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Evaluation Of Rice Extract As A Potential Stabilizer In Plain Yogurt

Sangeetha Nurani Viswanathan
North Carolina Agricultural and Technical State University

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Sangeetha Nurani Viswanathan

North Carolina A&T State University

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Sangeetha Nurani Viswanathan

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Approved by:

Dr. Salam A. Ibrahim Major Professor	Dr. Abolghasem Shahbazi Committee Member
Dr. Ipek Goktepe Committee Member	Dr. Valerie L. Giddings Department Chairperson

Dr. Sanjiv Sarin
Associate Vice Chancellor for Research and Graduate Dean

Biographical Sketch

Sangeetha Nurani Viswanathan was born in Chennai, India. She received her Bachelor's degree in Nutrition and Dietetics from the University of Madras, India. She moved to New Zealand where she pursued a Post Graduate Diploma in Food science from the University of Auckland, and also gained valuable work experience working for a beverage manufacturing company. Her keen interest in food science motivated her to enroll at North Carolina A&T State University for her graduate study. During her study, she served as a Graduate Research Assistant under the close supervision of Dr. Salam A. Ibrahim in the Department of Family and Consumer Sciences. Her research was focused on product development with emphasis on probiotics. Ms. Viswanathan was also a recipient of Wadran Kennedy Scholar award (2010 and 2011) for excellence in academic achievement. She also reviewed book chapters and scientific articles, under the direction of Dr. Salam A. Ibrahim and Dr. Mehrdad Tajkarimi. She is also a member of the Institute of Food Technologists (IFT).

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Nomenclature

ºC	Celsius
CFU	Colony Forming Units
CFU/ml	Colony Forming Units per Milliliters
h	Hour(s)
µL	Microliters
MRS	de Man, Rogosa and Sharpe
WPC	Whey Protein Concentrate
MPC	Milk Protein Concentrate
FAO	Food and Agriculture Organization
WHO	World Health Organization
GME	Gelatin Manufacturers of Europe
ANOVA	Analysis of Variance
RE	Rice Extract
Spp	Species

Abstract

Quality and consumer acceptability are essential parameters in dairy products. The objectives of the present study were to examine the microbiological (viability of *L. bulgaricus*, *S. thermophilus*, and bifidobacteria), chemical (pH, titratable acidity, and total solids), and physical (syneresis) qualities of yogurt enriched with rice extract as a stabilizer during 28 days of storage at 4°C. Additionally, viscosity measurements and consumer acceptability were determined for yogurt enriched with rice extract. Results showed that the viability of *L. bulgaricus* was maintained with the addition of rice extract. Population of *Streptococcus thermophilus* and bifidobacteria decreased significantly ($p<0.05$) in the control sample but the addition of rice extract at 15% concentration supported the viability of bifidobacteria in yogurt over storage. The addition of rice extract did not alter the pH or titratable acidity of yogurt. Viscosity, total solids and syneresis values slightly changed with the addition of rice extract. Yogurt prepared with rice extract ranked higher in terms of texture and appearance by the panelists. Our results indicated that the addition of rice extract could improve the quality characteristics and consumer acceptability of plain yogurt. Hence, rice extract has a promising potential to be used as an alternative stabilizer in dairy products.

CHAPTER 1

Introduction

Yogurt is a product formed by the fermentation of lactic acid in milk by the addition of a starter culture containing *Lactobacillus delbrueckii* spp. b*ulgaricus* and *Streptococcus thermophilus*. The versatility of yogurt, along with its acceptance as a healthy and nutritious food, has led to its widespread popularity across all population subgroups (Mckinley, 2005).

Body, texture, flavor, and shelf-life play a pivotal role in the marketability of any food product. The most frequent defects related to yogurt texture that may lead to consumer rejection are apparent viscosity variations and the occurrence of syneresis. To combat these defects, stabilizers and hydrocolloids have been added to yogurt (Keogh & O'Kennedy, 1998). Some of the common ingredients used are dry dairy ingredients (nonfat dry milk, whey protein concentrates, milk protein concentrates), gelatin and pectin. Due to their low cost and availability, starch and its derivatives are very popular ingredients in dairy systems (Hunt & Maynes, 1997).

In recent years, rice, especially rice flour, because of its unique functional properties, is being used in increasing numbers of novel foods such as tortillas, beverages, processed meats, puddings, salad dressing, and gluten-free breads (Kadan & Ziegler, 1989; McCue 1997; Kadan, Robinson, Thibodeux, & Pepperman, 2001). Therefore, the overall objective of this research was to study the effect of rice extract as a potential stabilizer in dairy products. The specific objectives of this research were: (a) to examine the microbiological, chemical and physical quality of yogurt prepared with rice extracts as stabilizer during 28 days of storage at 4°C, (b) to measure the viscosity of yogurt made with rice extract and (c) to determine consumer acceptability of yogurt made with rice extract.

CHAPTER 2

Literature Review

2.1 Functional Foods

Consumer interest in the relationship between diet and health has increased substantially. Over the last two decades, changing concepts in nutrition have led to the birth of functional foods. Today, as the field of nutritional science advances, there is increasing scientific evidence to support the hypothesis that some foods and food components have beneficial psychological effects over and above the provision of the basic nutrients. Though there has not been a legislative definition coined for the term functional food, it is generally referred to as those foods intended to be consumed as part of the normal diet and that contain biologically active components which offer the potential of enhanced health or reduced risk of diseases (Roberfroid, 1999).

Progress in biosciences indicates that diet could modulate various health relevant functions in the body beyond providing basic nutrition, thus emphasizing the promising use of foods to promote a state of well-being, better health and reduction of the risk of disease. The concept of functional foods is becoming popular among consumers as interest in achieving and maintaining good health has become a priority among consumers. Advances in food science and technology has presented the food and nutrition industry with a challenge to provide a wide array of healthy, processed or ready-to-eat foods for the busy consumer (Mollet & Rowland, 2002).

Hilliam (2000) reported that the global market of functional food is estimated to be at least $33 billion, based on a definition of functional food by which ingredients with an additional health-value have been added to foods (and this is announced to the consumers). A study done by Sloan (2002), indicated that the global functional food market rose from around $30 billion in

1995 to $47.6 billion in 2002, with the United States being the largest market segment, followed by Europe and Japan.

Functional foods usually contain one or more beneficial compounds such as prebiotic, probiotic, antioxidant polyphenols and sterols, carotenoids, and others (Shah, 2001). Foods fortified with vitamins and/or minerals such as vitamin C, vitamin E, folic acid, zinc, iron, and calcium were the earliest developments of functional foods (Sloan, 2000). Later on, the focus shifted to foods fortified with various micronutrients such as omega-3 fatty acid, phytosterol, and soluble fiber to promote good health or to prevent diseases such as cancers (Sloan, 2002).

Recently, food companies have put together efforts to develop food products that offer multiple health benefits in a single food (Sloan, 2004). Table 1 summarizes the different methods by which functional property can be incorporated into food products (Spence, 2006).

Table 1

Different ways of incorporating functional properties to food products

Type	Description	Examples
Fortified products	Increasing the content of existing nutrients	Grain products fortified with folic acid, fruit juices fortified with vitamin C
Enriched products	Adding new nutrients or components not normally found in a particular food	Orange juice with added calcium, plant sterol esters in margarines, foods with probiotics and prebiotics
Altered products	Harmful or undesirable components replaced by beneficial components	Grain-based high fiber fat replacers
Enhanced Commodities	Changes in the raw commodities that have altered nutrient composition	High lysine corn, golden rice, carotenoid containing potatoes

Source: Spence, 2006

Based on consumer health concerns and product preferences, functional products have been mainly launched in the dairy, confectionery, soft-drinks, bakery, and baby-food markets

(Menrad, 2003).

2.1.1 Functional dairy products. Milk and dairy products represent one of the major food groups that make up a balanced diet. Milk is an excellent source of nutrients, and milk-derived components have many beneficial physiological properties. Some of the dairy components and their health claims have been illustrated in Table 2 (Shortt, Shaw, & Mazza, 2004).

Table 2

Dairy components and ingredients in functional foods and their health claims

Ingredients	Sources	Claim Areas
Minerals	Calcium Casein peptides	Optimum growth and development, dental health, osteoporosis
Fatty Acids	CLA	Heart disease, cancer prevention, weight control
Prebiotics/carbohydrates	Galactooligosaccharides Lactulose Lactose	Digestion, pathogen prevention, gut flora balance, immunity, lactose intolerance
Probiotics	Lactic acid bacteria Bifidobacteria	Digestion, immunity, vitamin, production, heart disease, antitumor activity, remission of inflammatory bowel disease, prevention of allergy, alleviation of diarrhea.
Proteins/peptides	Caseins, whey proteins, immunoglobins, lactoferrin, glycoproteins, specific peptides	Immunomodulation, growth, antibacterial activity, dental health, hypertension, regulation (angiotensin inhibitors)

Source: Shortt et al., 2004

Functional dairy products could include a wide variety of products that are based on milk that is enriched with a functional component, or the product is based on ingredients originating from milk. Therefore, functional dairy products using milk as base or using dairy-derived components have great potential to contribute to the functional food market. Yogurt, which

contains probiotic bacteria and quite frequently enriched with prebiotics, is the most common functional dairy product (Saxelin, Korpela, & Mäyrä-Mäkinen, 2003). Market analyst Datamonitor has evaluated the yogurt market in the United States to be about $7 billion and expected to grow further. The key factor driving sales growth could be attributed to the increasing demand from consumers for dairy products with functional properties.

In Europe, dairy products account for approximately 60% of the functional food market (Shortt et al., 2004). In the U.S., with consumers spending $5.0 billion on functional dairy products in 2004, they were the second most popular category of functional foods (Vierhile, 2006).

2.2 Probiotics

Huis in't Veld and Havenaar (1991) defined probiotics as being 'a mono- or mixed-culture of live microorganisms which, applied to man or animal (e.g. as dried cells or as a fermented product), beneficially affect the host by improving the properties of the indigenous microflora'. This definition indicates that the live microorganisms found in probiotic products like yogurt, has beneficial effects in the gastrointestinal tract and boosts the health status of the host. Many other definitions of the term probiotic have been published (Sanders, 2003); however, the most widely accepted definition is that "probiotics are live microorganisms, administrated in certain quantities that confer health benefits to the host" (FAO/WHO, 2001).

Various strains of lactic acid bacteria have been described as probiotic, although relatively few meet the standards of the United Nations of having clinical trial documentation, and many die en route to the gut due to their sensitivity to intense acidity and presence of bile salts in the gastrointestinal tract (Hekmat & Reid, 2006). Among the probiotic products available in the market, majority of them contain *Lactobacillus* and *Bifidobacterium* species, and have

been characterized as probiotics (FAO/WHO, 2001). Of the common probiotics, lactic acid bacteria of the genera *Lactobacillus* and *Bifidobacterium* are widely used in the food industry of which, *Lactobacillus acidophilus, L. casei, L. johnsonii, L.rhamnosus, L. thermophilus, L. reuteri, L. delbrueckii* subsp. *bulgaricus, Bifidobacterium bifidum, B. longum, B. brevis, B. infantis,* and *B.animalis* are commonly used species (Vasiljevic & Shah, 2008).

The microorganisms *Propionibacterium freudenreichii* and *Saccharomyces cerevisiae* are now regarded as non-lactic microorganisms associated with probiotic activities, especially in pharmaceutical and animal products, while other lactic acid bacteria with probiotic properties are: *Enterococcus faecalis, E. faecium*, and *Sporolactobacillus inulinus* (Holzapfel & Schillinger, 2002). Even though the yogurt starter cultures (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*) have been linked to improved lactose digestion and immune enhancement, they fail to fulfill the criteria for a probiotic microorganism as they are sensitive to conditions in the digestive tract and do not survive in very high numbers in the gut. Hence, there is still a disagreement whether or not yogurt starter culture should be considered as probiotics (Tejada-Simon, Lee, Ustunol, & Pestka, 1999). However, the results of *in vivo*, *in vitro*, clinical, and animal studies indicate that *Lactobacillus* and *Bifidobacterium* species are the ones that present more available data about their mechanisms of action and efficiency (Reid, 1999).

For organisms to achieve probiotic status, they must fulfill a number of criteria such as: be non-pathogenic, non toxic and generally recognized as safe (GRAS), acid tolerant, bile tolerant, viable and present in sufficient quantity during consumption, survive passage through the gastrointestinal tract, colonize at the target site, and survive during processing conditions and prolonged periods of storage (Saarela, Mogensen, Fondén, Mättö, & Mattila-Sandholm, 2000). It is imperative that the probiotic culture be present in a dairy food to a minimum level of 10^6

CFU/g or the daily intake should be about 10^8 CFU/g so as to compensate for the loss en route to the gut (Shah, 2007).

A number of food products have been developed to enhance their usage as probiotics, and dairy products have been used as the most common vehicle. Some examples include: fermented milk (Tamime, Marshall, & Robinson, 1995; Mital & Garg, 1992), cheese (Dinakar & Mistry, 1994), cottage cheese (Blanchette, Roy, Bélanger, & Gauthier, 1996), and ice cream (Hekmat & McMohan, 1992).

2.2.1 Consumption of probiotics and beneficial effects. The human intestinal tract harbors a complex ecosystem of microorganisms. 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). Beneficial health effects of probiotics are specific to the strain. Even strains of the same species will not exert the same health benefits (Schrezenmeir & de Vrese, 2001); hence, a study done on one strain cannot be extrapolated to a related strain.

The consumption of probiotic products is helpful in maintaining good health, restoring body vigor, and in combating intestinal and other disease orders (Mital & Garg, 1992). Figure 1 shows the health benefits attributed to the ingestion of probiotic-containing foods. Additional benefits related to probiotics include: antimicrobial (Forestier, De Champs, Vatoux, & Joly, 2001), antimutagenic activities (Lankaputhra & Shah, 1998), anticarcinogenic properties (Burns & Rowland, 2000), antihypertension properties (Lye, Kuan, Ewe, Fung, & Liong, 2009), beneficial effects on mineral metabolism, especially regarding bone stability (Arunachalam,

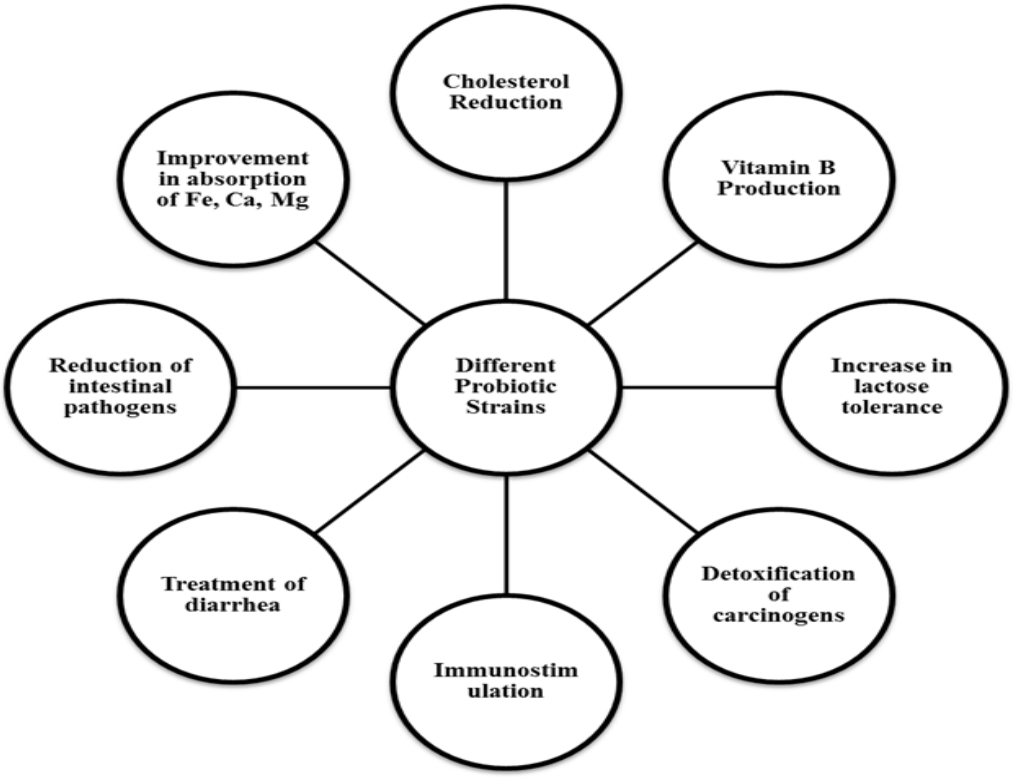


Figure 1. Different probiotic strains and beneficial effects

1999), attenuation of inflammatory bowel disease (Damaskos & Kolios, 2008), reduction of food allergies symptoms (Majamaa & Isolauri, 1997), and reduction of LDL-cholesterol levels (Sindhu & Khetarpaul, 2003). Some Lactobacillus strains have also shown to inhibit pathogenic microorganisms such as *Salmonella enteritidis, Escherichia coli, Shigella sonnei*, and *Serratia marcescens* (Drago, Gismondo, Lombardi, Haen, & Gozzoni, 1997).

To confer health benefits, the recommended concentration of probiotics in yogurt range from 6 to 8 log cfu/g (Güler-Akin & Akin, 2007; Vasiljevic & Shah, 2008; Vasiljevic, Kealy & Mishra, 2007). Although there is disagreement whether yogurt starter cultures should be considered probiotic, yogurt starter cultures fulfill all criteria (as mentioned above) to be considered as probiotics (Lomax & Calder, 2009; Guarner, Perdigon, Corthier, Salminen, Koletzko, & Morelli 2005; Salminen, Lahtinen, & Gueimonde, 2005) and have been reported to

confer health benefits (McKinley, 2005; Sarkar, 2008).

2.3 Yogurt

Yogurt was first introduced to the U.S. in the early 20th century and gained significant consumer popularity during the 1960's and 1970's. Popularity of yogurt is greatly attributed to Professor Elie Metchnikoff of the Pasteur Institute in Paris, who shared the Nobel Prize in Physiology and Medicine in 1908 and authored the book, "The Prolongation of Life" in which he advocated the health benefits of yogurt (Trachoo, 2002).

A vast array of yogurts is now available in the market to suit all palates and meal occasions. Yogurts are available in a variety of textures (e.g. liquid, set, smooth), fat contents (regular, low-fat, fat-free) and flavors (natural, fruit, cereal). The versatility of yogurt, along with its acceptance as a healthy and nutritious food, has led to its widespread popularity across all population subgroups (Mckinley, 2005). Yogurt is a product formed by the fermentation of lactic acid in milk by the addition of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. In some countries less traditional microorganisms, such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii* ssp. *lactis*, are sometimes mixed with the starter culture (McKinley, 2005).

The nutritional profile of yogurt can be attributed to that of milk from which it is made but will vary somewhat if fruit, cereal or other components are added. Since yogurt is often supplemented with milk solids, it is therefore a good source of protein, calcium, phosphorus, riboflavin (vitamin B2), thiamin (vitamin B1) and Vitamin B12, and a valuable source of folate, niacin, magnesium and zinc. The protein it provides contains all essential amino acids (high biological value), and the vitamins and minerals found in milk and dairy foods including yogurt are available for absorption and use by the body (bioavailable). Consuming dairy products, such

as yogurt, helps to improve the overall quality of the diet and increases the chances of achieving nutritional recommendations (Mckinley, 2005). It is also interesting to note that per capita consumption of yogurt has increased drastically because many consumers associate yogurt with good health (Hekmat & Reid, 2006).

In practice, commercial yogurts are obtained by the acidification of milk by bacterial cultures, which ferment lactose to lactic acid. The primary proteins in milk (casein) exist as micelles made of the four types namely α_{s1}, α_{s2}, β, and κ casein. It is proposed that the proteins are held together by hydrophobic interactions and by calcium phosphate bridges. A "hairy" layer made of κ-casein imparts a strong, repulsive, steric interaction that prevents casein micelle aggregation at the surface of the casein micelle. As the pH is lowered to 4.6, the isoelectric point of casein, the net electrostatic charge and repulsive steric interactions are diminished, resulting in the aggregation of the casein micelles and the formation of a protein network. Thus, yogurt gels are formed by this process (Considine, Noisuwan, Hemar, Wilkinson, Bronlund, & Kasapis, 2010). The physical attributes of yogurt, including whey separation play an important role in quality and consumer acceptance. Therefore, an understanding of the mechanisms involved during the yogurt formation along with the impact of processing conditions may be helpful in improving the quality and texture of yogurt (Lee & Lucey, 2004; Lee & Lucey, 2010).

2.4 Stabilizers in Yogurt

Yogurt texture is a very important characteristic that affects its quality (appearance, mouthfeel, and overall acceptability). The most frequent defects related to yogurt texture that may lead to consumer rejection are apparent viscosity variations and the occurrence of syneresis (Kroger, 1975). In an attempt to increase firmness and prevent syneresis, stabilizers and hydrocolloids have been added to yogurt (Keogh & O'Kennedy, 1998). Stabilizers induce

smoothness in body and texture, impart gel structure and help in preventing syneresis. They also form gel structures in water, thereby leaving less water for syneresis and in addition to that, some stabilizers can also form complexes with casein. Stabilizers may also increase shelf life and provide consistency in the product. Ideally, a yogurt stabilizer should not impart undesirable flavor, should be effective at low pH values, easily soluble, display good water holding capacity, and should promote gelation and adhesion. While choosing a stabilizer some points need to be considered: type of yogurt to be produced (set, stirred, drinkable etc.), formulation (fat content, total solids), firmness and consistency desired for the product, the type of ingredient (natural, organic, kosher) and possible masking effect on the flavoring system (Chandan & O'Rell, 2006). Some common stabilizers used in yogurt are discussed below.

2.4.1 Dry dairy ingredients. During yogurt processing, one of the most important steps is to increase the amount of total solids to provide better consistency, creaminess and texture. This involves milk fortification with dairy ingredients to increase protein content from 3.5% to 4–5%. Depending on legal standards, the sources of dry matter added in yogurt include skim milk powder, whey protein concentrate (WPC) or sodium caseinate (Lucey & Singh, 1998). It is also a common practice to add nonfat dry milk (NDM) by some manufacturers, although the amount of NDM that can be added to provide a firm body is limited, because too much NDM can lead to a powdery taste in the yogurt, and too much lactose from added NDM can cause excessive acid development, especially during storage (Mistry & Hassan, 1992). Quality control of dry dairy products may be difficult to achieve since the composition of commercial milk protein products is subject to variation due to differences in milk composition, processing methods and conditions strongly affecting protein composition (Karleskind, Laye, Mei, & Morr, 1995).

2.4.1.1 Whey Protein Concentrates (WPCs). WPCs are produced by ultrafiltration and drying of whey, and contain 34–88% protein. They are commonly used to substitute skim milk powder due to their availability and low cost, which make them desirable in yogurt formulation (Sodini, Mattas, & Tong, 2006). In addition, whey proteins offer functional properties such as gelation, foam formation, solubility and emulsification (Sodini, Montella, & Tong, 2005; Schmidt, Packard, & Morris, 1984). Whey protein concentrate has been added as an ingredient during yogurt preparation to reduce whey separation, increase firmness and enhance viscosity (Lucey, Munro, & Singh, 1999).

A number of scientists studied the use of WPC, in comparison to skim milk powder (SMP), in yogurt manufacture and a range of effects have been reported. Whey protein concentrates at 1.0 and 1.5% of protein addition produced yogurts generally superior to casein-based products for both appearance and smoothness (Modler, Larmond, Lin, Froehlich, & Emmons, 1983). When milk was enriched with WPC, a higher level of cross-linking within the gel network was observed, thereby increasing viscosity (Remeuf, Mohammed, Sodini, & Tissier, 2003). Substituting 20% of the skim milk solids with WPC produced yogurt with increased gel strength and viscosity (Augustin, Cheng, Glagovskaia, Clarke, & Lawrence, 2003). Whey protein concentrate and gum tragacanth, at various concentrations, as fat replacers in nonfat yogurt was studied. Yogurts stabilized with WPC showed more compact structure with more firmness and lower water drainage than control nonfat yogurt. It was stated that the use of WPC can provide a nonfat yogurt with good physical properties that bear resemblance to that of full fat yogurt (Aziznia, Khosrowshahi, Madadlou, & Rahimi, 2008).

Conversely, replacement of skimmed milk by dry dairy products such as whey protein concentrates (WPCs), milk protein concentrates (MPCs) and skim milk powder (SMP) was

studied. Set yogurts prepared with SMP, skim milk concentrate (SMC) and MPC exhibited higher values of viscosity and more syneresis than yogurts prepared with WPCs. Thus, set yogurts fortified with WPCs were softer and suffered less syneresis than control yogurts. The authors recommended that WPC may be useful for drinking yogurt production (Guzmán-González, Morais, Ramos, & Amigo, 1999). In a study comparing the physical and sensory characteristics of yogurt prepared from casein and whey based products, the casein-based yogurts were firmer with less syneresis than yogurts based on whey protein (Modler et al., 1983).

Excessive heat treatment of milk and the addition of high levels of whey proteins have contributed to textural defects. In yogurt samples where ~20% of milk solids-non-fat (SNF) was replaced with whey protein concentrate (WPC), a 'grainy' texture was observed (Greig & Van Kan, 1984). Substituting WPC for SMP to elevate the total solids content of yogurt mixes increased 'lumpiness' or 'graininess' (Guirguis, Hickey, & Freeman, 1988) while replacement of casein by WPC resulted in a yogurt with a 'less smooth and clumpy' appearance (Jelen, Buchheim, & Peters, 1987). Substitution of milk protein (casein) with a WPC solution (protein content 3.1%) up to 10 - 15% level had no effect on the final viscosity or sensory attributes of yogurt, but at high levels of substitution, flocculation occurred during heat treatment of the mix (Greig & Van Kan, 1984). The firmness of yogurt gels made from milk with various casein to whey protein ratios was similar (Jelen et al., 1987).

Morris, Ghaleb, Smith, and Bastian (1995) found that at similar protein concentrations, yogurt fortified with both SMP and WPC were not significantly different in firmness compared to yogurt fortified with SMP only. The addition of WPC to milk and heat treatment resulted in increased pH of gelation, reduction in gelation time and increase in storage modulus (G') for acid milk gels. It was suggested that during heat treatment, whey proteins were almost completely

denatured and some of the denatured whey proteins associate with the casein micelles. During acidification, the denatured whey proteins may aggregate, resulting in increased cross-linking or bridging within the gels (Lucey et al., 1999).

Possible reasons for these apparent conflicts could be the variations in the WPC preparations used for the production of the yogurts and the wide range of different instruments and tests used to obtain data. Additionally, differences in starters used to ferment the milk and also the variations in the functional properties of commercial WPCs may explain some of the inconsistencies between studies that could influence yogurt properties (Sodini et al., 2005).

Modler and Kalab (1983) reported that the casein micelles in yogurt form different matrices depending upon the concentration of the other proteins. When milk was heat treated, the denatured β-lactoglobulin reacted with α-casein to form an insoluble complex. When milk was fortified with WPC, the concentration of β-lactoglobulin greatly exceeded the concentration of α-casein. As a result, other protein complexes such as β-lactoglobulin and α-lactalbumin complexes would be formed. The stabilization mechanism in yogurt when fortified with WPC could be due to the β-lactoglobulin and α-lactalbumin complex rather than the casein complex, resulting in different consistency. Fortification of yogurt milk with WPC resulted in yogurt with better texture and consistency. Yogurts fortified with casein or SMP often have a firmer gel, but yogurts fortified with WPC tend to be smoother and have a better appearance.

Another study (Penna, Baruffaldi, & Oliveira, 1997) examined the effects of demineralized whey powder, lactic culture concentration and mix treatment temperature on yogurt quality characteristics. The results indicated that the addition of WPC to milk caused considerable changes in yogurt composition, increasing acidity and influencing some taste properties. Fermentation time depended on demineralized whey concentration; it decreased in

line with an increase in demineralized whey powder. Consistency increased as mix treatment temperature increased and demineralized whey powder decreased. Application of WPC, microparticulated whey protein (MWP), and modified tapioca starch in reduced-fat yogurts and their effect on the microstructure and texture of yogurt was studied by Sandoval-Castilla, Lobato-Calleros, Aguirre-Mandujano, and Vernon-Carter (2004). The authors reported that supplementation with WPC and blends of WPC and MWP, provided yogurts with textural characteristics resembling those of full fat yogurt.

2.4.1.2 Milk Protein Concentrates (MPCs). Milk protein concentrates, used as functional ingredients, are obtained by the ultrafiltration of skim milk to raise the protein level during yogurt manufacture. Another main reason for its use is to reduce the lactose content in the yogurt mix (Chandan & O'Rell, 2006).

Some authors have studied the replacement of skim milk powder by MPC for yogurt manufacture. Modler et al. (1983) mentioned that at constant protein levels, the replacement of skim milk powder by MPC did not alter the firmness, syneresis and flavor of the yogurt. In another study, Guzmán-González et al. (1999) reported that set yogurts manufactured with MPC exhibited higher values of viscosity.

The amount of powder required for fortification is much less due to the high protein content of MPC (50-85%), in comparison to skim milk powder (34-36%). Also, MPC can be directly used as the yogurt milk (Sodini & Tong, 2006). The viscosity and firmness of yogurts produced from ultrafiltered milks were higher due to the higher protein content, when compared to yogurts produced from milk fortified with SMP (Becker & Puhan, 1989; Biliaderis, Khan, & Blank, 1992; Lankes, Ozer, & Robinson, 1998). Savello and Dargan (1995) noticed a higher viscosity (100%) and higher gel strength (50%) in the yogurts made from ultra-filtered milk

while comparing yogurts produced from ultrafiltered milk and SMP fortified milk at a constant protein level.

2.4.2 Gelatin. Gelatin is a protein derived from the partial hydrolysis of collagen. Collagen is a structural protein found in bone, tendon, skin and the connective tissue of various organs of the animal body (Morrison, Sworn, Clark, Chen, & Talashek, 1999). The collagen molecule is comprised of a helical structure consisting of a sequence of amino acid chains. The composition of these chains is generally Glycine-Proline-hydroxyproline (Haug & Draget, 2009).

For the manufacture of gelatin, after a series of preliminary treatments, the raw material is treated with an acid (type A gelatin) or alkali (type B gelatin). The aim of both the acid and alkali treatments is to break the chemical cross-linkages in the fibers of the collagen, thereby creating a product that is soluble in water. It is perceived that the breakdown is largely dependent on the three factors: temperature, time, and pH. High temperatures and long periods of exposure to heat accelerate the process (Schreiber & Gareis, 2007).

The higher content of imino acids (proline and hydroxyproline) corresponds to the stability of the collagen structure. Collagen denatures at temperatures above 40°C, where the helical structure is broken down and random-coils single, double and triple strands are formed. Upon controlled cooling, the helical structure is re-formed. This re-formation leads to the formation of junction zones, which are required for gelation. It is generally believed that the junction zones in gelatin are stabilized by hydrogen bonds similar to those in native collagen and are interconnected through flexible peptide chains, forming a gel network (Haug & Draget, 2009; Wong, 1989).

The properties of this gel are very important in terms of the application for food use. The main attribute to be considered is the gel strength, also referred to as the bloom or the bloom

strength. This is defined as the weight in grams needed to produce a four millimeter deep depression by a plunger (12.7 mm in diameter) in the surface of a gel (6.67% concentration that has been set for 16 to 18 hours at 10 °C) (Haug & Draget, 2009). Gelatin of bloom strength of 225 or 250 is commonly used. The gelatin level in yogurt should be decided according to the consistency standards for yogurt. Usually, the amount of gelatin above 0.35%, results in yogurt that has a curdy and lumpy appearance upon stirring (Chandan & O'Rell, 2006). The strength of these gels can be affected by several factors, including pH, temperature, setting time, and interactions with other ingredients. There are also other factors that may affect other characteristics of a gelatin gel. (Haug & Draget, 2009). Similarly, viscosity may be affected, particularly by temperature, pH, and concentration. Mouthfeel and other sensory characteristics could be affected due to the changes in melting point and viscosity. Processing at ultra-high temperatures tend to degrade gelatin gels. The yogurt acquires a pudding like consistency at temperatures below 10°C (Chandan & O'Rell, 2006).

The unique organoleptic properties and flavor release by gelatin is achieved by forming thermally reversible gels with water and the gel-melting temperature (<35°C) is below body temperature. The thermoreversibility of this process gives the gelatin gel an inimitable 'melt-in-mouth' quality. Starch, alginate, pectin, agar, and carrageenan are all polysaccharides from plant sources used as gelling agents, but their gels lack the melt-in-the-mouth, elastic properties of gelatin gels as their melting points are significantly higher than gelatin gels (Karim & Bhat, 2008). Gelatin is versatile and multi-functional which can be used as a gelling, thickening, water-binding, emulsifying, foaming, film-forming agent. Gelatin is notable for its gelling properties and clean flavor profile (Schrieber & Gareis, 2007). The gelatin gel has been observed to have sheen like and clear appearance with clean melt-in-the mouth texture that has not yet been

imitated by any other polysaccharide (Baziwane & He, 2003). Gelatin is easy to use as it gels within the normal pH range of most foods and does not require the addition of salts, sugars or acids to set while other gelling hydrocolloids often require the addition of salts, food acids or sugars to form a gel (GME, 2008).

Fiszman, Lluch, and Salvador (1999) reported that gelatin over a great range of concentrations was able to improve the rheological and textural properties of skim yogurt and hindered the syneresis defect. Keogh and O'Kennedy (1998) reported that gelatin, xanthan, and locust bean increased the consistency of stirred yogurt, whereas the addition of wheat starch did not. Jawalekar, Ingle, Waghmare, and Zanjad (1993) also examined the use of gelatin and other stabilizers related to yogurt rheology and sensory quality, as well as whey separation. The addition of gelatin to yogurt made with either cow or buffalo milk demonstrated an improvement in body, texture, viscosity, and curd tension. Whey separation was also reduced, likely due to the stabilizer binding free water in the yogurt.

With its many advantages, there are some drawbacks that exist in the use of gelatin. Since most commercial gelatins are obtained from either pigskin or cow hides, for many years the vegetarian, halal and kosher markets have been reluctant to consume gelatin. Also, increased concerns in the last decade, particularly within Europe with the occurrence of bovine spongiform encephalopathy (''mad cow disease'') in the 1980s have led to considerable interest in finding and using alternative substitutes for gelatin. As a result, food scientists have been striving for many years to develop alternatives to gelatin that possess most or all of the unique functional properties. Driven by the foreseeable demand for halal/kosher gelatin, industries are now trying to develop gelatin-free products in which mammalian gelatin is no longer used, either as a processing aid or as an ingredient (Karim & Bhat, 2008). In addition, various studies have been

done on the development of gelatin alternatives or substitutes from plant hydrocolloids such as starch/modified starch, pectin, carrageenan and agar.

2.4.3 Pectin. Pectin is a polysaccharide found in the cell wall of most plants. They form gels to stabilize acidified milk beverages or to simply enhance the viscosity of beverages. Pectin is generally thought to be comprised of 1,4-linked α-D-galacturonic acid. The degree of esterification (DE) or degree of methylation (DM) can be defined when the D-galacturonic acid units are partially esterified with methanol. Pectins with a DE of higher than 50% are called high methylester pectins (HM pectins), and pectins with a lower DE than 50% are called low methylester pectins (LM pectins). These variations in the degree of esterification influence the properties of commercial pectins. One of the main aspects of pectins is their gelling property. HM pectin gels in total soluble solids higher than 55% and pH values below pH 3.5, whereas LM pectin may gel independent of the total soluble solids content and pH value, but requires the presence of cations, usually calcium (Endress & Mattes, 2009).

The gelling mechanism of HM pectin is postulated to rely on hydrogen bonding between non-dissociated carboxyl groups and secondary alcohol groups along with hydrophobic interactions between methyl ester groups. The ability to gel is enhanced with an increased degree of methyl esterification and low pH. A three-dimensional network is formed by the interaction between the pectin polymers to create a so-called 'junction zone'. Therefore, high methylester pectins are used as gelling agent for traditional jams, jellies, and marmalades. In contrast, low methoxy pectins, gel by forming structures referred to as 'egg boxes' in the presence of calcium ions. A low degree of methyl esterification enhances the ability to gel and the more calcium sensitive the pectin becomes. The many non-esterified carboxylic acids in LMP prevent the structure from being dehydrated enough to gel (Endreû & Christensen, 2009).

When drinks containing protein are heated at acidic pH, the proteins tend to precipitate and form larger clusters that impart a sandy mouthfeel. High ester pectin molecules are negatively charged at the actual pH and can bind to the protein particles and protect them from aggregation. The pectin also creates a weak molecular network throughout the drink that further contributes to stability. Thus, HMP is useful by providing a drinkable yogurt that exhibits good mouthfeel characteristics, is not chalky, and does not sediment (Endreû & Christensen, 2009).

These two types of pectin also differ in setting. Low methoxy pectin will set almost as soon as appropriate conditions are met. HM pectin, based on setting time and temperature, have been classified into ultra rapid set, rapid set, medium rapid set, slow set and extra slow set pectin, according to the degree of methyl esterification (Endress & Mattes, 2009). In general, the higher the degree of esterification, the faster the gel is set.

Towler (1984) examined the effects of propylene glycol alginate (PGA), carboxy methyl cellulose (CMC), and pectin on the viscosity and sedimentation of a cultured milk beverage. Higher amounts of stabilizers added resulted in a rapid increase in viscosity. Sedimentation of the milk protein increased with lower levels of stabilizers, but decreased once the level of stabilizer increased beyond the level of minimum viscosity. PGA and pectin were determined to be better stabilizers for this use as products made with CMC sedimented greatly.

Shukla and Jain (1991) studied the effects of gelatin, CMC, pectin, and other stabilizers on the organoleptic quality and the amount of whey separation in yogurt made from buffalo milk. The use of 0.1-0.3% gelatin improved the appearance, body, texture, and flavor of the yogurts. Similarly, pectin (0.2-0.3%) improved these quality attributes and reduced whey separation. CMC, however, negatively impacted the quality of the yogurt and these samples were deemed unacceptable in sensory analysis. The authors recommended that the usage of CMC not to

exceed 0.1%. Hydrocolloids and some of their mixtures were used to prepare spray-dried yogurt. Acetaldehyde retention and microbial viability were evaluated in addition to the structural analysis. From the micrographs, it was indicated that κ-carrageenan and κ-carrageenan-locust bean gum gave more protection to the casein matrix, leading to greater acetaldehyde retention (92% and 89%, respectively). Microbial viability was improved when pectin was used as the encapsulating agent (Rascón-Díaz, Tejero, Mendoza-Garcia, García, & Salgado-Cervantes, 2010). Significant improvement of the rheological profile of flavored yogurt was observed by Ramaswamy and Basak (1992) when 0.3 to 0.4% pectin was added.

However, due to health, dietary restrictions, and religious reasons or in an attempt to reduce cost, there is an increasing demand for the use of natural ingredients as stabilizers in yogurt. In addition to gelatin, CMC, and pectin, numerous other stabilizers, such as starches, agar, locust bean gum, alginates, and guar gum have been studied for their use in yogurt (Tamime & Robinson, 1999). The properties, functionality, and quantity of these ingredients may affect the mouthfeel and acceptance of a yogurt product.

2.4.4 Starch. Starch is the most widely used thickening and gelling agent in the food industry because of the wide variety of texture and mouthful sensations it provides. Starch is a typical ingredient of foodstuffs such as sauces, soups, and many other processed foods. In these products the method of preparation such as water content, temperature and the presence of other organic/inorganic materials is an important factor that determines the rheological behavior of starch dispersions (Abu-Jdayil, Mohamed, & Eassa, 2004). Starches are used extensively in yogurts as stabilizers, to increase viscosity and to reduce syneresis (Lucey, 2002). Schmidt, Herald, and Khatib (2001) mentioned that for a fermented dairy product like yogurt, the ideal starch stabilizer would be one that is cross-linked and substituted. The cross-linking reinforces

the hydrogen bonds in the starch granule with chemical bonds which act as bridges between molecules.

Starch is a polymer of D-glucopyranose that are linked together by α-1,4 and α-1,6 glycosidic bonds. Amylose, a linear polymer and amylopectin, a branched chain polymer constitute the major components of starch. The amount of amylose and amylopectin differs depending on the starch type. Starch granules in their native state are insoluble in cold water. When starch is heated in the presence of water, the molecules swell or gelatinize and the textural properties develop. A rapid onset in the development of viscosity is observed and at this point, the structural changes that occur in the granule are irreversible. Changes that occur during the processing of native starch are caused by the ratio of amylose to amylopectin, the concentration of lipid material, and other factors such as the presence of phosphate groups. Viscosity can be lost with continued heating due to rupturing and collapse of the granule (Mitolo, 2006). During this process the amylose and amylopectin molecules will begin to solubilize and eventually leach out of the granule. This leads to a viscous dispersion of starch fragments that are swollen, hydrated aggregates and dissolved molecules. Upon cooling, a firm gel can be formed. It is also possible for the released amylose to complex with lipids which is the origin of a discontinuity that can be seen in the viscosity from the pasting experiments after holding at high temperatures. The gelled paste becomes opaque and cloudy over time as water is eventually released, resulting in a rubber-like consistency. It is important to note that the rate of gelling and texture that results upon cooling is dependent upon the starch source and level of amylose (Mitolo, 2006; Taggart, 2009).

The property of starch depends on many factors: the botanical source of the starch, the presence or otherwise of chemical modifications (modified or native starch), the starch

concentration, the cooking procedure (temperature, pH, heating time, shearing time and intensity, among others) and the presence of other ingredients or additives. Corn and wheat starches have much higher amylose contents (about 28%) followed by potato and tapioca starches (which contain about 20% amylose) and then rice starch (which contains about 17% amylose). The fat and protein contents also vary among the different botanical sources of starch. Cereal starches like wheat, corn, barley, or rice contain more lipids (0.6–1% w/w) than tubers (potato-0.05%), roots (tapioca-0.1%), and waxy mutant cereal starches. The same trend is found in the protein content: 0.25–0.6% for cereal starches compared with 0.06% for potato and 0.1% for tapioca. The lipid/protein content of starch has been correlated with swelling behavior and shear sensitivity. Starches that swell rapidly on heating tend to be more shear sensitive and contain less protein and lipid than starches that display a more controlled swelling. Native starches are not preferred in industrial applications due to their high thermal and shearing instability and their tendency to retrograde during cooling or/and freezing, causing a decrease in food product quality. Nevertheless, the current trend towards natural, clean-label food has promoted the use of native starches (Debet & Gidley, 2006).

Williams, Glagovskaia, and Augustin (2004) reported that yogurts made with the addition of 1% (w/w) modified waxy maize starch made from SMP, at 10% dairy solid, markedly increased the viscosity of yogurt but developed a grainy texture. However, increasing the concentration of SMP or the level of replacement of SMP with WPC reduced the graininess but had little or no effect on the viscosity of yogurt. Keogh and O'Kennedy (1998) showed that the addition of wheat starch had an insignificant effect on the syneresis of stirred yoghurt, but did affect the viscosity of the stirred yogurt. Schmidt et al. (2001) evaluated the effect of gelatin; native wheat starch; and modified wheat starches in yogurt and proposed that characteristics of

yogurt formulated with native wheat starch and gelatin were similar and native wheat starch may be used as an alternative stabilizer.

Sandoval-Castilla et al. (2004) reported the use of tapioca starch as a fat replacer along with other whey-protein based fat replacers and observed that even though a more loose structure was obtained, yogurt with tapioca starch provided greater firmness than full-fat yogurt. However, it was suggested that the loss of network strength might be due to the phase separation. Oh, Anema, Wong, Pinder, and Hemar (2007) investigated the effect of potato starch on acidified skim milk, heated to 85ºC for 30 min. They reported that the storage modulus increased linearly with an increase in the potato starch concentration. The results from confocal laser scanning microscopy (CLSM) showed that the acidified milk gels were made of swollen potato starch granules embedded in the protein network, and that an increase in the starch concentration resulted in an increase in the density of the protein network.

The effect of addition of starches of different botanical origin on the yogurt gel properties was investigated by Najgebauer-Lejko, Grega, Sady, Faber, Domagała, and Machaczka (2007). The authors observed that yogurt fortified with waxy maize starch had the best sensory properties and was found to maintain the highest acetaldehyde level after 3 weeks of storage. Yogurt produced with maize and tapioca starches demonstrated the highest resistance to whey separation.

Recently, the dynamic rheological behavior of skim milk gels containing 2% normal rice starch granules pasted to different temperatures was investigated (Zuo, Hemar, Hewitt, & Saunders, 2008). It was found that the complex modulus G' was maximal when the starch granules were pasted to the temperature of maximum swelling and not to the temperature of maximum viscosity or when the starch was fully pasted. The authors suggested that, in these

systems, the starch granules behave as inactive fillers and that their main effect is to increase the milk protein concentration during swelling by absorbing water from the continuous phase. Thus, the rheological properties of yogurt can also be modified by fortifying the milk with dairy-based ingredients, non-dairy ingredients or a combination of both.

2.5 Starch and Milk Protein Interaction

The protein and polysaccharide behavior determines the structure and other physicochemical properties in food systems (Tolstoguzov, 1991). Although there are a lot of systems in which starch and milk protein co-exist, and have been studied separately, the literature is scarce on the mechanisms, interaction and synergistic effects of both. The electrostatic interactions between starch and protein were emphasized by Takeuchi (1969) and reported that only potato starch provided such interactions due to its anionic properties.

A milk-based system containing starch used by Ling (1984) demonstrated that the changes in viscosity was a result of protein and starch entanglement rather than individual protein effect on starch swelling. The graininess observed in stirred yogurt could be due to the specific and non-specific between modified waxy maize starch and milk protein (Willimas, Glagovskaia, & Augustin, 2003). A synergistic effect was reported in a mixed system containing cassava starch and whey proteins, at low starch concentration (Aguilera & Rojas, 1996). Additionally, the microstructure of yogurt with added tapioca starch illustrated some soluble starch integrated into the casein network along with starch gel fragments forming independent structures (Korolczuk, Breten-Dollet, Tissier, & Maingonnat, 1996). Such complexities in a mixed system of milk and starch during heat treatment may lead to different characteristics in the final yogurt gel compared with yogurt gels made from milk and bacterial cultures alone.

2.6 Rice Flour

Many food based applications use rice flour and starch due to their qualities of being hypo allergenic, gluten free, bland in flavor, and in its native forms it exists with many different functional characteristics. Some broken rice kernels during harvesting, handling, drying and milling are used for grinding into rice flour or for brewing. Usually, rice flours have the same composition as their parent grain. The difference between rice flour and starch is that most of the native proteins and lipids are removed from starch (Bao & Bergman, 2004). In recent years, rice, especially rice flour, because of its unique functional properties, is being used in increasing numbers of novel foods such as tortillas, beverages, processed meats, puddings, salad dressing, and gluten-free breads (Kadan & Ziegler, 1989; McCue, 1997; Kadan et al., 2001). Proteins and starch are the two major components of rice, with approximately 8% and 80%, respectively. Rice protein is valuable because it has unique hypoallergenic properties and ranks high in nutritive quality (rich in the essential amino acid lysine) among the cereal proteins (Ju, Hettiarachchy, & Rath, 2001).

In a study done by Chun and Yoo (2004), the steady and dynamic rheological properties of Korean rice flour dispersions were evaluated at different concentrations and found that the apparent viscosity increased with increase in concentration. A model was also proposed for expressing the relationship between concentration and apparent viscosity. Therefore, studies on rheological properties of rice flour dispersions are important in producing rice flour products with the desirable qualities. Also, use of rice flour was evaluated in the production of vanilla ice cream. It was found that though samples still deteriorated in textural properties under the experimental temperature abuse conditions, rice flour reduced the negative impact of temperature abuse on textural properties. In addition, rice starch lowered the perceived sweetness and the

authors added that the use of rice flour appeared to be most advantageous for low fat ice cream samples (Cody, Olabi, Pettingell, Tong, & Walker, 2007). However, cooked rice flour dispersions have not been investigated much in dairy based products.

Furthermore, previous work done in our laboratory indicated that addition of rice extract to banana flavored yogurt improved quality characteristics and was well accepted in sensory analysis.

Therefore, the overall objective of this research was to study the effect of rice extract as a potential stabilizer in dairy products. The specific objectives of this research were:

1. To examine the microbiological, chemical, and physical quality of yogurt prepared with rice extracts as stabilizer during 28 days of storage at 4°C,
2. To measure the viscosity of yogurt made with rice extract and,
3. To determine consumer acceptability of yogurt made with rice extract.

CHAPTER 3

Materials and Methods

3.1 Rice Extract Preparation

Rice flour (5%, 10%, 15% wt/vol.) was mixed with tap water and cooked on a stove top. For the 5% wt/vol. extract, 25g of rice flour was mixed with 500 ml of tap water and was cooked for approximately 70 minutes until a smooth gel-like consistency was obtained. For the 10% wt/vol. extract, 50g of rice flour was mixed with 500 ml of tap water and was cooked for approximately 45 minutes until a smooth gel-like consistency was obtained. For the 15% wt/vol. extract, 75g of rice flour was mixed with 500 ml of tap water and was cooked for approximately 25 minutes until a smooth gel-like consistency was obtained. The extracts were then stored at 4°C for 24 h until the yogurt samples were prepared.

3.2 *Bifidobacterium* Growth and Activation

Three types of commercially available *Bifidobacterium* supplements were weighed equally and mixed with 10 ml of sterilized MRS broth. The samples were incubated under anaerobic conditions for 48 h at 37°C. Incubated samples were then centrifuged for 10 minutes at 8000 rpm at 4°C (Thermo Electron Scientific, Sorvall RC 6 Plus, Asheville, NC). The supernatant was discarded and to the pellet, approximately 40 ml of sterilized milk was added and mixed well. The samples were incubated anaerobically overnight at 37°C. After incubation, the samples were mixed well with the commercial yogurt cultures that were used for preparing the yogurt. Seven hundred microliter of the sample was added to 300 µl of sterilized glycerol and stored at -80°C and this served as the stock culture.

3.3 Yogurt Preparation

Two percent organic milk was purchased from a local grocery store in Greensboro, NC. Rice flour was purchased from a local Indian grocery store. Two types of store bought Greek style yogurt were used as cultures; one containing 0% milk fat and the other containing 2% milk fat and both of them contained active cultures of *L.bulgaricus* and *S.thermophilus*. The two types of commercial cultures, along with the activated *Bifidobacterium*, were mixed together and this served as the starter culture for preparing the yogurt samples.

Yogurt was prepared by placing milk in a water bath set at 40ºC and simultaneously checked on an instant read thermometer until it reached that temperature. For the treatments using gelatin and rice extract, the stabilizers were added to the milk prior to heating and mixed well until incorporated. Once the temperature reached 40ºC, yogurt culture was inoculated into the milk mixture and stirred well. The mixture was stored in sterile screw-capped glass bottles and incubated at 42ºC for about 5h until a pH range of 4.4 to 4.6 and a titratable acidity of 0.85 to 0.95% were attained. The yogurt samples were then placed immediately in a 4ºC refrigerator until further testing. Table 3 describes the yogurt formulation and Figure 2 shows the flowchart for yogurt preparation.

Table 3

Yogurt formulation

Control	**Gelatin treatment**	**Rice Extract (5, 10, and 15%)**
2% milk – 1500 ml	2% milk – 1500 ml	2% milk – 1500 ml
Yogurt culture – 75 g	Yogurt culture – 75 g	Yogurt culture – 75 g
	Gelatin – 6 g	Rice extract – 75 g

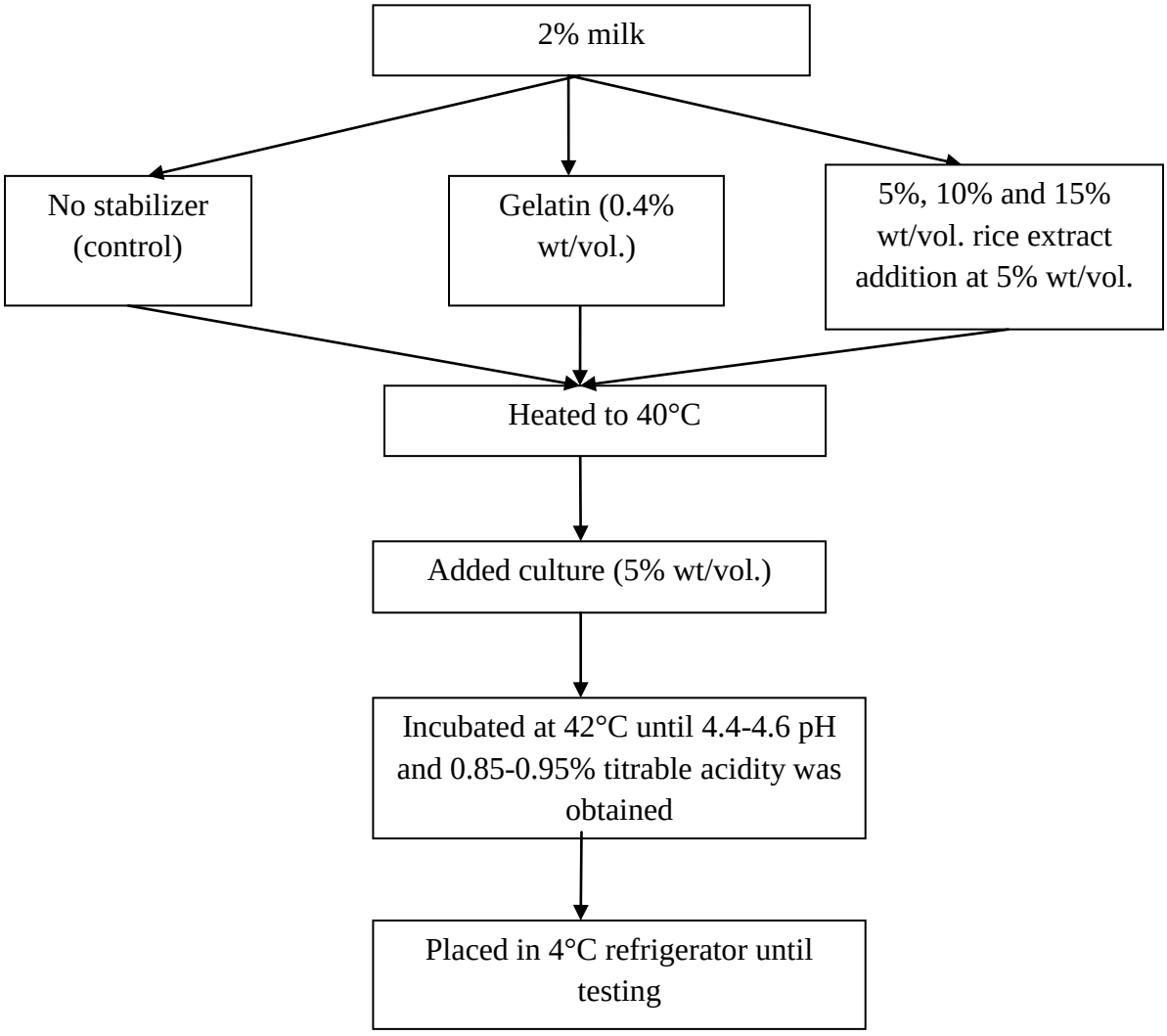


Figure 2. Flowchart for the preparation of yogurt

3.4 Storage Study

Objective 1: To examine the microbiological, chemical, and physical quality of yogurt prepared with rice extracts as stabilizer during 28 days of storage at 4°C.

For the storage study, yogurt samples were stored in the refrigerator at 4°C. Samples were drawn at day 1, day 7, day 14, day 21, and day 28 to ascertain the microbiological, physical, and chemical quality.

3.4.1 Microbiological analysis. MRS and glucose M-17 agar were used for the

enumeration of *L.delbrueckii spp. bulgaricus* and *Streptococcus thermophilus*, respectively. Seven consecutive dilutions were prepared by homogenizing one milliliter of each sample in 9 ml of sterile peptone water using a vortex. The fifth, sixth and seventh dilutions were plated onto the respective agar plates. After 48 h of incubation at 37ºC, visible colonies were counted and data were expressed as log CFU/ml.

Modified BIM-25 agar was used for the enumeration of bifidobacteria. Five consecutive dilutions of samples were prepared by weighing 11 ml of each sample into a screw-capped bottle that contained 99 ml of sterile peptone water. Samples were then mixed well by shaking for 45 s. The third, fourth and fifth dilutions were plated onto the agar. After 72 h of anaerobic incubation at 37ºC, visible colonies were counted and data were expressed as log CFU/ml.

3.4.2 Titratable Acidity (TA) and pH. Samples were warmed to 25°C and mixed well with a stirring rod. Nine ml sample was taken with a pipette and placed in a 100 ml beaker. Eighteen ml of distilled water was added to the mixture and mixed gently. A 0.1 NaOH solution was used for titration and titrated until a pH of 8.6-8.8 was obtained.

TA (%) = (Volume of 0.1 NaOH x Normality of NaOH used x 9)/9

pH was measured with a pH meter (Accument Excel XL15, Thermo Fisher Scientific, Pittsburgh, PA) that was calibrated with standardized pH buffer solutions 4.0 and 7.0 prior to the analysis.

3.4.3 Total solids. Approximately 5 g of yogurt sample was placed in a pre-weighed, pre-dried aluminum pan, and transferred to an atmospheric oven at 85 ºC for 3 h. Samples were cooled in a desiccator for 30 minutes before final weights were recorded.

$$\textbf{Total solids (\%)} = \frac{\textbf{Wt. of sample + pan after drying (g) – Wt. of empty pan (g)}}{\textbf{Wt. of sample before drying (g)}} \times 100$$

3.4.4 Syneresis. Syneresis is contraction of a gel without the application of any external forces and is related to instability of the gel network, resulting in the inability to entrap all the

serum phase (Lucey & Singh, 1998). A modified method of Kumar and Mishra (2004) was used for determining syneresis in the yogurt samples. Equal amounts of samples were weighed and placed in a centrifuge (Thermo Electron Scientific, Sorvall RC 6 Plus, Asheville, NC) for 10 minutes at 5000 rpm at 4°C. The supernatant was collected and measured in a graduated cylinder.

Syneresis (%) = (ml. of drained whey/Total g of sample) x 100

3.5 Viscosity Measurements

Objective 2: To measure the viscosity of yogurt made with rice extract.

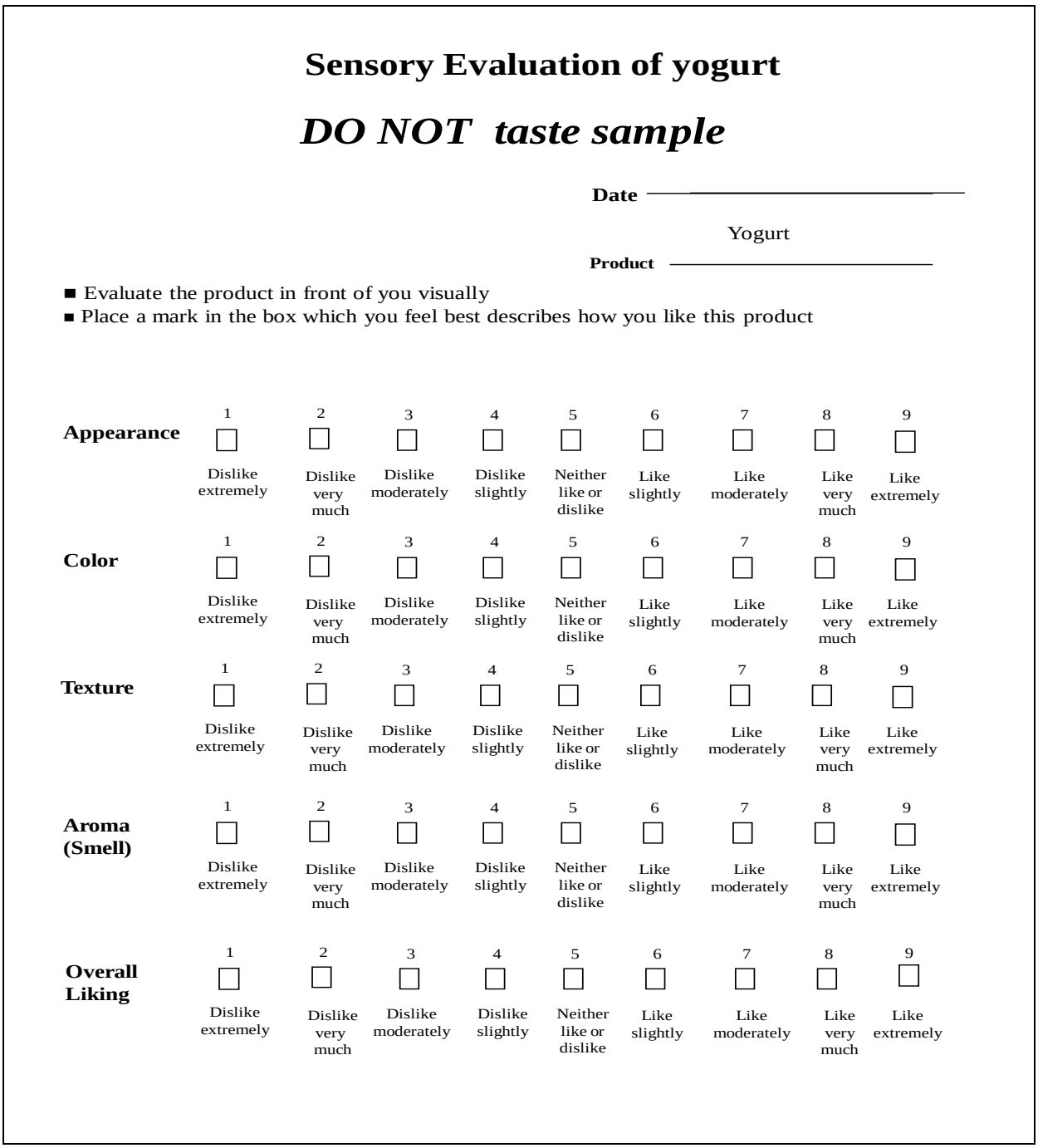
Viscosity measurements were carried out by a method stated by Milani and Koocheki, (2011) with slight modifications. The apparent viscosity of the yogurt samples were measured by Haake 7 plus viscotester at 10 rpm with the aid of L3 spindle at ambient temperature. The volume and also the immersion depth of the spindle were kept constant throughout the experiment.

3.6 Sensory Analysis

Objective 3: To determine consumer acceptability of yogurt made with rice extract.

A convenient group of 10 untrained panelists evaluated the liking of the samples with respect to appearance, color, texture, aroma and overall acceptability. The sensory attribute tests were carried out on a laboratory scale using panelists with a background in food science and their familiarity with the product. Subjects were requested not to consume the sample. The following samples were evaluated: a control with no stabilizer, yogurt stabilized with gelatin, and yogurt stabilized with 10% rice extract. The 10% rice extract, which exhibited suitable microbiological and physicochemical properties, was chosen based on the results from the storage study. Figure 3 shows an example of the sensory evaluation form and the hedonic scale that was used. Subjects

rated their liking for each item on a 9-point hedonic scale (1=dislike extremely, 2=dislike very



Sensory Evaluation of yogurt

DO NOT taste sample

Date _____

Yogurt

Product _____

■ Evaluate the product in front of you visually
■ Place a mark in the box which you feel best describes how you like this product

	1	2	3	4	5	6	7	8	9
Appearance	☐ Dislike extremely	☐ Dislike very much	☐ Dislike moderately	☐ Dislike slightly	☐ Neither like or dislike	☐ Like slightly	☐ Like moderately	☐ Like very much	☐ Like extremely
Color	☐ Dislike extremely	☐ Dislike very much	☐ Dislike moderately	☐ Dislike slightly	☐ Neither like or dislike	☐ Like slightly	☐ Like moderately	☐ Like very much	☐ Like extremely
Texture	☐ Dislike extremely	☐ Dislike very much	☐ Dislike moderately	☐ Dislike slightly	☐ Neither like or dislike	☐ Like slightly	☐ Like moderately	☐ Like very much	☐ Like extremely
Aroma (Smell)	☐ Dislike extremely	☐ Dislike very much	☐ Dislike moderately	☐ Dislike slightly	☐ Neither like or dislike	☐ Like slightly	☐ Like moderately	☐ Like very much	☐ Like extremely
Overall Liking	☐ Dislike extremely	☐ Dislike very much	☐ Dislike moderately	☐ Dislike slightly	☐ Neither like or dislike	☐ Like slightly	☐ Like moderately	☐ Like very much	☐ Like extremely

Figure 3. Sample of sensory evaluation form for yogurt samples

much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely) to determine the sensory characteristics among

the samples. The samples were presented in color coded plastic containers. The key used was: pink-for control, blue-for yogurt stabilized with gelatin and white-for yogurt stabilized with 10% rice extract, and this was not revealed to the participants. Samples were stored at 4°C in a refrigerator to maintain integrity during sensory analysis.

3.6 Statistical Analysis

All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). An ANOVA was performed using the general linear models procedure to determine significant differences among samples at $p<0.05$. Means were compared by using Duncan's multiple range test.

CHAPTER 4

Results and Discussion

4.1 Storage Study

The purpose of this study was to ascertain the microbiological, chemical, and physical quality of yogurt samples prepared using different stabilizers (control, gelatin, rice extract 5, 10 and 15%) over storage for 28 days at 4°C. Samples were drawn on day 1, day 7, day 14, day 21, and day 28.

4.1.1 Microbiological analysis. For the microbiological analysis, *L.delbrueckii spp. bulgaricus*, *Streptococcus thermophilus*, and bifidobacteria were enumerated on selective media to determine viability. Table 4 shows the viability of *L.delbrueckii spp. bulgaricus* in yogurt samples prepared using different stabilizers during 28 days of storage at 4°C. At 1 day storage, the mean bacterial population among the yogurt samples ranged from 7.67-7.94 log CFU/ml. The bacterial population in all samples ranged between 8.03-8.24 log CFU/ml at 7 day storage period, showing a significant increase ($p<0.05$) from day 1. The bacterial population peaked at the 14 day storage period, ranging between 8.12-8.32 log CFU/ml for all samples, and declined subsequently thereafter. The mean bacterial population was almost similar among all yogurt samples until 21 days of storage. However, on day 28, the bacterial population for control, gelatin, rice extract at 5, 10 and 15% supplementation was 7.50, 7.81, 7.70, 7.83 and 7.88 log CFU/ml respectively, compared to their initial bacterial population of 7.76, 7.86, 7.67, 7.83 and 7.94 log CFU/ml.

Overall, the population of *L.delbrueckii spp. bulgaricus* during the 28 day storage period when compared to day 1, showed a significant decrease ($p<0.05$) in the control sample, while

Table 4

Population of L.delbrueckii spp. bulgaricus in yogurt samples prepared with different stabilizers during 28 days of storage at 4°C

	Log CFU/ml				
Samples	**1 day**	**7 days**	**14 days**	**21 days**	**28 days**
Control	$7.76^{hi} \pm 0.028$	$8.03^{bcdefg} \pm 0.148$	$8.12^{abcd} \pm 0.084$	$7.98^{cdefgh} \pm 0.0063$	$7.50^{j} \pm 0.134$
Gel	$7.86^{fghi} \pm 0.084$	$8.12^{abcd} \pm 0.056$	$8.21^{abc} \pm 0.247$	$8.07^{bcdef} \pm 0.021$	$7.81^{ghi} \pm 0.035$
RE 5%	$7.67^{ij} \pm 0.021$	$8.22^{ab} \pm 0.035$	$8.26^{ab} \pm 0.000$	$8.10^{abcde} \pm 0.028$	$7.70^{ij} \pm 0.063$
RE 10%	$7.83^{ghi} \pm 0.007$	$8.13^{abcd} \pm 0.134$	$8.25^{ab} \pm 0.190$	$7.86^{fghi} \pm 0.042$	$7.83^{ghi} \pm 0.084$
RE 15%	$7.94^{defgh} \pm 0.176$	$8.24^{ab} \pm 0.014$	$8.32^{a} \pm 0.091$	$8.09^{abcde} \pm 0.063$	$7.88^{efghi} \pm 0.063$

Note. Means (± standard deviation) within the same column or row followed by different letters are significantly different ($p < 0.05$). Gel=Gelatin, RE=Rice extract.

samples containing gelatin and rice extract at various concentrations (5, 10 and 15%) maintained viability at the end of the 28 day storage period. Bacterial populations in all samples remained above 7.00 log CFU/ml, irrespective of the storage period.

The growth pattern of *L.delbrueckii spp. bulgaricus* in this study was similar to those obtained by Kim, Lee, Palanivel, and Kwak (2011), who examined the effect of yam supplementation on physiochemical, microbial, and sensory properties of yogurt. The authors found that the counts of lactic acid bacteria increased from 9.32 to 9.65 log CFU/ml as the concentration of powdered yam increased from 0.2% to 0.8% during storage at 4°C for 16 days. The survival of lactic acid bacteria in our study could be attributed to increased availability of starch, which the lactic acid bacteria hydrolyze to sugars and subsequently to lactic acid.

Table 5 shows the viability of *Streptococcus thermophilus* in yogurt samples prepared using different stabilizers during 28 days of storage at 4°C. At 1 day storage, the mean bacterial population among the yogurt samples ranged from 8.74-8.95 log CFU/ml, indicating no significant difference ($p>0.05$). The bacterial population in all samples ranged from 8.30-8.74 log CFU/ml at the 7^{th} day storage period, showing a slight decrease from day 1, except in control sample a significant ($p<0.05$) drop from 8.73 to 8.30 log CFU/ml was observed. At the 14 day storage period, there was a significant decrease ($p<0.05$) in bacterial population compared to day 1, for all samples and subsequent decrease thereafter. At the 28 day storage period, the bacterial population for control, gelatin, rice extract at 5, 10 and 15% supplementation was 7.00, 7.80, 7.68, 7.83 and 7.95 log CFU/ml respectively, compared to their initial bacterial population of 8.74, 8.95, 8.79, 8.82 and 8.87 log CFU/ml.

Overall, the bacterial population of *Streptococcus thermophilus* showed a significant decrease ($p<0.05$) in all samples by almost 1 log CFU/ml, compared to day 1. The survival

Table 5

Population of Streptococcus thermophilus in yogurt samples prepared with different stabilizers during 28 days of storage at 4°C

	Log CFU/ml				
Samples	**1 day**	**7 days**	**14 days**	**21 days**	**28 days**
Control	$8.74^{ab} \pm 0.098$	$8.30^{de} \pm 0.332$	$8.28^{ef} \pm 0.049$	$8.16^{efg} \pm 0.000$	$7.00^{j} \pm 0.000$
Gel	$8.95^{a} \pm 0.049$	$8.68^{abc} \pm 0.212$	$8.09^{efgh} \pm 0.028$	$7.97^{fghi} \pm 0.035$	$7.80^{hi} \pm 0.049$
RE 5%	$8.79^{ab} \pm 0.275$	$8.62^{bcd} \pm 0.014$	$8.37^{cde} \pm 0.021$	$8.17^{efg} \pm 0.183$	$7.68^{i} \pm 0.304$
RE 10%	$8.82^{ab} \pm 0.042$	$8.74^{ab} \pm 0.042$	$8.36^{cde} \pm 0.063$	$8.23^{efg} \pm 0.091$	$7.83^{hi} \pm 0.091$
RE 15%	$8.87^{ab} \pm 0.063$	$8.74^{ab} \pm 0.028$	$8.41^{cde} \pm 0.077$	$8.34^{de} \pm 0.028$	$7.95^{ghi} \pm 0.261$

Note. Means (± standard deviation) within the same column or row followed by different letters are significantly different ($p < 0.05$). Gel=Gelatin, RE=Rice extract

pattern of the bacterial population in all samples followed the same trend over the storage period, regardless of the stabilizer used. Overall, the bacterial population remained above 7 log CFU/ml for all of the samples, irrespective of the storage day.

These results coincide with a study conducted by Rosburg, Boylston and White (2010), who examined the viability of yogurt containing mixed strains with added oat beta-glucan and corn starch during cold storage. The authors found that *S. thermophilus* and *L. bulgaricus* survived at a level well above the therapeutic level of 10^7 CFU/ml and starch addition was found to favor the growth of these organisms. Survival of yogurt cultures in our study is consistent with another research work (Saccaro, Tamime, Pilleggi & Oliveira, 2009) which showed that *S. thermophilus* and *L. bulgaricus* strains survive well during cold storage at lowered pH. In the current study, however, we speculate that the cultures positively benefitted from the addition of rice extract or gelatin.

Table 6 shows the viability of bifidobacteria in yogurt samples prepared using different stabilizers during 28 day storage at 4°C. At 1 day storage, the mean bacterial population among the yogurt samples ranged from 7.29-7.53 log CFU/ml, indicating no significant difference ($p>0.05$). The bacterial population ranged between 5.83-6.56 log CFU/ml for all samples after the 7 days of storage, showing a significant decrease ($p<0.05$) by approximately 1 log CFU/ml, compared to those of the first day findings. At the end of 14 days, the bacterial population was not different for the control sample and samples containing gelatin, compared to the results obtained on day 7. However, there was a significant increase ($p<0.05$) in the bacterial population of the samples containing rice extract at 5, 10, and 15% supplementation (5.83, 5.95, 6.51 at day 7 to 7.14, 7.21, 7.33 log CFU/ml at day 14, respectively). The bacterial population remained the same at the 21st day for all samples, except for samples containing gelatin which had a

Table 6

Population of bifidobacteria in yogurt samples prepared with different stabilizers during 28 days of storage at 4°C

	Log CFU/ml				
Samples	**1 day**	**7 days**	**14 days**	**21 days**	**28 days**
Control	$7.29^{ab} \pm 0.091$	$6.45^{ef} \pm 0.212$	$6.52^{ef} \pm 0.106$	$6.67^{def} \pm 0.197$	$6.76^{de} \pm 0.106$
Gel	$7.53^{a} \pm 0.162$	$6.56^{ef} \pm 0.049$	$6.35^{f} \pm 0.042$	$7.26^{ab} \pm 0.176$	$6.90^{dc} \pm 0.190$
RE 5%	$7.26^{ab} \pm 0.021$	$5.83^{g} \pm 0.091$	$7.14^{bc} \pm 0.169$	$7.18^{abc} \pm 0.028$	$6.49^{ef} \pm 0.275$
RE 10%	$7.32^{ab} \pm 0.028$	$5.95^{g} \pm 0.000$	$7.21^{abc} \pm 0.254$	$7.39^{ab} \pm 0.000$	$6.73^{de} \pm 0.374$
RE 15%	$7.35^{ab} \pm 0.049$	$6.51^{ef} \pm 0.106$	$7.33^{ab} \pm 0.084$	$7.39^{ab} \pm 0.000$	$7.36^{ab} \pm 0.035$

Note. Means (± standard deviation) within the same column or row followed by different letters are significantly different ($p < 0.05$). Gel=Gelatin, RE=Rice extract

significant increase ($p<0.05$) from day 14 (6.56 to 7.26 log CFU/ml). After the 28 day storage period, the bacterial population for control, gelatin, rice extract at 5, 10 and 15% supplementation was 6.76, 6.90, 6.49, 6.73 and 7.36 log CFU/ml respectively, compared to their initial bacterial population of 7.29, 7.53, 7.26, 7.32 and 7.35 log CFU/ml

Overall, during the 28 day storage period, the population of bifidobacteria decreased significantly ($p<0.05$) in all samples, except for the sample treated with 15% rice extract. The bacterial population remained above 6 log CFU/ml for all samples at the end of the 28^{th} day storage. This indicates that rice extract at 15% concentration could be used to support the growth and viability of bifidobacteria cultures in yogurt.

The results were similar to a study conducted by Rosburg et al. (2010), who examined the viability of bifidobacteria strains in yogurt with added oat beta-glucan and corn starch during cold storage. It was found that *B. breve* counts remained above 7 log CFU/mL over the 5 week storage, in the presence of β-glucan. It was concluded that the addition of beta-glucan or corn starch could enhance the survival of *B. longum* in yogurt during cold storage.

Though there are gaps in the literature about the survival mechanism of bifidobacteria in yogurt, it is evident from the results of this study that bifidobacteria could not remain viable during refrigerated storage at 4°C. Therefore, the protective effect of rice extract (at 15% concentration) may cause physical changes in the environment surrounding the probiotic, or it could be due to the cytoplasmic buffering capacity (pH 3.72–7.74) which may allow the bacteria to resist changes in cytoplasmic pH and gain stability under acidic conditions (Kailasapathy & Chin, 2000). It has also been reported that the survival of bifidobacteria under acidic conditions is strain specific. The survival of nine strains of *Bifidobacterium* spp. in acidic conditions (pH 1.5–3.0) was studied; *B. longum* showed the greatest survival and *B. adolescentis, B. infantis, B.*

bifidum and *B. breve* survived poorly at all highly acidic pH levels (Kailasapathy & Chin, 2000). Since a mixture of strains was used in this study, the survival probability could be from one specific strain. Thus, the impact could be more of an encapsulating effect, rather than a prebiotic effect. The survival of bifidobacteria with respect to possible post-acidification was not investigated and; thus, no conclusions can be made regarding the effect of acidity on the survival of bifidobacteria.

4.1.2 Titratable acidity and pH. Yogurt samples were withdrawn from the incubator when a pH of 4.4-4.6 and a titratable acidity of ~0.85% was attained, which occurred within 5±0.5 h of incubation at 42°C. Measurements were also carried out throughout the storage period of 28 days.

Figures 4 and 5 represent the titratable acidity and pH values respectively for yogurt samples treated with different stabilizers at 4°C during 28 days of storage. The titratable acidity for control, gelatin, and rice extract at 5, 10, and 15% supplementation after 5h of incubation (0 day) was 0.66, 0.64, 0.66, 0.73, and 0.60, respectively and reached to a level of 1.55, 1.70, 1.91, 1.91 and 1.96% on day 28, indicating a significant increase ($p<0.05$). Usually, the normal pH of commercial yogurt products ranges from 4.0 to 4.4 (Seo, Lee, Chang, & Kwak, 2009). The pH values for control, gelatin, and rice extract at 5, 10, and 15% supplementation after 5h incubation period (0 day) was 4.58, 4.83, 4.59, 4.63, and 4.68, respectively and existed in the range of 3.61, 3.55, 3.52, 3.56 and 3.62 at 28 days of storage, indicating a significant decrease ($p<0.05$). It was observed that over time, the yogurt gels became more acidic, as indicated by a gradual increase in the titratable acidity and a decrease in pH from day 1 to 28.

Kim et al. (2011) also reported the pH and titratable acidity of yogurt samples supplemented with yam powder decreased and increased respectively, during 16 day storage at

4°C. The findings of the current study indicated that the addition of rice extract at various levels provided less adverse effects on the pH and titratable acidity of yogurt during the 28 day storage period.

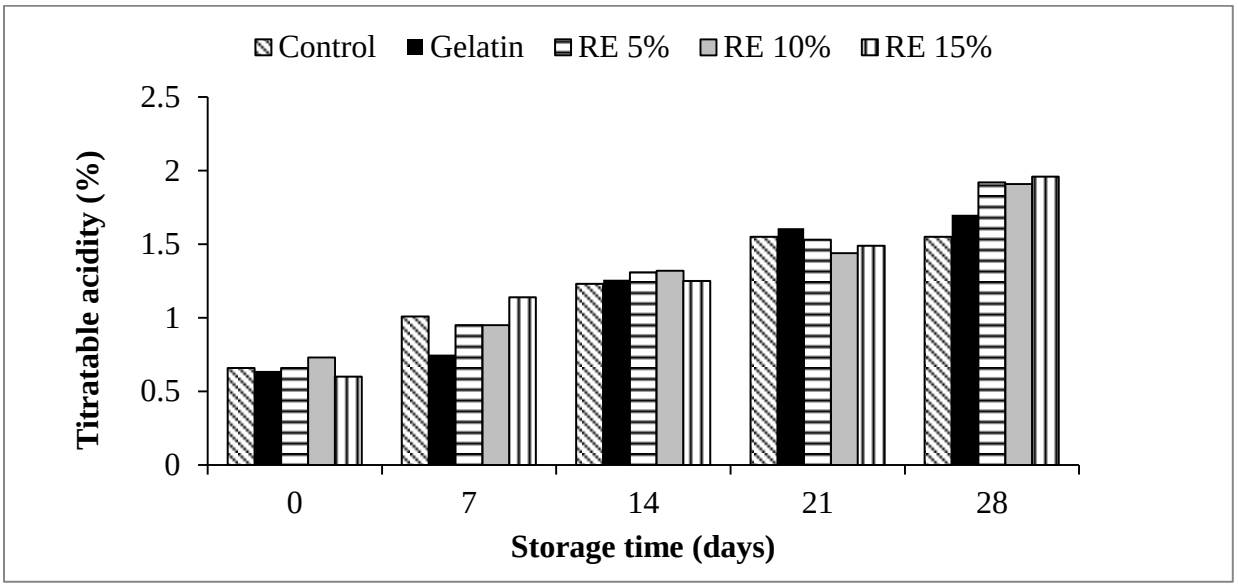


Figure 4. Titratable acidity (%) of yogurt samples prepared with different stabilizers during 28 days of storage at 4°C

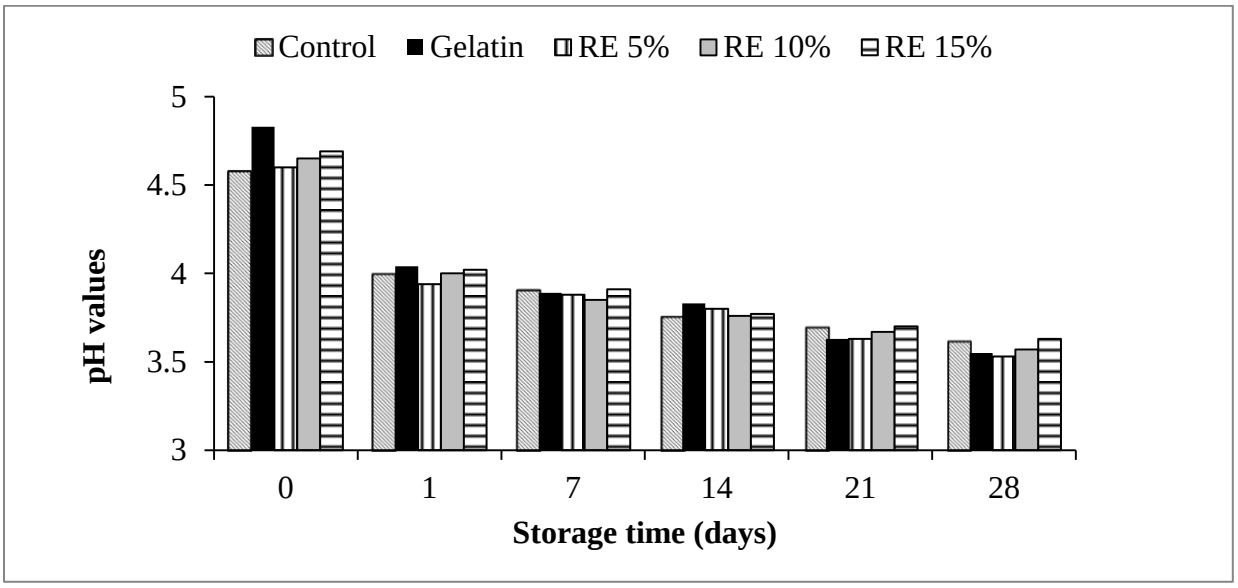


Figure 5. pH values of yogurt samples prepared with different stabilizers during 28 days of storage at 4°C

4.1.3 Total solids. The total solids (%) of yogurt samples prepared with different stabilizers at 4°C during 28 days of storage are represented in Figure 6. On day 1, the total solids of all samples ranged from 11.75-13.5%. The total solid contents of all samples showed a significant decrease ($p<0.05$) in the first 2 weeks of storage after which no significant decrease was observed between samples, except for control (11.75-9.42%). The total solids at the end of the 28 day storage period were 9.42, 10.43, 10.20, 10.76, and 10.74% for control, gelatin and rice extract (5, 10 and 15%) respectively.

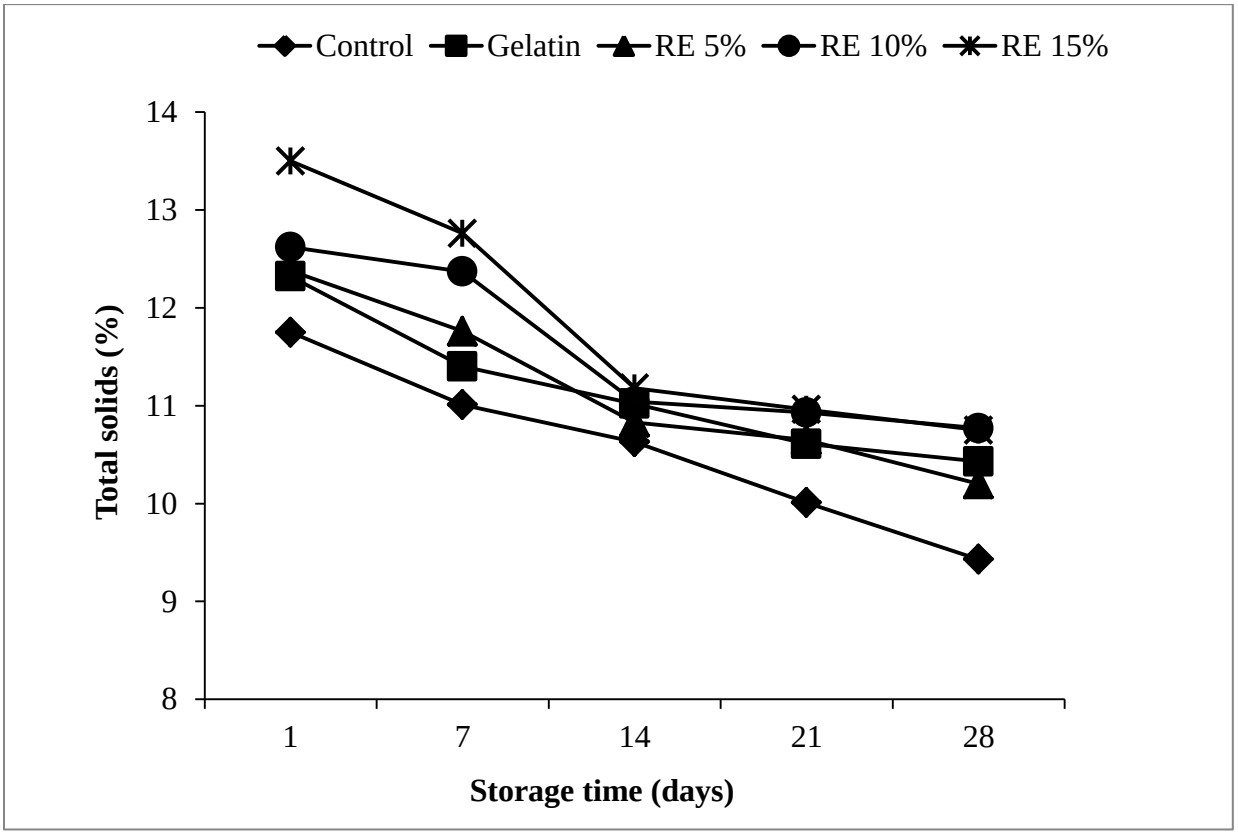


Figure 6. Total solids (%) of yogurt samples prepared with different stabilizers during 28 days of storage at 4°C

The results obtained in this study were consistent with those obtained by Obi, Henshaw, and Atanda (2010) who evaluated the quality of plain-stirred probiotic yogurt produced from skim and whole milk powder during refrigerated storage. They found that the total solid contents

of both samples showed a significant decrease in the first 2 weeks of storage after which no further decrease was observed. The total solids (%) for the skim milk probiotic yoghurt decreased from 14.25 – 12.50% while a decrease in total solids content from 14.20 – 11.75% was observed for the whole milk probiotic yogurt.

However, our results are not in agreement with the results of Khalifa, Elgasim, Zaghloul, and Mahfouz (2011), who tested the application of inulin and mucilage as stabilizers in yogurt production during a storage period of 10 days. In their study they found that there was a significant increase in total solids over the storage period and partially attributed that to the increase in titratable acidity and total carbohydrates. Generally, during the preparation of yogurt, milk is standardized to contain total solids at concentrations of 14-15% by adding milk powder, whey powder, milk protein concentrate, whey protein concentrate, or sodium caseinate. Increasing the total solid content, particularly the amount of protein in yogurt, generally increases the density of the protein network and decreases the pore sizes. Decrease in total solids content in this study could be due to the lack of milk standardization with some of the ingredients mentioned above.

4.1.4 Syneresis. Figure 7 shows the syneresis values of yogurt samples prepared with different stabilizers at 4°C during 28 days of storage. Syneresis of yogurts after centrifugation ranged from 8.90-17.88% on day 1. As the storage time increased, syneresis values increased for all samples substantially ($p<0.05$). However, samples prepared with gelatin showed less syneresis. Samples containing 5% rice extract expelled whey as much as the control sample at the 28 day storage period. The syneresis values at the end of the 28^{th} day were 41.88, 8.48, 40.40, 37.91, and 32.30% for control, gelatin and rice extract (5, 10 and 15%) respectively.

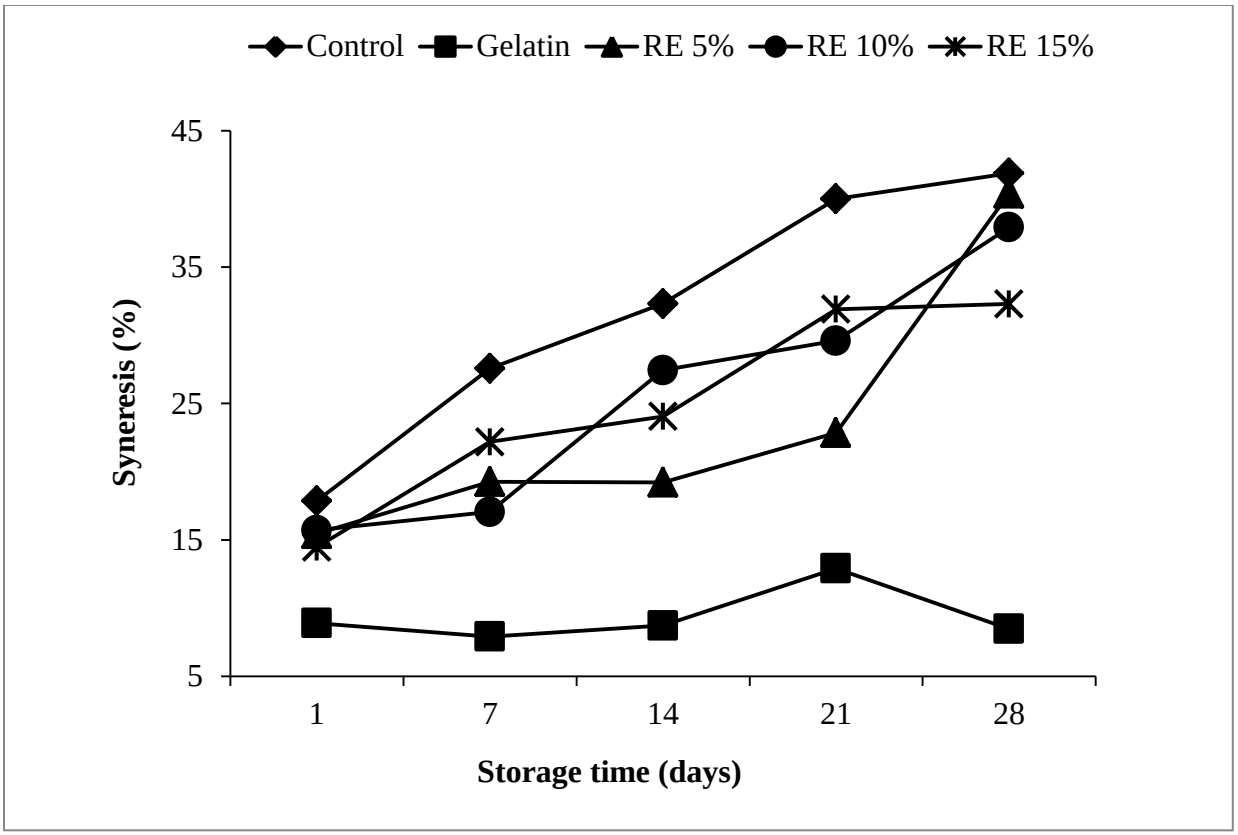


Figure 7. Syneresis (%) of yogurt samples prepared with different stabilizers during 28 days of storage at 4°C

The results obtained in this study were similar to those obtained by Vasiljevic et al. (2007), who studied the effect of addition of β-glucan from 2 different cereal sources (oat and barley) on the growth and metabolic activity of *Bifidobacterium animalis* ssp. *lactis* (Bb-12TM) in yogurt during prolonged cold storage. The syneresis values were also assessed and it was found that samples containing barley and oat β glucan expelled substantial amounts of whey even on the 1st day of storage (41.7 and 48.6% respectively) and postulated that it could be due to the presence of a long chain polysaccharide likely interfered with a development of a 3-dimensional structure of casein, leading to a weaker gel incapable of retaining water.

Another study was done on the effect of thickeners on the texture of stirred yogurt, in which samples were prepared with two concentrations of gelatin (3000 and 6000 ppm), three

with starch (1000, 5000, 10000 ppm) and a sample without thickener. The syneresis (%) measured by centrifugation at 1100 rpm for 10 minutes showed that gelatin was more efficient in reducing syneresis than starch. Syneresis decreased with increasing levels of gelatin. However, samples manufactured with the addition of 5000 or 10000 ppm of starch reduced syneresis by 18% (Gonçalvez, Pérez, Reolon, Segura, Lema, Gámbaro, Varela, & Ares, 2005). Although the addition of rice extract did not prevent syneresis, the findings from our study revealed that rice extract at higher concentrations could help in reducing syneresis in yogurt over storage.

4.2 Viscosity Measurements

Fermented yogurt samples were stored in a refrigerator overnight at 4°C and analyzed for viscosity the following day using L3 spindle at 10 rpm. Based on the results obtained from the storage study, a concentration of 10% rice extract was chosen and samples that were evaluated included: a yogurt sample with no stabilizer (control), yogurt sample stabilized with gelatin and yogurt sample stabilized with 10% rice extract.

Figure 8 shows the mean viscosity measurements for yogurt samples. It was observed that the initial viscosity of yogurt samples were 5850, 9700 and 9820 mPas for control, gelatin and rice extract stabilized yogurts, respectively. The viscosities gradually decreased with time and at the end of 14 min, the values were 970, 3860 and 3850 mPas for control, gelatin and rice extract stabilized yogurts, respectively. The control samples had the least viscosity measurements and gelatin and rice extract stabilized yogurts were almost similar.

Milani and Koocheki (2011) analyzed the effects of date syrup and guar gum on the physical and sensory properties of low fat frozen yogurt dessert. Their findings showed that as the gum and date syrup concentration increased, the viscosity increased in a linear manner. It was suggested that the higher solid contents due to the molecular movements and interfacial film formation could be attributed to the increase in viscosity. The viscosity of yogurt was improved

with the addition of yam powder (without the removal of mucilage and starch) when compared to the viscosity of yogurt with yam powder after removing mucilage and starch. The results confirmed that yam powder mucilage and starch was associated with the viscosity of yogurt (Kim et al., 2011). Another study conducted by Amaya, Martínez-Alegría, Zazueta-Morales, and Martínez-Bustos (2008) reported that the viscosity of yogurt formulated with acid thinned jicama and maize starch did not show differences among samples.

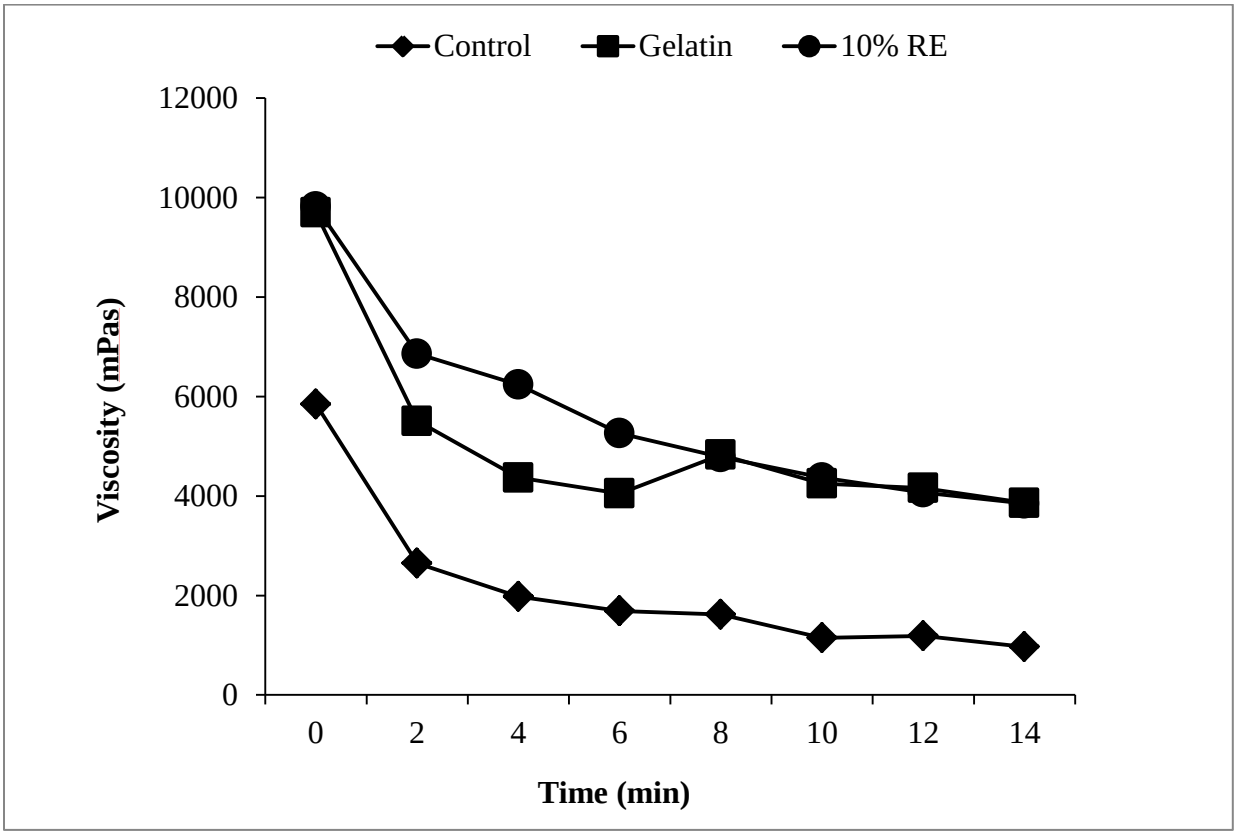


Figure 8. Viscosity measurements (mPas) for yogurt samples at 10 rpm at 25°C

Viscosity development is an indication of the aggregation of casein micelles and consequently leading to the biochemical and physiochemical changes during the fermentation of milk (Singh & Kim, 2009). Some studies also proposed that casein and starch interaction led to increase in viscosity, but promoted phase separation (Williams et al., 2004). Thus, in the present

study, the addition of 10% rice extract in yogurt may favor the interaction of the rice starch with casein in yogurt, which could eliminate static repulsion and aid in viscosity development.

4.3 Sensory Analysis

The yogurt samples were evaluated by 10 untrained participants on a 9 point hedonic scale. Based on the results obtained from the storage study, a concentration of 10% rice extract was chosen and samples that were evaluated included: a yogurt sample with no stabilizer (control), yogurt sample stabilized with gelatin and yogurt sample stabilized with 10% rice extract. Fermented samples were stored in a refrigerator overnight at 4°C and analyzed the following day for sensory characteristics including appearance, color, texture, aroma and overall liking.

Figure 9 represents the average sensory ratings obtained for yogurt samples on a 9 point hedonic scale. With respect to appearance, yogurt samples prepared with rice extract ranked the highest (6.8), placing it more on the “like moderately” category, followed by gelatin (6.3) and control (4.8). Since texture is one of the critical aspects in yogurt, the maximum score was obtained for yogurt stabilized with 10% rice extract (6.5), which correlated with our viscosity measurements. All yogurt samples obtained almost similar scores with respect to color 6.5, 6.9 and 6.70 for the control, gelatin, and 10% rice extract samples, respectively. The yogurt samples stabilized with gelatin were ranked higher in terms of aroma 7.3, followed by yogurt stabilized with rice extract (6.3), and control (6.2). Overall, the scores of yogurt stabilized with rice extract (6.9) and gelatin (7.0), were almost similar. However, the control samples ranked the least in all of the sensory characteristics tested.

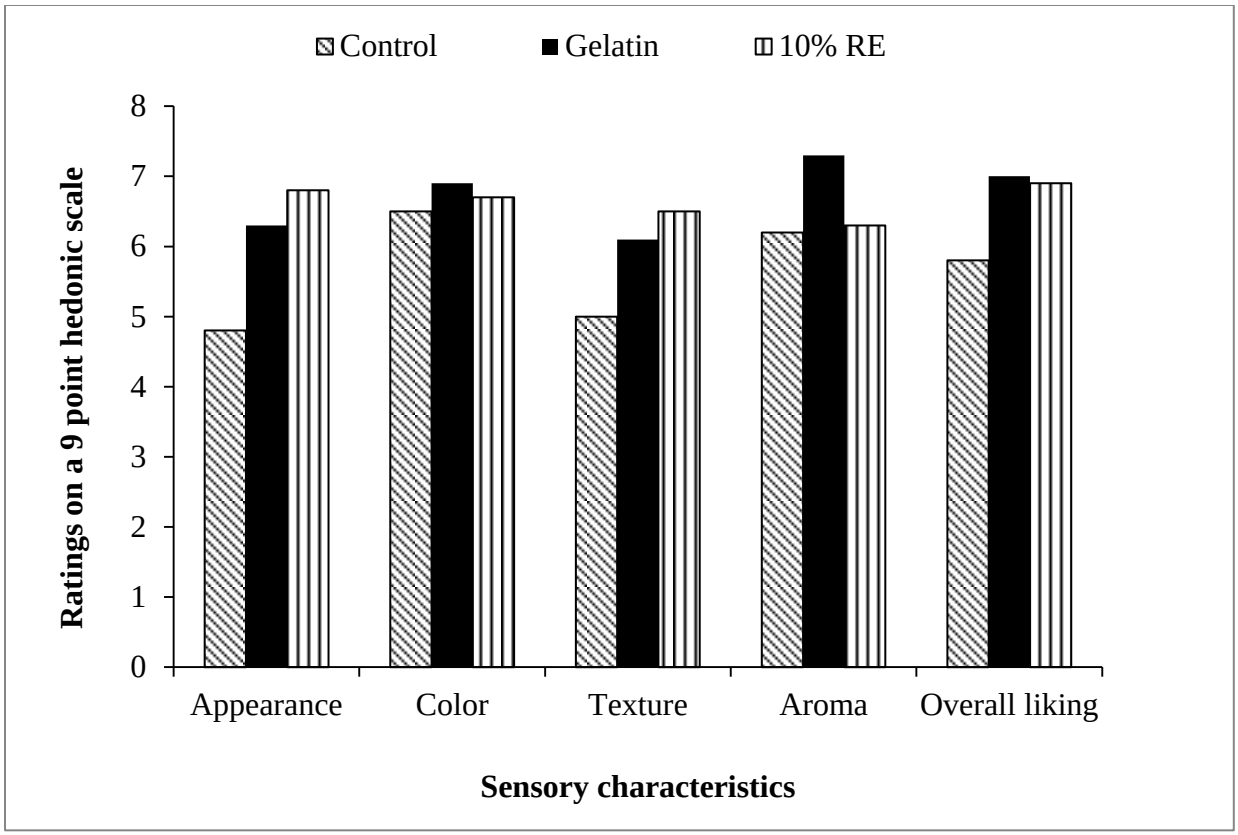


Figure 9. Average sensory ratings of yogurt samples at 4°C on a 9 point hedonic scale

It is possible that the thickness of the product influenced the acceptability of yogurt samples. In many food products, appearance or eye appeal is the first indicator of quality and may contribute significantly to the decision of the consumer to accept or reject the product (Alakali, Okonkwo, & Iordye, 2008). The tendency of starch to impart a good body as well as smooth and glossy appearance in some foods may not be ruled out in explaining why rice extract containing yogurt had the best appearance. The present study also indicated that the addition of rice extract did not influence some of the sensory attributes and was preferred by the consumer panel.

CHAPTER 5

Conclusions

In the present study, the microbiological, chemical, and physical properties of yogurt prepared with rice extract were ascertained over storage for a period of 28 days at 4°C. Furthermore, the viscosity and consumer acceptability were determined.

The results from the microbiological study showed that the population of *L.delbrueckii spp. bulgaricus* at the 28 day storage period when compared to day 1, showed a significant decrease ($p<0.05$) in the control sample. However, samples containing gelatin and rice extract at various concentrations maintained viability at the end of the 28 day storage period. The bacterial population of *Streptococcus thermophilus* showed a significant decrease ($p<0.05$) in all samples by almost 1 log CFU/ml after 28 days. The population of bifidobacteria also decreased significantly ($p<0.05$) in all samples, except for the sample treated with 15% rice extract, which indicated that rice extract at 15% concentration could be used to support the viability of bifidobacteria in yogurt. The addition of rice extract did not alter the pH and the titratable acidity of yogurt over storage. The total solids showed a gradual decrease and the addition of rice extract helped in reducing syneresis.

The viscosity measurements revealed that yogurt containing 10% rice extract showed higher viscosity and that the starch present in rice could be associated with the increased viscosity. Results from the sensory analysis demonstrated that yogurt samples containing 10% rice extract scored higher in texture, appearance, and was also preferred by the consumer panel.

The findings of this study indicated that rice extract could be used as a potential stabilizer for its clean label, stable characteristics, and reasonable cost. Further studies should be carried out to determine the rheological aspect of rice extract addition into yogurt and the stabilizing

mechanism of rice extract under different conditions. Additionally, the gel network could be investigated using confocal scanning electron microscopy to obtain further information on the starch and milk protein interaction. For enhanced quality and texture attributes, rice extract could be supplemented with a variety of gums and other polysaccharides and explored in different food systems.

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