7	380.4	372.2	380.5
	R2	390.3	390.3	389.2	392.2	389.5	391.9	391	393.9	382.6
	R3	376.3	376.6	383.6	376.2	376.1	374.1	372.7	392.4	373.4
43	R1	392	392.9	388.9	388.9	389.2	389.8	388.2	386.3	391.7
	R2	376.8	380.2	383.1	387.6	373.1	384.8	385.3	386	387
	R3	389.7	385.2	385.8	389.8	393.8	393.8	389.9	378.7	388.1
50	R1	387	385.7	391	388.3	375.8	391.5	374.9	392.1	389.7
	R2	391.1	390.9	392.1	393.4	389.9	387.3	392.1	389	390.8
	R3	374.6	376.8	375.4	376.7	379.3	284.8	378.8	377.3	384.3

[y] NS-BA, non-supplemented yogurt inoculated with *B. animalis*; NS-LA, non-supplemented yogurt inoculated with *L. acidophilus*; NS-P, non-supplemented yogurt inoculated with both probiotics; PE-BA, 0.5% plant extract supplemented yogurt inoculated with *B. animalis*; PE-LA, 0.5% plant extract supplemented yogurt inoculated with *L. acidophilus*; PE-P, 0.5% plant extract supplemented yogurt inoculated with both probiotics; SA-BA, 0.25% sodium acetate supplemented yogurt inoculated with *B. animalis*; SA-LA, 0.5% sodium acetate supplemented yogurt inoculated with *L. acidophilus*; SA-P, 0.5% sodium acetate supplemented yogurt inoculated with both probiotics

I.V. Initial values before fermentation

Table C.18 Syneresis (%) of various yogurts[y] during storage

Day	Replication	NS-P	PE-P	SA-P	NS-BA	PE-BA	SA-BA	NS-LA	PE-LA	SA-LA
1	R1	2.91	2.26	2.96	2.31	3.4	2.61	2.15	3.2	2.44
	R2	3.04	2.46	3.96	2.02	2.94	2.21	1.91	4.2	2.8
	R3	2.68	3.69	3.25	3	2.74	3.01	2.63	3.64	2.44
8	R1	2.7	2.46	2.82	2.42	3.28	2.99	2.16	3.53	3.12
	R2	2.9	2.92	3.21	3.56	3.79	2.4	2.54	5.16	2.78
	R3	2.04	3.28	3.21	2	4.38	3.37	3.2	3.5	3.82
15	R1	3.2	4	3.64	3.02	3	4.52	2.54	4.24	2.38
	R2	1.9	2.33	2.69	3.71	3.54	2.85	2.62	4.1	2.94
	R3	2.49	3.32	2.9	2.17	3.86	3.51	2.57	3.85	3.74
22	R1	2.43	2.68	3.77	2.9	7.1	4.05	3.34	4.54	2.88
	R2	3.16	2.4	2.34	2.2	2.92	2.38	2.6	3.54	3.51
	R3	1.98	3.22	3.1	1.76	4.16	2.92	3.08	2.78	3.62
29	R1	2.42	3.06	3.4	2.64	3.86	4.99	2.82	4.75	2.48
	R2	2.49	3.58	3.18	2.21	3.05	3.17	3.06	3.29	3.64
	R3	1.94	3.83	3.1	1.92	3.58	4.56	2.88	4.29	4.15
36	R1	2	2.58	3.18	2.01	3.19	3.8	2.14	3.63	2.65
	R2	2.62	2.6	2.76	2.57	3.46	3.2	3.48	4.38	2.95
	R3	2.55	3.82	3.48	2.28	3.56	3.24	3.04	4.25	2.6
43	R1	3.08	2.46	3.03	2.72	4.9	3.6	3	3.78	2.48
	R2	2.49	2.06	3.81	3.19	2.3	2.33	2.23	3.71	3.33
	R3	1.72	3.55	3.56	2.9	3.28	3.17	4.51	3.91	3.86
50	R1	3.26	3.07	3.31	1.9	2.94	2.81	3.03	4.48	1.98
	R2	1.63	2.04	3.05	3.35	3.36	2.47	2.14	4.38	3.3
	R3	1.63	3.04	3.4	1.46	3.78	3.6	4.1	3.52	3.24

[y] NS-BA, non-supplemented yogurt inoculated with *B. animalis*; NS-LA, non-supplemented yogurt inoculated with *L. acidophilus*; NS-P, non-supplemented yogurt inoculated with both probiotics; PE-BA, 0.5% plant extract supplemented yogurt inoculated with *B. animalis*; PE-LA, 0.5% plant extract supplemented yogurt inoculated with *L. acidophilus*; PE-P, 0.5% plant extract supplemented yogurt inoculated with both probiotics; SA-BA, 0.25% sodium acetate supplemented yogurt inoculated with *B. animalis*; SA-LA, 0.5% sodium acetate supplemented yogurt inoculated with *L. acidophilus*; SA-P, 0.5% sodium acetate supplemented yogurt inoculated with both probiotics

Table C.19 *S. thermophilus* counts (log cfu mL^{-1}) of various yogurts[y] during storage

Day	Replication	NS-P	PE-P	SA-P	NS-BA	PE-BA	SA-BA	NS-LA	PE-LA	SA-LA
I.V.	R1	7.69	7.73	6.61	7.08	7.73	7.07	7.75	6.71	7.48
	R2	6.66	6.76	6.58	6.5	6.67	6.42	6.88	6.46	6.76
	R3	7.33	7.37	6.9	7.08	6.8	6.57	7.01	6.6	6.73
0	R1	8.72	8.88	8.66	8.87	8.93	8.62	9.11	8.87	8.76
	R2	8.29	8.87	8.88	8.47	8.57	9.01	8.88	8.79	8.61
	R3	8.83	8.69	8.7	8.54	8.52	8.52	8.35	8.76	8.52
1	R1	8.5	8.56	8.67	8.67	8.66	8.72	8.94	8.39	8.71
	R2	8.49	8.48	8.55	8.89	8.92	8.85	8.19	8.73	8.61
	R3	8.33	8.07	8.3	8.07	8.32	8.83	8.09	8.62	8.44
8	R1	7.77	7.55	8.25	7.41	8.06	7.43	7.45	7.36	6.74
	R2	7.18	7.6	8.34	7.72	8.53	8.27	7.53	8.1	7.66
	R3	7.9	7.76	7.62	8.06	8.48	8.02	7.76	8.06	8.36
15	R1	6.7	6.16	7.46	5.46	7.92	7.95	7.56	7.57	6.09
	R2	6.74	8.34	6.89	7.99	8.34	7.33	7.44	7.42	7.93
	R3	8.39	8.25	7.44	8.32	8.44	7.42	8.41	7.97	7.26
22	R1	7.06	7.44	6.93	7.62	7.95	7.23	7.52	7.45	7.21
	R2	7.9	7.66	7.1	8.26	7.1	8.3	6.95	8.21	7.38
	R3	7.65	7.45	8.39	7.24	7.76	8.37	8.41	8.35	8.45
29	R1	7.17	7.3	8.33	8.24	7.04	6.97	7.04	7.14	7.09
	R2	7.97	8.42	8.36	6.83	8.29	6.99	8.4	6.9	8.38
	R3	7.78	6.56	8.73	7.89	8.31	8.66	8.55	7.88	8.42
36	R1	8.3	7.4	6.66	8.38	8.1	6.79	8.36	6.58	8.38
	R2	8.23	8.28	8.22	7.84	8.3	7.71	8.4	7.2	8.38
	R3	7.26	6.51	8.04	7.02	7.61	7.39	8.45	6.6	7.63
43	R1	8.32	8.09	7.27	8.49	7.46	7.29	8.41	6.33	8.47
	R2	7.65	6.48	7.02	7.48	7.56	6.88	8.2	7.13	7.65
	R3	8.16	8.06	8.68	8.25	8	7.83	8.66	7.76	8.64
50	R1	8.26	6.91	7.33	6.89	7.59	7.11	8.01	5.71	7.16
	R2	8.3	6.47	7.93	8.32	6.55	7.48	7.99	7.48	8.45
	R3	7.64	7.06	6.39	6.7	6.33	6.31	6.78	6.5	6.63

[y] NS-BA, non-supplemented yogurt inoculated with *B. animalis*; NS-LA, non-supplemented yogurt inoculated with *L. acidophilus*; NS-P, non-supplemented yogurt inoculated with both probiotics; PE-BA, 0.5% plant extract supplemented yogurt inoculated with *B. animalis*; PE-LA, 0.5% plant extract supplemented yogurt inoculated with *L. acidophilus*; PE-P, 0.5% plant extract supplemented yogurt inoculated with both probiotics; SA-BA, 0.25% sodium acetate supplemented yogurt inoculated with *B. animalis*; SA-LA, 0.5% sodium acetate supplemented yogurt inoculated with *L. acidophilus*; SA-P, 0.5% sodium acetate supplemented yogurt inoculated with both probiotics

I.V. Initial values before fermentation

Table C.20 *L. bulgaricus* counts (log cfu mL^{-1}) of various yogurts[y] during storage

Day	Replication	NS-P	PE-P	SA-P	NS-BA	PE-BA	SA-BA	NS-LA	PE-LA	SA-LA
I.V.	R1	7.11	7.19	6.77	6.76	6.93	6.78	6.83	6.96	6.98
	R2	7.09	7	6.91	6.81	6.89	6.66	7.17	6.89	7.07
	R3	7.3	7.24	7.06	6.78	7.06	6.96	7.12	6.67	6.93
0	R1	8.67	8.95	8.84	8.48	9.02	8.82	8.52	8.9	8.66
	R2	8.58	9.02	9.06	8.77	8.83	9.06	8.19	9.11	8.88
	R3	8.4	8.75	8.67	8.39	8.82	8.82	8.63	9.02	8.67
1	R1	8.36	8.56	8.76	8.43	8.64	8.7	8.63	8.38	8.63
	R2	8.78	9.1	8.91	8.39	9.04	8.65	8.19	8.41	8.6
	R3	8.37	8.55	8.74	7.55	8.38	8.62	8.62	8.78	8.87
8	R1	8.01	8.07	7.22	7.2	8.22	7.88	7.82	7.65	8.12
	R2	7.7	7.94	7.11	6.57	8.37	7.91	7.07	7.88	8.11
	R3	8	7.89	8.29	6.66	8.27	7.48	8.15	8.26	8.12
15	R1	6.57	6.23	6.86	5	8.08	6.55	6.1	7.59	7.1
	R2	7.18	7.62	7.55	6.87	7.72	6.97	7.26	6.45	7.51
	R3	7.18	8.3	7.49	5.48	7.54	5.88	7.3	7.72	7.68
22	R1	6.36	7.07	5.57	4.78	7.42	6.81	5.65	7.53	6.52
	R2	6.78	7.09	7.08	6.5	7.56	6.99	6.04	7.24	7.05
	R3	7.28	6.96	7.12	5.8	7.88	5.16	5.91	7.38	6.12
29	R1	6.19	6.97	5.32	3.91	7.54	6.57	3.42	6.92	4.81
	R2	5.05	7.29	7.44	5.01	7.67	7.14	5.06	6.49	6.31
	R3	6.26	5.46	6.29	4.59	7.75	5.19	3.79	5.88	5.82
36	R1	5.81	7.01	6.39	4.49	7.03	6	2.44	6.77	5.72
	R2	5.71	6.75	6.65	4.85	6.95	6.03	2.46	5.92	5.26
	R3	5.72	5.76	6.39	3.58	6.1	4.38	3.94	5.38	5.99
43	R1	5.48	6.62	5.07	2.42	6.76	5	2.11	5.8	3.94
	R2	4.5	4.38	5.57	3.93	7.66	3.42	1.95	5.74	5.01
	R3	4.72	5.95	5.5	4.34	5.01	3.21	1.74	5.05	2.48
50	R1	4.88	6.96	3.48	3.14	6.5	4.66	0.5	5.21	2.35
	R2	3.71	5.89	4.48	0.5	5.49	3.31	0.5	3.24	2.1
	R3	4.45	5.35	5.08	4.36	5.61	3.48	2	3.46	1.78

[y] NS-BA, non-supplemented yogurt inoculated with *B. animalis*; NS-LA, non-supplemented yogurt inoculated with *L. acidophilus*; NS-P, non-supplemented yogurt inoculated with both probiotics; PE-BA, 0.5% plant extract supplemented yogurt inoculated with *B. animalis*; PE-LA, 0.5% plant extract supplemented yogurt inoculated with *L. acidophilus*; PE-P, 0.5% plant extract supplemented yogurt inoculated with both probiotics; SA-BA, 0.25% sodium acetate supplemented yogurt inoculated with *B. animalis*; SA-LA, 0.5% sodium acetate supplemented yogurt inoculated with *L. acidophilus*; SA-P, 0.5% sodium acetate supplemented yogurt inoculated with both probiotics

I.V. Initial values before fermentation

Table C.21 *B. animalis* counts (log cfu mL^{-1}) of various yogurts[y] during storage

Day	Replication	NS-P	PE-P	SA-P	NS-BA	PE-BA	SA-BA
I.V.	R1	6.92	6.86	6.77	6.74	6.68	6.88
	R2	6.84	6.84	6.7	6.91	6.96	6.86
	R3	6.8	6.67	6.81	6.73	7.08	6.79
0	R1	7.03	6.39	6.61	6.5	6.93	6.87
	R2	7.53	6.26	7.65	7.42	6.16	7.61
	R3	7.99	8.37	8.04	7.23	8.04	7.85
1	R1	7.21	5.54	6.82	6.53	6.24	6.55
	R2	7.26	6.43	7.23	6.35	6.39	6.38
	R3	7.41	7.06	7.42	7.11	7.94	7.48
8	R1	6.64	5.52	5.75	6.15	5.37	5.92
	R2	5.47	6.41	5.67	6.83	5.53	5.48
	R3	5.51	5.49	7.26	5.36	5.51	6.46
15	R1	5.35	4.36	6.13	4.8	5.49	6.42
	R2	5.52	5.42	6.85	4.82	5.5	6.46
	R3	5.4	5.14	6.08	5.22	5.19	6.03
22	R1	4.64	4.34	4.52	5.37	5.08	5.62
	R2	5.44	4.57	6.83	5.42	5.04	5.69
	R3	6.33	3.98	5.8	5.48	4.58	3.89
29	R1	4.72	4.65	4.38	4	5.09	5.55
	R2	4.79	4.94	6.35	5.48	5.02	6.31
	R3	4.09	3.64	6.43	5.51	4.63	3.77
36	R1	4.56	4.5	5.33	4.59	4.54	5.03
	R2	5.5	3.54	5.96	4.03	4.44	6.12
	R3	4.27	3.7	5.88	4.26	4.26	3.29
43	R1	5.47	4.46	4.21	< 1	4.68	4.25
	R2	3.52	3.48	5.05	4.58	4.62	3.59
	R3	3.95	3.03	4.48	3.92	4.41	2.83
50	R1	3.77	3.26	3.07	3.78	4.31	3.95
	R2	3.99	3.15	2.87	4.21	5.75	3.41
	R3	3.63	3.12	3.79	4.04	3.74	2.24

[y] NS-BA, non-supplemented yogurt inoculated with *B. animalis*; NS-LA, non-supplemented yogurt inoculated with *L. acidophilus*; NS-P, non-supplemented yogurt inoculated with both probiotics; PE-BA, 0.5% plant extract supplemented yogurt inoculated with *B. animalis*; PE-LA, 0.5% plant extract supplemented yogurt inoculated with *L. acidophilus*; PE-P, 0.5% plant extract supplemented yogurt inoculated with both probiotics; SA-BA, 0.25% sodium acetate supplemented yogurt inoculated with *B. animalis*; SA-LA, 0.5% sodium acetate supplemented yogurt inoculated with *L. acidophilus*; SA-P, 0.5% sodium acetate supplemented yogurt inoculated with both probiotics

I.V. Initial values before fermentation

Table C.22 *L. acidophilus* counts (log cfu mL^{-1}) of various yogurts[y] during storage

Day	Replication	NS-P	PE-P	SA-P	NS-LA	PE-LA	SA-LA
I.V.	R1	7.15	7.19	6.72	6.71	6.99	7.02
	R2	6.75	7.11	6.83	6.84	7.11	7.38
	R3	7.14	7.14	7.04	6.95	7.1	6.83
0	R1	8.51	8.97	8.86	8.54	8.85	8.56
	R2	8.41	9.02	9.11	8.14	9.07	8.93
	R3	8.48	8.78	8.66	8.69	9.02	8.74
1	R1	8.5	8.67	8.76	8.58	8.6	8.66
	R2	8.61	9.04	8.81	8.58	8.56	8.75
	R3	8.2	8.29	8.63	8.47	8.8	8.86
8	R1	8.04	8.08	7.3	7.86	8.02	8.01
	R2	8.12	7.97	7.93	7.72	7.88	8.3
	R3	7.64	7.46	7.28	7.52	8.08	8.1
15	R1	7.3	7.22	6.83	7.36	7.59	7.18
	R2	6.52	7.18	7.15	7.14	6.43	6.31
	R3	7.11	8.3	7.19	7.16	7.7	7.66
22	R1	6.49	7.12	5.24	5.96	7.36	6.23
	R2	6.87	7.07	7.09	6.09	7.39	7.12
	R3	7.24	6.92	7.09	5.93	7.39	6.16
29	R1	4.09	7.09	5.35	3.69	6.99	4.84
	R2	6.18	7.39	7.36	5.14	5.79	6.35
	R3	5.94	5.55	5.74	4.13	5.81	6.02
36	R1	5.94	7.34	5.72	2.46	6.9	6.04
	R2	5.65	6.77	6.42	2.47	6.04	5.5
	R3	5.93	6.29	4.92	3.99	5.3	5.94
43	R1	5.42	5.7	5.15	3.83	5.68	3.98
	R2	4.02	6.11	5.08	3.66	6.56	5.23
	R3	4.29	6	5.14	3.73	4.9	3.71
50	R1	2.74	7.08	3.53	3.78	5.72	4.26
	R2	3.62	5.85	3.72	3.56	3.24	3.8
	R3	4.48	5.64	4.48	3.86	2.56	3.53

[y] NS-BA, non-supplemented yogurt inoculated with *B. animalis*; NS-LA, non-supplemented yogurt inoculated with *L. acidophilus*; NS-P, non-supplemented yogurt inoculated with both probiotics; PE-BA, 0.5% plant extract supplemented yogurt inoculated with *B. animalis*; PE-LA, 0.5% plant extract supplemented yogurt inoculated with *L. acidophilus*; PE-P, 0.5% plant extract supplemented yogurt inoculated with both probiotics; SA-BA, 0.25% sodium acetate supplemented yogurt inoculated with *B. animalis*; SA-LA, 0.5% sodium acetate supplemented yogurt inoculated with *L. acidophilus*; SA-P, 0.5% sodium acetate supplemented yogurt inoculated with both probiotics

I.V. Initial values before fermentation

Table C.23 Total solids (% w/w) of various yogurts[y] during storage

Replication	NS-P	PE-P	SA-P	NS-BA	PE-BA	SA-BA	NS-LA	PE-LA	SA-LA
R1	14.61	14.65	14.69	14.47	14.81	14.82	14.7	14.86	14.68
R2	14.4	14.5	14.3	14.5	14.56	14.28	14.56	14.55	14.71
R3	14.48	14.53	14.29	14.43	14.81	14.56	14.54	14.44	14.7

[y] NS-BA, non-supplemented yogurt inoculated with *B. animalis*; NS-LA, non-supplemented yogurt inoculated with *L. acidophilus*; NS-P, non-supplemented yogurt inoculated with both probiotics; PE-BA, 0.5% plant extract supplemented yogurt inoculated with *B. animalis*; PE-LA, 0.5% plant extract supplemented yogurt inoculated with *L. acidophilus*; PE-P, 0.5% plant extract supplemented yogurt inoculated with both probiotics; SA-BA, 0.25% sodium acetate supplemented yogurt inoculated with *B. animalis*; SA-LA, 0.5% sodium acetate supplemented yogurt inoculated with *L. acidophilus*; SA-P, 0.5% sodium acetate supplemented yogurt inoculated with both probiotics

Table C.24 Firmness (g) of various yogurts[y] during storage

Replication	NS-P	PE-P	SA-P	NS-BA	PE-BA	SA-BA	NS-LA	PE-LA	SA-LA
R1	138.63	138.94	152.702	134.2	123.68	147.98	147.31	121.34	133.91
R2	148.54	136.26	136.87	128.19	140.07	131.18	144.22	124.82	144.18
R3	148.93	130.68	130.817	142.34	148.06	135.57	132.15	123.717	135.9

[y] NS-BA, non-supplemented yogurt inoculated with *B. animalis*; NS-LA, non-supplemented yogurt inoculated with *L. acidophilus*; NS-P, non-supplemented yogurt inoculated with both probiotics; PE-BA, 0.5% plant extract supplemented yogurt inoculated with *B. animalis*; PE-LA, 0.5% plant extract supplemented yogurt inoculated with *L. acidophilus*; PE-P, 0.5% plant extract supplemented yogurt inoculated with both probiotics; SA-BA, 0.25% sodium acetate supplemented yogurt inoculated with *B. animalis*; SA-LA, 0.5% sodium acetate supplemented yogurt inoculated with *L. acidophilus*; SA-P, 0.5% sodium acetate supplemented yogurt inoculated with both probiotics

C.4 Experiment-II: Fermentation study

Table C.25 Various parameters of non-supplemented yogurt fermented with *B. animalis* and *L. acidophilus* cultures (NS-P) during fermentation

Time (h:min)	Replication	pH	TA	Eh	Buffering capacity	*S. thermophilus* counts (log cfu mL^{-1})	*L. bulgaricus* counts (log cfu mL^{-1})	*B. animalis* counts (log cfu mL^{-1})	*L. acidophilus* counts (log cfu mL^{-1})
0:00	R1	6.5	0.21	276	0.032	7.23	7.1	6.64	7.1
	R2	6.48	0.2	276.8	0.025	7.07	7.15	6.96	7.23
1:00	R1	5.95	0.32	291.5	0.032	6.81	6.86	6.71	6.89
	R2	5.74	0.36	260.9	0.035	7.24	7.41	7.09	7.37
2:00	R1	5.58	0.43	285.1	0.04	6.85	6.86	6.73	6.89
	R2	5.55	0.45	272.7	0.042	7.34	7.47	6.75	7.43
3:00	R1	5.1	0.65	289.7	0.044	7.94	7.01	6.97	7.12
	R2	5.19	0.61	285.6	0.046	8.02	7.75	7.27	7.73
4:00	R1	4.83	0.75	299.1	0.047	7.71	7.31	7.41	7.31
	R2	4.94	0.78	292.6	0.053	8.22	8.33	7.46	8.3
5:00	R1	4.67	0.92	302.8	0.054	7.87	7.58	7.68	7.74
	R2	4.68	0.94	310.3	0.062	8.39	8.45	7.73	8.38
5:48	R1	4.5	0.96	306.7	0.056	8.47	7.92	7.6	7.96
5:40	R2	4.5	1.02	322.5	0.064	8.47	8.57	7.86	8.67

TA Titratable acidity (% lactic acid)
Eh Redox potential (mV)

Table C.26 Various parameters of non-supplemented yogurt fermented with *B. animalis* culture (NS-BA) during fermentation

Time (h:min)	Replication	pH	TA	Eh	Buffering capacity	*S. thermophilus* counts (log cfu mL^{-1})	*L. bulgaricus* counts (log cfu mL^{-1})	*B. animalis* counts (log cfu mL^{-1})
0:00	R1	6.48	0.2	288	0.021	7.15	7.07	6.78
	R2	6.47	0.2	274.1	0.024	7.06	7.11	6.91
1:00	R1	5.9	0.32	291.8	0.028	7.18	7.15	6.96
	R2	5.93	0.34	276.6	0.031	7.19	7.26	7.09
2:00	R1	5.5	0.43	297.1	0.039	7.91	7.73	7.15
	R2	5.7	0.4	279.7	0.037	7.21	7.49	7.14
3:00	R1	5.08	0.69	300.8	0.04	8.39	8.2	7.33
	R2	5.36	0.55	278.9	0.041	7.57	7.69	7.32
4:00	R1	4.73	0.83	303.1	0.05	8.61	8.38	7.8
	R2	4.97	0.77	307	0.051	7.87	7.86	7.38
5:00	R1	4.56	0.94	309.4	0.055	8.38	8.2	7.63
	R2	4.71	0.9	316.4	0.056	8.08	8.17	7.43
5:10	R1	4.5	0.97	311	0.056	8.46	8.28	7.58
5:33	R2	4.5	0.98	326.8	0.06	8.11	8.16	7.21

TA Titratable acidity (% lactic acid)
Eh Redox potential (mV)

Table C.27 Various parameters of non-supplemented yogurt fermented with *L. acidophilus* culture (NS-LA) during fermentation

Time (h:min)	Replication	pH	TA	Eh	Buffering capacity	*S. thermophilus* counts (log cfu mL^{-1})	*L. bulgaricus* counts (log cfu mL^{-1})	*L. acidophilus* counts (log cfu mL^{-1})
0:00	R1	6.5	0.21	274.4	0.024	7.14	7.06	7.14
	R2	6.49	0.2	280.8	0.024	7.17	7.17	7.2
1:00	R1	5.93	0.31	288.6	0.032	7.17	7.22	7.22
	R2	5.9	0.33	286.4	0.032	7.2	7.41	7.42
2:00	R1	5.54	0.47	287.2	0.038	7.81	7.26	7.24
	R2	5.7	0.39	284.9	0.033	7.87	7.4	7.43
3:00	R1	5.01	0.69	291.6	0.042	8.3	7.35	7.35
	R2	5.38	0.48	282.9	0.036	8.26	7.66	7.62
4:00	R1	4.73	0.76	293.9	0.047	8.36	7.37	7.4
	R2	4.93	0.8	288.7	0.042	8.37	7.9	7.83
5:00	R1	4.68	0.84	300	0.05	8.2	8.11	8.13
	R2	4.72	0.96	298.1	0.046	8.38	8.19	7.98
6:20	R1	4.5	0.94	305.5	0.056	8.31	8.59	8.68
6:18	R2	4.5	1.01	324.2	0.05	8.46	8.39	8.38

TA Titratable acidity (% lactic acid)
Eh Redox potential (mV)

Table C.28 Various parameters of 0.5% plant extract supplemented yogurt fermented with *B. animalis* and *L. acidophilus* culture (PE-P) during fermentation

Time (h:min)	Replication	pH	TA	Eh	Buffering capacity	*S. thermophilus* counts (log cfu mL^{-1})	*L. bulgaricus* counts (log cfu mL^{-1})	*B. animalis* counts (log cfu mL^{-1})	*L. acidophilus* counts (log cfu mL^{-1})
0:00	R1	6.52	0.21	228.9	0.028	7.04	7.09	6.99	7.18
	R2	6.49	0.19	230.1	0.027	7.07	7.34	7.05	7.1
1:00	R1	5.91	0.36	261.3	0.042	7.14	7.06	7.06	7.28
	R2	5.83	0.39	262.4	0.04	7.15	7.21	7.02	7.14
2:00	R1	5.71	0.45	260.1	0.054	7.3	7.32	7.12	7.38
	R2	5.63	0.47	268.9	0.048	7.34	7.33	7.09	7.31
3:00	R1	5.4	0.58	260.9	0.064	7.67	7.39	7.24	7.41
	R2	5.32	0.61	272.3	0.056	7.57	7.41	7.3	7.52
4:00	R1	5.16	0.77	262.4	0.062	8.2	7.77	7.31	7.8
	R2	5.13	0.75	284.5	0.061	8.19	7.9	7.22	7.88
5:00	R1	4.97	0.92	277	0.072	8.4	7.97	7.34	8.05
	R2	4.94	0.95	295.6	0.066	8.35	8.06	7.26	8.38
6:00	R1	4.86	1.06	297.7	0.078	8.49	8.17	7.36	8.26
	R2	4.86	1.04	301.1	0.069	8.45	8.49	7.23	8.53
7:00	R1	4.72	1.11	305.7	0.083	8.69	8.34	7.45	8.41
	R2	4.74	1.16	311.8	0.069	8.54	8.56	7.26	8.62
8:07	R1	4.5	1.28	313.6	0.089	8.76	8.38	7.59	8.52
8:42	R2	4.5	1.33	320.6	0.075	8.59	8.7	7.34	8.73

TA Titratable acidity (% lactic acid)
Eh Redox potential (mV)

Table C.29 Various parameters of 0.5% plant extract supplemented yogurt fermented with *B. animalis* culture (PE-BA) during fermentation

Time (h:min)	Replication	pH	TA	Eh	Buffering capacity	*S. thermophilus* counts (log cfu mL^{-1})	*L. bulgaricus* counts (log cfu mL^{-1})	*B. animalis* counts (log cfu mL^{-1})
0:00	R1	6.5	0.2	230.4	0.025	7.16	7.13	6.97
	R2	6.49	0.21	227.7	0.026	7.18	7.22	7.03
1:00	R1	6.02	0.3	260.7	0.036	7.05	7.09	6.88
	R2	6.01	0.36	264.1	0.036	7.18	7.18	7.02
2:00	R1	5.82	0.41	272.8	0.038	7.16	7.23	7.11
	R2	5.84	0.41	268.5	0.046	7.5	7.61	7.11
3:00	R1	5.45	0.53	276.8	0.054	7.97	7.88	7.23
	R2	5.53	0.51	268.5	0.052	8.22	8.19	7.2
4:00	R1	5.12	0.74	281.1	0.058	8.61	8.33	7.34
	R2	5.22	0.71	272.2	0.058	8.63	8.67	7.17
5:00	R1	4.92	0.98	305.2	0.076	8.88	8.88	7.37
	R2	4.99	0.9	281.1	0.058	8.85	8.87	7.32
6:00	R1	4.72	1.14	308.9	0.082	9.02	9.04	7.6
	R2	4.79	1.1	301.1	0.062	8.91	8.9	7.41
7:05	R1	4.5	1.32	314.5	0.083	8.94	8.98	7.7
7:34	R2	4.5	1.35	326.8	0.085	9	9.04	7.34

TA Titratable acidity (% lactic acid)
Eh Redox potential (mV)

Table C.30 Various parameters of 0.5% plant extract supplemented yogurt fermented with *L. acidophilus* culture (PE-LA) during fermentation

Time (h:min)	Replication	pH	TA	Eh	Buffering capacity	*S. thermophilus* counts (log cfu mL^{-1})	*L. bulgaricus* counts (log cfu mL^{-1})	*L. acidophilus* counts (log cfu mL^{-1})
0:00	R1	6.52	0.21	235.4	0.028	7.08	7.23	7.26
	R2	6.49	0.2	226.1	0.028	7.08	7.26	7.17
1:00	R1	6.11	0.33	262	0.038	7.17	7.24	7.35
	R2	6	0.33	255.8	0.037	7.13	7.39	7.43
2:00	R1	5.88	0.39	267.8	0.046	7.15	7.31	7.33
	R2	5.83	0.38	270.8	0.046	7.17	7.57	7.55
3:00	R1	5.6	0.51	268.4	0.053	7.28	7.35	7.37
	R2	5.64	0.47	279.9	0.055	7.28	7.72	7.6
4:00	R1	5.31	0.7	280	0.058	7.96	7.44	7.56
	R2	5.4	0.63	288.5	0.061	7.56	7.9	7.95
5:00	R1	5.11	0.84	287.1	0.064	8.34	7.8	7.89
	R2	5.23	0.74	294.7	0.061	8.01	8.16	8.27
6:00	R1	4.99	0.94	293.4	0.07	8.15	8.06	8.05
	R2	5.05	0.84	317.3	0.066	8.17	8.42	8.37
7:00	R1	4.87	1.05	297.9	0.074	8.32	8.23	8.21
	R2	4.9	0.9	323.4	0.075	8.41	8.58	8.54
8:00	R1	4.69	1.14	304.3	0.076	8.36	8.51	8.5
	R2	4.78	1.08	327	0.073	8.38	8.71	8.7
8:40	R1	4.5	1.28	308.9	0.082	8.36	8.5	8.53
8:55	R2	4.5	1.27	333.8	0.077	8.44	8.74	8.71

TA Titratable acidity (% lactic acid)
Eh Redox potential (mV)

Table C.31 Various parameters of 0.25% sodium acetate supplemented yogurt fermented with *B. animalis* and *L. acidophilus* culture (SA-P) during fermentation

Time (h:min)	Replication	pH	TA	Eh	Buffering capacity	*S. thermophilus* counts (log cfu mL^{-1})	*L. bulgaricus* counts (log cfu mL^{-1})	*B. animalis* counts (log cfu mL^{-1})	*L. acidophilus* counts (log cfu mL^{-1})
0:00	R1	6.52	0.2	239.9	0.023	6.93	7.05	6.95	7.02
	R2	6.52	0.2	226.7	0.025	7.12	7.25	7.1	7.19
1:00	R1	5.89	0.35	256.8	0.033	7.21	6.95	6.96	7.2
	R2	5.78	0.37	279.8	0.037	7.11	7.45	7.1	7.68
2:00	R1	5.68	0.44	268.1	0.038	7.14	7.09	7.21	7.41
	R2	5.56	0.49	276.8	0.046	7.18	7.71	7.24	7.82
3:00	R1	5.37	0.57	272.4	0.048	7.26	7.25	7.39	7.29
	R2	5.33	0.61	280.1	0.05	8.3	7.83	7.12	7.78
4:00	R1	5.06	0.77	280.4	0.049	7.72	7.8	7.63	8.21
	R2	5.11	0.75	296.3	0.05	8.35	8.38	7.1	8.42
5:00	R1	4.86	0.93	291.6	0.055	7.72	8.14	7.58	8.36
	R2	4.87	0.96	302.6	0.056	8.29	8.68	7.16	8.42
6:00	R1	4.69	1.06	311	0.061	8.72	8.5	7.71	8.82
	R2	4.72	1.07	298.2	0.06	8.57	8.72	7.2	8.62
6:50	R1	4.5	1.18	322.7	0.064	8.83	8.51	7.73	8.88
7:08	R2	4.5	1.16	310.8	0.064	8.67	8.72	7.33	8.78

TA Titratable acidity (% lactic acid)
Eh Redox potential (mV)

Table C.32 Various parameters of 0.25% sodium acetate supplemented yogurt fermented with *B. animalis* culture (SA-BA) during fermentation

Time (h:min)	Replication	pH	TA	Eh	Buffering capacity	*S. thermophilus* counts (log cfu mL^{-1})	*L. bulgaricus* counts (log cfu mL^{-1})	*B. animalis* counts (log cfu mL^{-1})
0:00	R1	6.5	0.2	223.2	0.022	7.11	6.99	6.92
	R2	6.48	0.2	235.5	0.028	7.04	7.1	7.1
1:00	R1	6.01	0.3	244	0.032	7.14	7.06	6.91
	R2	5.98	0.35	276.1	0.035	7.11	7.05	7.12
2:00	R1	5.72	0.39	256.8	0.04	7.36	7.38	7.14
	R2	5.74	0.41	280.2	0.048	7.46	7.42	7.25
3:00	R1	5.35	0.56	268.4	0.048	8.28	8.32	6.85
	R2	5.36	0.55	282.1	0.054	8.12	8.11	7.32
4:00	R1	4.94	0.85	289.1	0.055	8.81	8.65	7.34
	R2	5.12	0.73	283.1	0.056	8.39	8.5	7.48
5:00	R1	4.72	1	302.2	0.058	8.85	8.58	7.54
	R2	4.92	0.91	299.7	0.061	8.54	8.76	7.42
6:00	R1	4.52	1.2	316.8	0.068	8.77	8.68	7.64
	R2	4.64	1.12	319.7	0.071	8.63	8.72	7.35
6:10	R1	4.5	1.21	319.9	0.068	8.66	8.7	7.68
6:34	R2	4.5	1.22	323.1	0.078	8.69	8.71	7.38

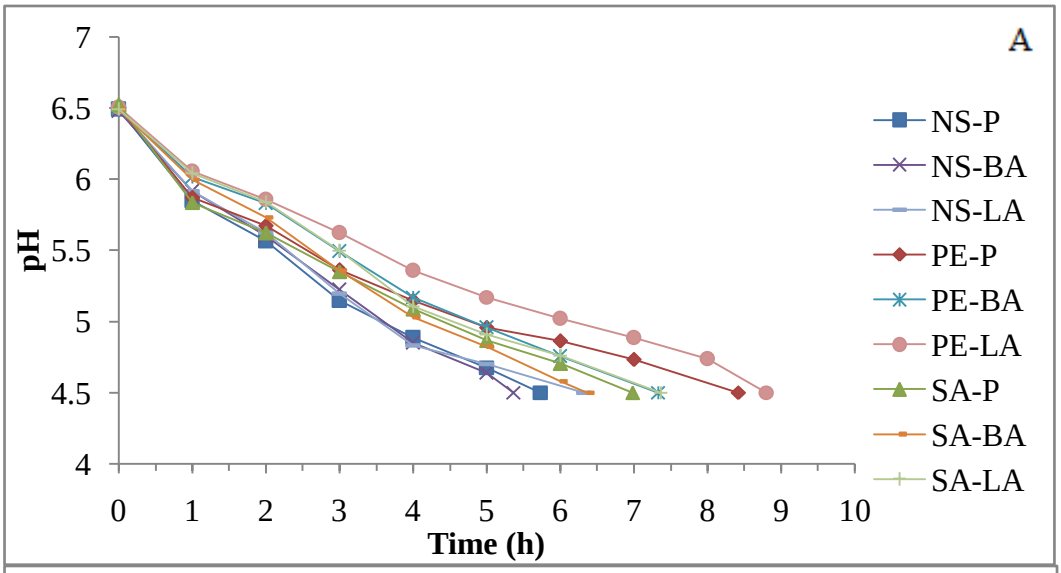
TA Titratable acidity (% lactic acid)
Eh Redox potential (mV)

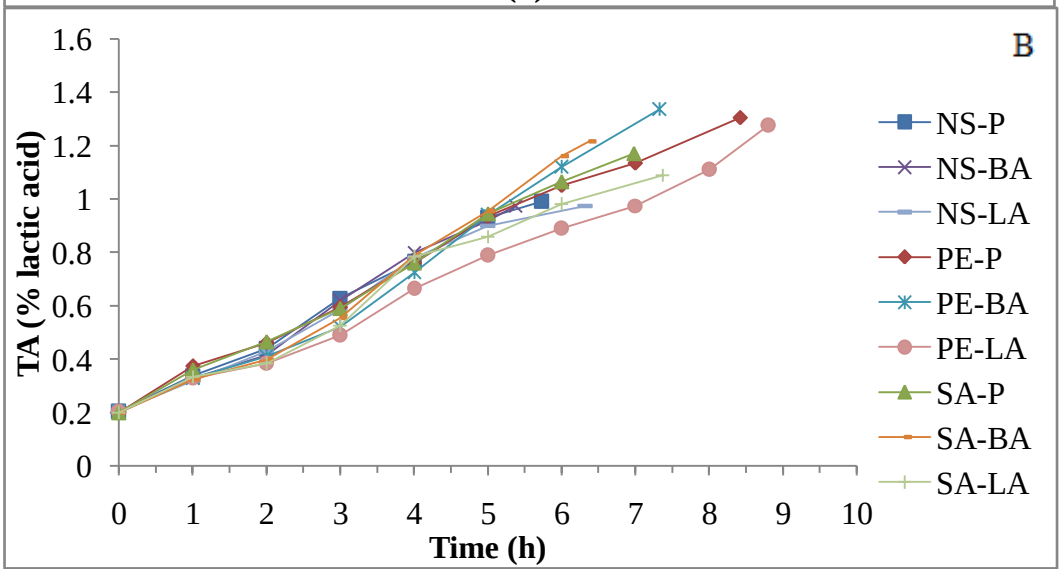
Table C.33 Various parameters of 0.25% sodium acetate supplemented yogurt fermented with *L. acidophilus* culture (SA-LA) during fermentation

Time (h:min)	Replication	pH	TA	Eh	Buffering capacity	*S. thermophilus* counts (log cfu mL^{-1})	*L. bulgaricus* counts (log cfu mL^{-1})	*L. acidophilus* counts (log cfu mL^{-1})
0:00	R1	6.5	0.2	230	0.023	7.15	7.11	7.21
	R2	6.48	0.2	229.6	0.026	7.08	7.19	7.2
1:00	R1	6.1	0.31	252.6	0.03	6.58	7.3	7.25
	R2	5.98	0.36	267	0.034	7.12	7.18	7.36
2:00	R1	5.82	0.38	268.4	0.039	7.67	7.38	7.33
	R2	5.86	0.39	272	0.038	7.44	7.45	7.3
3:00	R1	5.41	0.54	269.8	0.05	8.05	7.76	7.84
	R2	5.58	0.51	280.6	0.048	8.11	7.72	7.72
4:00	R1	5.04	0.82	281.7	0.052	8.64	7.86	7.87
	R2	5.17	0.75	280.7	0.05	8.45	7.78	8.08
5:00	R1	4.86	0.85	286.1	0.05	8.68	7.93	7.72
	R2	4.95	0.87	286.1	0.055	8.57	8.2	8.17
6:00	R1	4.72	1.01	306.2	0.058	8.72	8.28	8.27
	R2	4.8	0.95	295.1	0.063	8.74	8.4	8.42
7:00	R1	4.5	1.1	318.9	0.065	8.6	8.38	8.3
7:43	R2	4.5	1.08	318.3	0.075	8.79	8.58	8.43

TA Titratable acidity (% lactic acid)
Eh Redox potential (mV)

Appendix D - Graphs of fermentation study of experiment-II





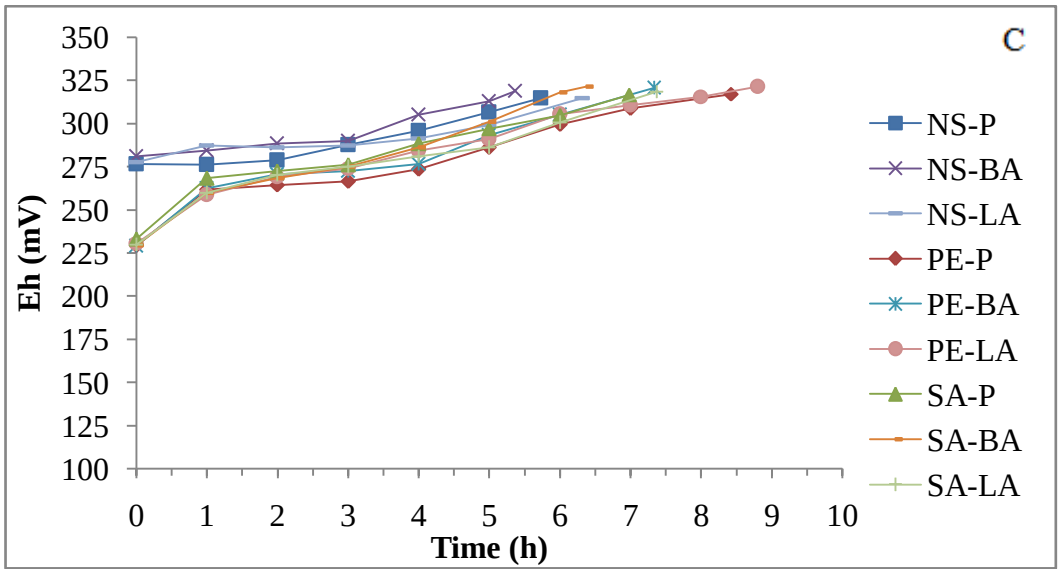


Figure D.1 pH, titratable acidity (TA) and redox potential (Eh) of yogurt mixes during fermentation: (A) mean pH (n = 2); (B) mean TA (n = 2); (C) mean Eh (n = 2). Non-supplemented yogurt fermented with starter cultures and both probiotics (NS-P), *B. animalis* (NS-BA) or *L. acidophilus* (NS-LA); 0.5% plant extract supplemented yogurt fermented with starter cultures and both probiotics (PE-P), *B. animalis* (PE-BA) or *L. acidophilus* (PE-LA); 0.25% sodium acetate supplemented yogurt fermented with starter cultures and both probiotics (SA-P), *B. animalis* (SA-BA) or *L. acidophilus* (SA-LA).

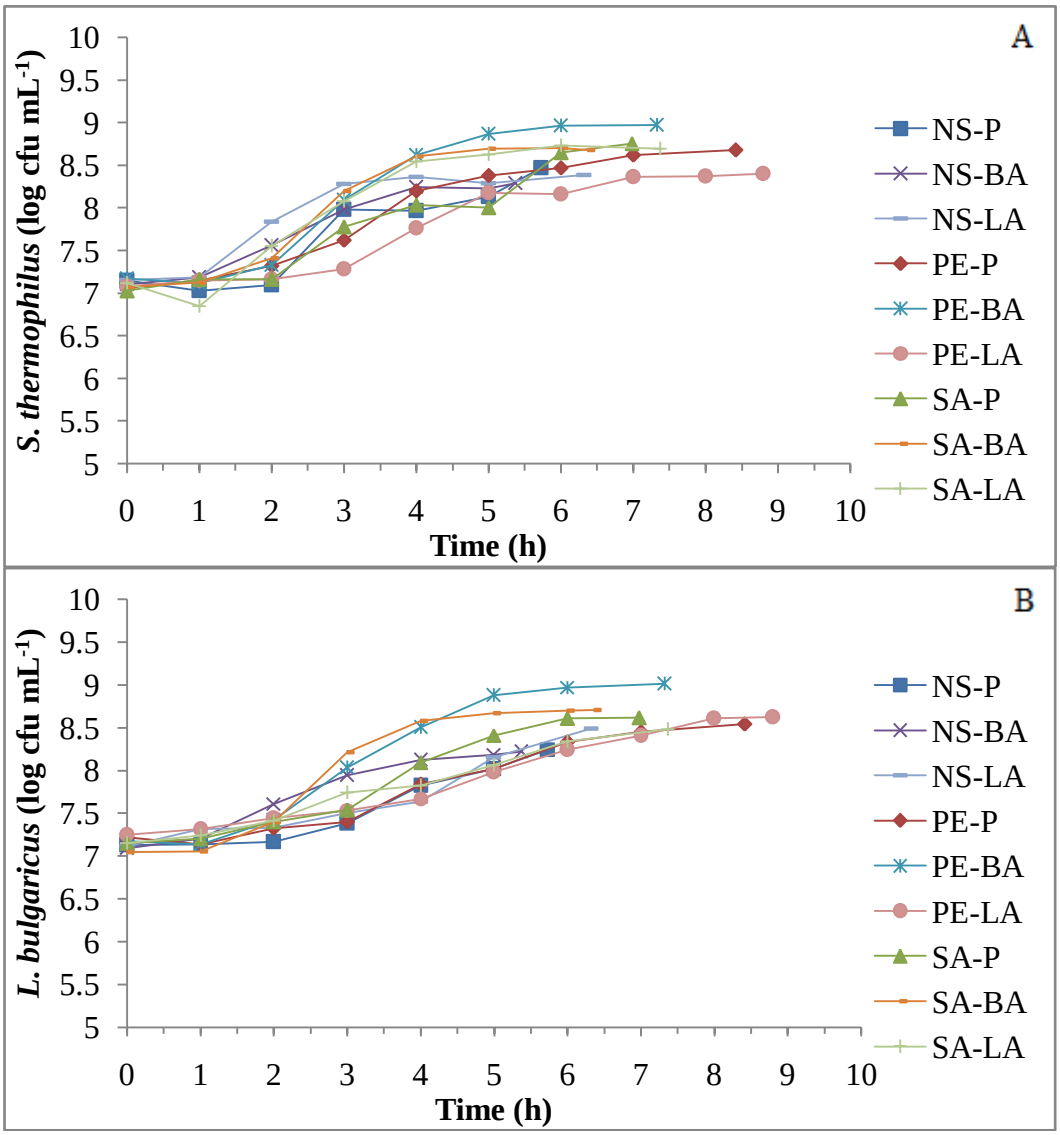


Figure D.2 Counts of yogurt starter bacteria of yogurt mixes during fermentation: (A) mean *S. thermophilus* counts (n = 2); (B) mean *L. bulgaricus* counts (n = 2). Non-supplemented yogurt fermented with starter cultures and both probiotics (NS-P), *B. animalis* (NS-BA) or *L. acidophilus* (NS-LA); 0.5% plant extract supplemented yogurt fermented with starter cultures and both probiotics (PE-P), *B. animalis* (PE-BA) or *L. acidophilus* (PE-LA); 0.25% sodium acetate supplemented yogurt fermented with starter cultures and both probiotics (SA-P), *B. animalis* (SA-BA) or *L. acidophilus* (SA-LA).

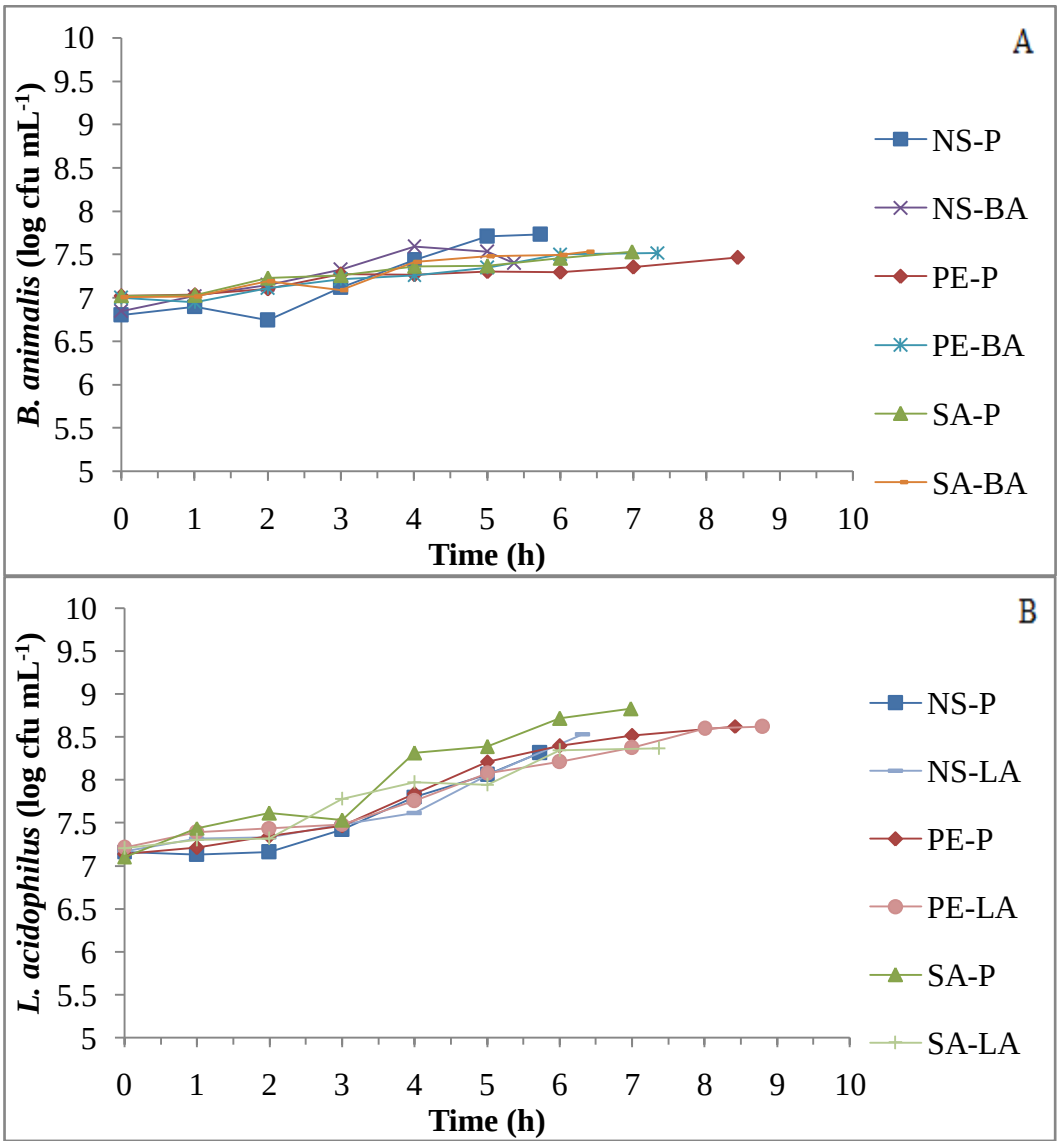


Figure D.3 Counts of probiotic bacteria of yogurt mixes during fermentation: (A) mean *B. animalis* counts (n = 2); (B) mean *L. acidophilus* counts (n = 2). Non-supplemented yogurt fermented with starter cultures and both probiotics (NS-P), *B. animalis* (NS-BA) or *L. acidophilus* (NS-LA); 0.5% plant extract supplemented yogurt fermented with starter cultures and both probiotics (PE-P), *B. animalis* (PE-BA) or *L. acidophilus* (PE-LA); 0.25% sodium acetate supplemented yogurt fermented with starter cultures and both probiotics (SA-P), *B. animalis* (SA-BA) or *L. acidophilus* (SA-LA).