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Functional Plain Yogurt Enhanced with Inulin and Aloe Vera

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FUNCTIONAL PLAIN YOGURT ENHANCED WITH INULIN AND ALOE VERA

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ABSTRACT

Yogurt has been recognized as a healthy food. Nutritional value of yogurt can be increased with the addition of aloe vera juice (AVJ) and inulin. People are interested in consuming products without artificial ingredients and with high content of bioactive compounds. The aims of this study were to develop a functional plain yogurt enhanced with inulin and AVJ, to evaluate the physicochemical properties, the enumeration of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, inulin content, and consumer acceptability. Six treatments were evaluated at day 1, 7, 14 and 30 at 4°C. Treatments were prepared using 2% fat milk, ABY culture, 5 and 15% of AVJ, and 1.5 % of inulin. Yogurts were analysed for syneresis, pH, color, titratable acidity (TA), viscosity, inulin content, enumeration of starter cultures. A consumer test was also conducted. Triplicate experiments were conducted, data were analyzed at $\alpha = 0.05$ using a factorial arrangement with repeated measures in time. Results show pH decreased and TA increased over time. Addition of inulin and increase levels of AVJ decreased pH and TA. Luminosity (L*) was reduced significantly ($P<0.05$) in treatments with AVJ. Treatments with 15% AVJ and inulin decreased a* and increased b* values significantly ($P<0.05$). ΔE^* was less than 3 in all treatments. Syneresis increased and viscosity decreased significantly ($P<0.05$) over time. Treatments with 15% of AVJ and inulin was significantly higher for syneresis and lower in viscosity ($P<0.05$) than the other treatments. *Streptococcus thermophilus* counts increased ($P<0.05$) over time with values higher than 10^9 CFU/g. *Lactobacillus bulgaricus* counts decreased at day 14 and increased significantly at day 30 with values higher than 10^6 CFU/g. Inulin content decreased over time. The treatment with 15% AVJ had the lowest inulin content. For sensory evaluation, treatments had significantly lower sensory scores. Purchase intent increased after the consumers are made aware of the health benefits. The study demonstrated that use high concentration of AVJ in combination with inulin

negatively affect important properties in yogurt; however, yogurt can be fortified with inulin and low concentrations of AVJ to provide health benefits.

CHAPTER 1. INTRODUCTION

Fermented milk products represent an important component of functional foods. Several studies have been conducted to develop different kinds of fermented dairy products with the incorporation of probiotics to give them an added value (Panesar 2011). Yogurt is a popular fermented dairy product. It has been considerate as a healthy food due to the beneficial action of its viable bacteria. Those bacteria can compete with other bacteria like pathogenic bacteria for nutrients and space in the gastrointestinal system. (Tamine and Robinson, 1985). The main strains used in yogurt starter cultures are *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Yogurt contains different natural compounds with a high nutritional value such as vitamins (mainly B_{12}, riboflavin and vitamin D); also, it contains proteins, peptides, and minerals (principally calcium, phosphorus, and potassium). Because of its nutritional value, yogurt is considered a foodstuff for daily consumption (Singh *et al*., 2012). Worldwide, yogurt has been considerated as the second most popular snack for children (Han *et al.*, 2016).

Of the many bioactive compounds that can be added to yogurt, probiotics is the most widely used. The most common commercially available probiotics are species of the genera *Bifidobacterium* and *Lactobacillus*, and other species such as *Streptococcus* (Gueimonde *et al*., 2011). Probiotics can improve human health when there is an ingestion of those. Other functional ingredients can be used to enrich yogurt. They can originate from nondairy and dairy sources. Among those functional ingredients are different types of fibers such as inulin, ingredients like phytosterols, peptides, β-glucans from various sources, vitamins, and polysaccharides (Ozer and Kirmaci, 2010). Aloe Vera has been used in traditional medicine for several thousand years. The attribution of several positive effects in aloe vera motivated the food industry to use aloe extracts as a supplement for functional foods (Newton 2004). Nell 2004, reported the use of aloe vera in functional and nutritive foods especially in dairy sectors and beverages. The main functional component in aloe vera is the polysaccharide acemannan (Salah *et al*., 2017). This component is the responsible of the beneficial properties attributed to Aloe Vera, these properties include the reduction in blood glucose, blood pressure and the improvement of lipid profile in diabetic patients, among many others (Fuentes *et al*., 2017). Aloe vera has been used in yogurt, since aloe has shown a good viability of probiotic cultures (Panesar and Shinde, 2012).

Another important functional ingredient used in yogurt are prebiotics. This term refers to selectively fermented ingredients which result in specific changes in composition and/or the activity of gastrointestinal flora (Abreu-Abreu 2012). When prebiotics are consumed, they provide a beneficial physiological effect on the host, they stimulate the favorable growth or metabolism of beneficial bacteria (Sanders *et al*., 2007). Prebiotics such as inulin have been found to stimulate absorption and retention of several minerals, particularly magnesium, calcium, and iron (Iannitti and Palmieri, 2010). Inulin belongs to a group called fructans, a type of carbohydrate. This prebiotic improves the survival and activity of the storage of other probiotics such as *Lactobacillus acidophilus* (Kaplan and Hutkins, 2003) and *Bifidobacterium spp.* (Alkalin *et al*., 2004). Plain yogurt enhanced with different concentrations of aloe vera (0, 5 and 15%) and inulin (0 and 1.5%) were manufactured to determine the influence and effect of inulin and aloe vera in the chemical and physical characteristics of yogurt during 30 days of storage at 4 ºC. The quantification of inulin content and the enumeration of Streptococcus thermophilus and Lactobacillus bulgaricus were conducted to determine the effects of these functional ingredients in the starter cultures and determine the acceptability of this functional yogurt. The main objective of this research was to develop a functional plain stirred yogurt enhanced with inulin and aloe vera.

CHAPTER 2. LITERATURE REVIEW

2.1 Fermented dairy products

2.1.1 Definition

Fermented milk has an important role in human nutrition. Fermented dairy products are nutritious because starter cultures predigest fats, carbohydrates, and proteins; therefore, improving the digestibility and absorption in the intestinal tract (Shahani 1979). Lactic acid bacteria are the main player during milk fermentation converting lactose to lactic acid. The most common lactic acid bacteria (LAB) used are species belonging to the genera *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Enterococcus* and *Lactococccus* (Quigley *et al*., 2011). LAB used in dairy fermentation are divided into two groups: mesophilic and thermophilic lactic acid bacteria. Mesophilic have an optimum growth temperature between 20ºC and 30ºC. Western and northern European countries use mesophilic bacteria in their fermented products. Thermophilic bacteria have their optimum growth between 30ºC and 45º. Sub-tropical countries use this bacteria for traditional fermented products (Wouters *et al*., 2001).

Fermented milk products represent an important component of functional foods. Much research has been conducted to develop different kinds of fermented dairy products with the incorporation of probiotics to give them an added value (Panesar 2011). Other health benefits from dairy products come from several nutrients that are present in milk. Other bioactive components are produced by probiotic bacteria during the fermentation process. Some of those nutrients and bioactive compounds are vitamins, proteins, bioactive peptides, fatty acids and oligosaccharides (Ebringer *et al*., 2008).

There are multiple fermented dairy products in which diverse microorganisms are involved like: curd, yogurt, cultured buttermilk, lassi, acidophilus milk, Bulgarian buttermilk, koumiss, kefir, Leben, cheese, and others (Panesar 2011). During fermentation, physical and chemical changes occur in dairy products due to the growth and fermentative activities of LAB used as a starter culture (Koutinas 2017).

2.1.2 Yogurt

It is a popular and recognized fermented dairy product. Yogurt is an important nutritional and healthy source. It has been considered as a healthy food due to the beneficial action of its viable bacteria. Those bacteria can compete with bacteria, like pathogenic bacteria, for nutrients and space in the gastrointestinal tract for maintaining this section of our body (Tamine and Robinson, 1985).

The main strains used as starter cultures are *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. During milk fermentation, yogurt gel is formed. Milk is usually heated at 85 ºC for 30 min, causing the denaturation of whey proteins interacting and cross linking with ƙ-casein. As the pH approaches 4.6 by the production of lactic acid from *Streptococcus thermophilus* and *Lactobacillus bulgaricus,* there is increased casein-casein attraction.

Yogurt can be stirred or set. Stirred yogurt is the result of a mechanical effect such as shearing after fermentation. The set yogurt usually is fermented in a pack, obtaining a firmer structure than the stirred yogurt (Loveday *et al.,* 2013). Other kinds of yogurt are plain yogurt, fruit-flavored yogurt (it could be in blended forms or with fruit on the bottom), whipped yogurt, granola topped yogurt, frozen yogurt, drinkable yogurt, and Greek yogurt. Those yogurts can have different fat contents (nonfat, low fat and regular fat) (Cassel 2014). According to the US Code of Federal Regulations in 21CFR131.206 and 21CFR131.203, nonfat yogurt contains less than 0.5% milkfat and low-fat yogurt between 0.5% and 2% milkfat. In recent years, people have increased the demand for low-calorie products increasing their consumption. That increased consumption may reduce the risk of type 2 diabetes. Consumption of 4 servings/d of low-fat dairy milk and yogurt products for 6 months may improve insulin resistance without negatively impacting bodyweight or lipid status (Rideout *et al.*, 2013).

2.1.3 Market of yogurt

In the world, yogurt has been the second most popular snack for children (Han *et al.*, 2016). Several trends are growing within cultured dairy markets especially with yogurt. The per capita consumption of yogurt in pounds per person has increased every year from 2000 to 2015. In 2015, the consumption was 14.4 pounds per person. In 2016, consumption decreased a bit to 13.7 pounds per person (USDA 2017).

Manufacturers are increasing the production of clean labels or less processed products. Sugar content is decreasing. Cultured products are becoming more popular and functional by the usage of probiotics A report from Chicago-based Mintel shows that spoonable yogurt had the most sector sales in the market with a value of $8.2 billion in 2016. This kind of yogurt represents 90% of all yogurts consumed and is expected that the sales will continually grow to $10 billion by 2021 (Kennedy 2017).

2.1.4 Health benefits of yogurt

Yogurt is widely consumed because of its beneficial effects, playing an important role in human health (Loveday *et al.*, 2013). Yogurt contains different natural compounds with a high nutritional value such as vitamins (mainly B_{12}, riboflavin and vitamin D). It also contains proteins, peptides, and minerals (principally calcium, phosphorus, and potassium). Because of this high nutritional value, yogurt is considered a foodstuff for daily consumption (Singh *et al*., 2012). There are different bioactive compounds that can be added to yogurt, specifically probiotics. However, there are other functional ingredients that can be used to enrich yogurt. These ingredients can be non-dairy and dairy sources, such as different types of fibers acting as prebiotics, phytosterols, peptides, β-glucans, polysaccharides, and vitamins (Ozer and Kirmaci, 2010).

2.2 Probiotics

Dairy products with probiotics can be considered "functional foods" because they demonstrate and confer certain health benefits on the host beyond basic nutritional functions when administrated in adequate amounts (FAO-WHO 2006). The most common commercially available probiotics are species of the genera *Bifidobacterium* and *Lactobacillus*, and other species such as *Streptococcus* (Gueimonde *et al*., 2011). Probiotics can improve human health when there is an ingestion of those, by inhibiting the growth of harmful bacteria. Also, they provide protection against pathogenic microorganisms in the intestine by producing known antimicrobial substances like hydrogen peroxide, organic acids, diacetyl, bacteriocins, and short chain fatty acids (Salminen 1999). Likewise, probiotic bacteria can induce mucosal regeneration by increasing the numbers of cells in the villi and increasing mitosis rate in the small intestine (Banasaz *et al*., 2002).

Streptococcus thermophilus and *Lactobacillus bulgaricus* are considered probiotics since they can help to lessen the symptoms of lactose intolerance (Gill and Guarner, 2005). However, *Streptococcus thermophilus* and *lactobacillus bulgaricus* are not bile- resistant or acid-tolerant and thus cannot survive under gastro intestinal conditions (Del campo *et al*., 2005). The term bio-yogurt is for some yogurt products that have been reformulated to include species of *Bifidobacterium* and strains of *L. acidophilus* also known as AB cultures which are added with the yogurt starter culture. The bio-yogurt contains live probiotic microorganisms, claiming to be beneficial for human health (Koutinas 2017). The minimal amount suggested for probiotic bacteria in dairy products is 10^6 CFU/g/day. The high dosage is required to compensate the loss of cells during the passage through the gastrointestinal tract (Granato *et al*., 2010). In the U.S., refrigerated yogurts displaying the "Live and Active Cultures" seal on the containers should have $\geq$ 8 log CFU/g yogurt bacteria at the time of manufacture (NYA, 2009).

2.2.1 *Streptococcus thermophilus*

Streptococcus thermophilus is gram-positive bacteria that is gram catalase negative. It is a small cocci in pair with long chains between 0.7 - 0.9 µm. It is a facultative anaerobe and its fermentation is homofermentative. Its optimum pH for growth is 6.5. This bacteria is identified among the lactic acid bacteria by its high-temperature tolerance, it can resist temperatures like 60 ºC for 30 minutes.

S. thermophilus can ferment in glucose, fructose, lactose and sucrose (Hui 2007). During yogurt fermentation, *S. thermophilus* grows optimally between 37 - 45 ºC and hydrolyzes lactose via β-galactosidase. This bacteria requires a complex of amino acids to grow and is nutritionally fastidious (Driessen, Kingma, and Stadhouders, 1982). *S. thermophilus* is of commercial interest because it builds up the texture in yogurt due to the secretation of exopolysaccharides (Delorme, 2008 and Broadbent *et al*., 2003).

2.2.3 *Lactobacillus delbrueckii subsp. bulgaricus*

Lactobacillus bulgaricus is a gram-positive bacteria that is catalase negative. *L. bulgaricus* is long, straight sided rods singly paired or in short chains about 0.5 - 0.9 × 2 -9 µm. It is facultative anaerobic with the optimum temperature for growth between 40 - 45 ºC. Also, *L. bulgaricus* is homofermentative and ferments glucose, fructose and lactose (Hui 2007).

This bacteria is considered one of the most important economically of the heterogeneous group of LAB in yogurt production (Heller 2001). *L. bulgaricus* is highly recognized because its competent proteolytic system consisting of peptidases and extracellular proteinase (Khalid and Marth, 1990).

2.2.4 Synergistic interactions between *S. thermophilus* and *L. bulgaricus*

During the processing of yogurt, there is a positive interaction between *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The interaction of the two strains change the physicochemical properties and the aroma compounds in yogurt, these changes differ when each single strain is used separately (Sieuwerts *et al*., 2010).

Streptococcus thermophilus and *Lactobacillus bulgaricus* have a symbiotic relationship during the processing of yogurt. During yogurt fermentation, *Streptococcus thermophilus* grows faster at the beginning of yogurt fermentation by the use of the essential amino acids produced by *Lactobacillus bulgaricus*. Due to this action, *S. thermophilus* produces lactic acid, reducing the pH and allowing the growth of *L. bulgaricus*. Also, the production of formic acid and carbon dioxide from lactose by *Streptococcus thermophilus* stimulates the growth of *Lactobacillus bulgaricus* (Driessen *et al*., 1982). *S. thermophilus* is inhibited around pH of 4.2-4.4. *L. bulgaricus* tolerates pH of 3.5 - 3.8 (Hamann and Marth 1983).

S. thermophilus declines when the yogurt fermentation increases, but *L. bulgaricus* continues to reduce the pH producing amounts of lactic acid. The distinctive yogurt flavor arises by the interaction of these two bacteria producing acetaldehyde. *S. thermophilus* produces acetaldehyde by its metabolism but is less active at normal fermentation than *L. bulgaricus* (Davis *et al*., 1971).

Another important compound in yogurt is folic acid. *S. thermophilus* is responsible for the production of this compound, but the level of the folic acid depends on the *L. bulgaricus* strain. The *L. bulgaricus* strain uses and degrades folic acid during its growth (Rao *et al*., 1984). Considering this, it is essential to determine the optimal combination of these strains. By not having an adequate combination, it can affect the organoleptic and physical acceptability of the yogurt. Furthermore, it can decrease the concentration of folic acid.

2.2.5 *Lactobacillus acidophilus*

Lactobacillus acidophilus is a gram-positive and non-spore forming and non-flagellated rod. It is a microaerophilic bacteria, meaning it requires oxygen to growth but at a lower concentration. It grows best under modified atmospheric conditions and is a homofermentative bacteria (Kurman and Rasic 1991). The growth of *L. acidophilus* may occur at 45 ºC, but the optimum growth is between 35- 40 ºC (Marshall and Cole 1983).

According to Modler 1990, it is considered a probiotic bacteria. It has been used in fermented dairy products such as yogurt. *L. acidophilus* provides various therapeutic benefits such as reduction in the level of cholesterol, prevention of cancer, management of gut flora, stimulating host immune mechanism, and enhancing nutrient bioavailability (Sandine 1979). *Lactobacillus acidophilus* help to inhibit *Campylobacter jejuni* and *Salmonella typhimurium* due this probiotic produces the bacteriocin CH5 (Lee and Salminen, 2008). Although *L. acidophilus* tolerates acidity, studies have shown a decrease in their number under acidic condition (Conway *et al*., 1987). The growth of this probiotic bacteria ceases below pH 4.0. Hydrogen peroxide produced by *Lactobacillus bulgaricus* also affects the growth of this bacteria (Shah 1997).

2.2.6 *Bifidobacterium animalis ssp. lactis* BB-12

Bifidobacteria are gram- positive actynomycetes. They grow strictly under anaerobic conditions and are branched rod-shaped bacteria. Bifidobacteria are found naturally in the human's gut and other mammals (Parche *et al*., 2007). There are around 30 species of Bifidobacterium, perhaps the most studied probiotic is *Bifidobacterium animalis ssp. lactis* BB-12 (Garrigues *et al*., 2010). According to Libera *et al*., 2015, *Bifidobacterium animalis* can be used as a health promoting starter culture. Products using this bacteria may be considered as a potential functional food.

2.3 Aloe vera

2.3.1 Definition and properties

The aloe vera plant has been used in traditional medicine for several thousand years. Its applications have been recorded in ancient cultures like India, Egypt, Greece, Rome and China (Ahlawat and Khatkar 2011). Actually, the aloe vera industry is flourishing, and the gel is used in many products such as fresh gel, juice and other formulations for health, medicinal and cosmetic purpose (Eshun and He 2004). The beneficial effects of aloe vera have motivated the food industry to start using the aloe extracts as a supplement for functional foods (Newton 2004).

Aloe vera juice is composed approximately of 99% water (Hamman 2008). The remainder is vital ingredients include vitamins, minerals, amino acids, polysaccharides, enzymes, plant steroids, saponins, lignin, anthraquinones, and salicylic acid (Keerthi *et al*., 2017). The polysaccharides contain glucomannans, mannans, and pectins of different molecular weights and are responsible for its biological activities *in vivo*, as well as, *in vitro* (Yaron 1993).

Some of the most common health benefits from aloe vera products include immunomodulatory activity, digestive aid, anti-diabetic activity, oral/gum health, anti-inflammatory, cholesterol/triglyceride reduction, antioxidant protection, and detoxification (Gruenwald 2009; Eshun 2004). Aloe vera also is related to pharmacological actions that include anti-inflammatory, antiproliferation, antimicrobial activity laxative and blood glucose lowering effects (Reynolds and Dweck 1999).

2.3.2 Acemannan

The main functional component in aloe vera is acemannan (Salah *et al*., 2017). It helps in the reduction of blood glucose, blood pressure and the improvement of lipid profile in diabetic patients, among many others (Minjares *et. al*., 2017).

Acemannan storage polysaccharide is located within the protoplast of the parenchymatous cells of the aloe vera gel (not a component of the cell walls) (Femenia *et al*., 1999). Acemannan is mainly composed of a glucose and mannose chain with β-(1-4) bounds and galactose branching (Pellizoni *et al*., 2011). The molecular weight of acemannan compound is around 40 - 50 kDa (Talmadge *et al*., 2004).

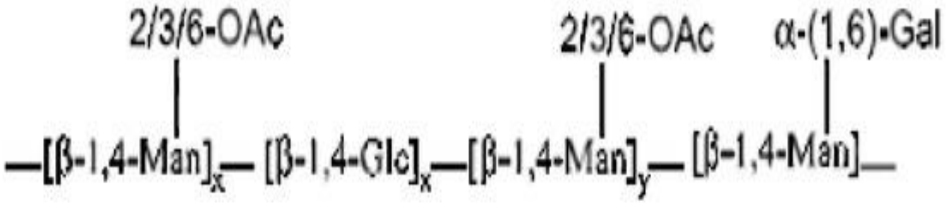

Figure 1. The structure of acemannan

2.3.3 Aloe vera in fermented milk products

Aloe vera can provide health advantages when it is used in food. Nell 2004, reported the use of aloe vera in functional and nutritive foods especially in dairy sectors and beverages. Aloe vera has been used in yogurt and has shown a good viability with probiotic cultures (Panesar and Shinde, 2012). According to Azari *et al*., 2017, the high concentrations of aloe vera in yogurt had a negative effect in the viscosity of the same. The aloe vera can make up a fragile coagulum due to the aloe containing a high number of polyphenols which are highly reactive. The use of aloe gel in yogurt maintain an acceptable range of *L. acidophilus* and *Bifidobacterium lactis* BB-12 between 10^6 - 10^7 CFU/g (Azari *et al*., 2017). According to Michael *et al*., 2010, when increasing the concentration of aloe vera gel in fermented products like yogurt, it can increase syneresis. This is due to the aloe vera decreasing the colloidal stability of casein micelles, therefore, increasing yogurt syneresis. Previous studies reported that syneresis increase over time owing to the decrease of pH and increase of acidity (Fox *et al*., 2000).

Dahi is a fermented milk product consumed in South-Asian countries. It is prepared using mixed strains of mesophilic lactic acid bacteria. By applying aloe vera juice in dahi, it decreases pH values and increases probiotics counts. According to Hussain *et al*., 2016, the addition of aloe vera into dahi increases the physiological and nutritional virtues. However, the usage of this functional ingredient in fermented milk products can affect different attributes such as texture and sensory attributes and affect consumer's acceptance.

Cultured buttermilk is a dairy beverage with a therapeutic and high nutritive value. According to Mudgil *et al*., 2016, the fortification of cultured buttermilk with aloe vera improves nutritive qualities by increasing vitamin C, dietary fiber, iron, desirable sensory characteristics, and physicochemical properties. The aloe vera added to cultured buttermilk does not affect the acidity nor the pH of the product. When the concentrations of aloe vera juice are increased, the phase separation decreases, and the viscosity increases proportionally.

Another fermented dairy product is Lassi. This product is made out of cow's milk and has a sour taste. Lassi is the left-over liquid after the extraction of butter from dahi. A distinct method to prepare Lassi is by blending yogurt with water, salt, and species. The addition of aloe vera in Lassi affected some physicochemical characteristics decreasing pH but increasing titratable acidity; and decreasing viscosity but increasing consumers' acceptability (Singh *et al*., 2012). Functional foods present and give valuable promises for developments in human nutrition. They can contribute by improving the food quality and consumer's health. Bioactive fermented milk products such as yogurt have many nutraceutical and nutritional values for consumers.

2.4 Prebiotics

2.4.1 Definition and health benefits

The term prebiotics refers to selectively fermented ingredients which result in specific changes in composition and/or the activity of gastrointestinal flora (Abreu-Abreu 2012). When prebiotics are consumed, they provide a beneficial physiological effect on the host. Prebiotics stimulate the metabolism and growth of beneficial bacteria (Sanders *et al*., 2007).

A food ingredient can be prebiotic if it resists gastric acidity of the stomach, bile salts and other hydrolyzing enzymes in the intestine. The absorption of the same should not occur in the upper gastrointestinal tract. It must be easily fermentable by the intestinal microflora. The prebiotics selectively stimulate the growth or activity of intestinal bacteria potentially associated with health and well-being (Gibson *et al*., 2004). Some common prebiotics include fructooligosaccharides, galactooligosaccharides (GOS), xylooligosaccharides pyrodextrins, inulin, isomaltooligosaccharides, lactulose, soy-derived, oligosaccharides (Oliveira and Gonzales, 2016).

Prebiotics such as inulin, oligofructose, glucooligosaccharide, and galacto-oligosaccharide have been found to stimulate absorption and retention of several minerals, particularly magnesium, calcium, and iron (Iannitti and Palmieri, 2010). Prebiotics reduce the prevalence and duration of infectious and antibiotic-associated diarrhea. They also reduce the inflammation and symptoms associated with inflammatory bowel disease, exert protective effects to prevent colon cancer and lower some risk factors for cardiovascular disease. Prebiotics promote satiety and are related to weight loss and prevent obesity (Slavin 2013).

2.5.2 Interaction with probiotics

Prebiotics and probiotics can be mixed together, forming a symbiotic system (Iannitti and Palmieri, 2010). When prebiotics and probiotics are mixed at the same time, the prebiotic can be used to increase intestinal survival of the probiotic organism. In other words, the prebiotics stimulates the growth and/or activity of the beneficial bacteria (Sanderson *et al*., 2012). Prebiotics can transit through the stomach and small intestines undigested because humans do not have the intestinal enzymes needed to digest them. They make their way intact to the colon, where they will be bacterially fermented, along with the unabsorbed nutrients. Particularly, prebiotics provide energy and simple sugars to the host through the gut microbiota (Shigwedha *et al*., 2014).

2.4.2 Inulin

Inulin belongs a group of carbohydrates called fructans. This carbohydrate is a polymer of fructose units (2-60 fructose units) built with β (2,1) linked fructosyl (Modzelewska and Klebukowska, 2009). Two species are currently used by the food industry to produce inulin.

These are Chicory root (*Cichorium intybus*) and Jerusalem artichoke (*Helianthus tuberosas*) (Debruyn *et al*., 1992). Inulin can be digested by human enzymes by virtue of the β-2-1 fructosyl link, but inulin can be fermented by the beneficial bacteria present in the colon (Mudannayake *et al*., 2015). Inulin has a variety of uses acting as a replacement for sugar, fat, texturizing agent, and low-calorie bulking agent. Also, it acts as a soluble and fermentable dietary fiber. In dairy products it is adapt for its prebiotic properties (Tungland and Meyer, 2002). Inulin is characterized by its biochemical and physiological features to provide and maintain a healthy gastrointestinal function (Robertfroid *et al*., 2011).

According Watson *et al*., 2013, some probiotic lactobacilli such as *Lactobacillus bulgaricus*, *Lactobacillus rhamnosus* GG®, *Lactobacillus plantarum* can not degrade inulin. However, some strains of *Lactobacillus paracasei subsp paracasei* and *Lactobacillus casei* can digest this prebiotic. Inulin improves the survival and activity during the storage of other probiotics such as *Lactobacillus acidophilus* (Kaplan and Hutkins, 2003) and *Bifidobacterium spp.* (Alkalin *et al*., 2004). According to Alves *et al*., 2013, inulin does not affect the viability and favourable interaction with probiotics, which can be an advantage. Inulin will be degraded in the intestinal tract by probiotic bacteria, modulating in a positive way the composition of the consumer's intestinal microflora.

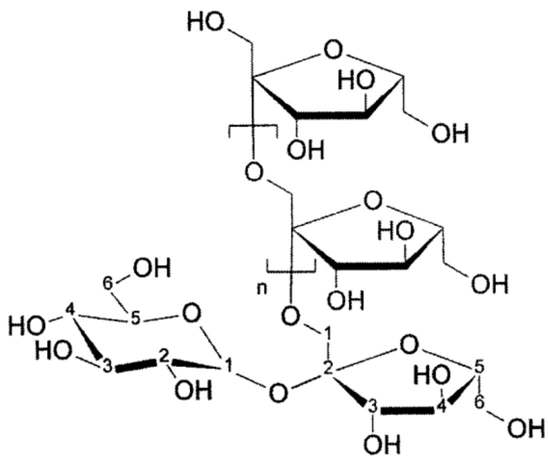

Figure 2. Inulin structure

2.4.3 Effects of inulin in yogurt

Prebiotics have different effects on the physical, chemical and microbial characteristics of dairy products. Inulin is a prebiotic that has been used in yogurt. Aryana *et al*., 2007, reported that the use of inulin with short, medium and long chains affects yogurt properties. The chains influence the physical, chemical and microbial properties, decreasing pH and resulting higher scores in flavour. The chain length does not affect viscosity, color, and product appearance. Ibrahin *et al*., 2004, reported that the curd tension and viscosity increased with inulin addition, but decreased syneresis. Guven *et al*., 2005, found that the whey separation increased in yogurt using different concentrations of Inulin with fat-free milk. Oliveira *et al*., 2012, reported that the time required for yogurt fermentation is shorter than without inulin usage. This prebiotic increases the levels of volatile compounds, acetic and lactic acid. Inulin causes many effects on the physical properties of yogurt. According to Paseephol *et al*., 2008, yogurts containing inulin have lower of firmness, viscosity, yield stress and loss modulus values. This is a result of the molecules of inulin being dispersed among the casein micelles, thus interfering with the protein matrix formation resulting in a weak gel structure.

Akin *et al*., 2006, used inulin to evaluate its effect in probiotic ice-cream. The addition of inulin improved viscosity and did not have any effect on sensory characteristics. Inulin stimulated the growth and improved the viability of *Bifidobacterium lactis* and *Lactobacillus acidophilus* but reduced the counts of *Lactobacillus bulgaricus* while maintaining counts of *Streptococcus thermophilus*.

Inulin has been used in sheep milk yogurts. According to Balthazar *et al*., 2016, they did not find significant difference in the enumeration of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in treatments with and without inulin. Counts decreased 1 to 2 cycles in both bacteria overtime. Due to inulin addition, lower post acidification was observed. Different inulin levels did not affect the fatty acids content in yogurt.

CHAPTER 3. MATERIALS AND METHODS

3.1 Experimental design

Functional stirred plain yogurt was manufactured using different concentrations of aloe vera juice with micro pulp at 0, 5 and 15%, with the incorporation of inulin at the rate of 0 and 1.5%. Six treatments were evaluated: PY = plain yogurt used like a control, YA5 = yogurt with 5% of aloe vera, YA15 = yogurt with 15% of aloe Vera, YI = yogurt with inulin, YIA5 = yogurt with inulin and 5% of aloe vera, YIA15 = yogurt with inulin and 15% of aloe vera. *Streptococcus thermophilus* counts, *Lactobacillus bulgaricus* (including *Lactobacillus acidophilus*) counts, pH, titratable acidity, viscosity, color, syneresis and determination of inulin content were analyzed at days 1, 7,14 and 30 after manufacture and were conducted three replications of each treatment. The experimental design was factorial arrangements with repeated measures over time. Sensory evaluation was conducted three days after manufacture.

3.2 Yogurt manufacture

All utensils, equipment and containers were sanitized before use with Chlorilizer plus (Afco Industries Inc, Alexandria, LA). Six treatments were manufactured following different formulations according Table 1. Ingredients information is shown in Table 2. For yogurt manufacture, the 2% fat milk was preheated at 45 ± 2 ºC. The dry ingredients (non-fat dry milk and / or inulin) were added to the milk and the mix was stirred vigorously to ensure efficient dissolution of the powders. The mix was heated treated at 85 ± 2 ºC for 30 minutes. This time was measured from the time the milk reached the required temperature. Aloe vera juice was then added to the mix and blended. The mix was cooled to a fermentation temperature at 43 ± 1 ºC in ice bath while it was stirred slowly with a spoon. For the inoculation, starter culture FD-DVS was made by adding 50 unit of FD-DVS to 500 g of pasteurized milk (85 ± 2 ºC for 30 minutes) and cooled to fermentation temperature (43 ± 1 ºC). The mixture was agitated gently until the culture was dissolved. From this dilution, 6 ml was added to 3 liters of tempered milk. Inoculated yogurt mix was placed in 8 L stainless steel containers and incubated at 40 ± 1 ºC, until pH 4.55 was reached. The yogurts were placed in a cooler and stored overnight at 4 ºC. Yogurt was stirred manually using a spoon following 43 full manual rotations around the container. The finished plain stirred yogurt was packed in polypropylene containers (Airlite Plastics Co, Omaha, NE), filled approximately with 170 g of yogurt using a lid of high-density polyethylene, containers were labeled with the manufacture day (1, 7, 14 and 30). Samples were refrigerated at 4 ºC. Each container was removed from the cooler only at the time of analysis.

Table 1. Yogurt mix formulation

Ingredient	Addition Rate	Treatments					
		PY	YA5	YA15	YI	YIA5	YIA15
Milk (Kg)		3	2.85	2.55	3	2.85	2.55
NFDM (g)	2% / 0.5%	60	60	60	15	15	15
Aloe Vera (g)	5% / 15%	-	150	450	-	150	450
Inulin (g)	1.5%	-	-	-	45	45	45
Culture (ml)		6	6	6	6	6	6

PY = plain yogurt (Control), YA5 = yogurt with 5% of aloe vera, YA15 = yogurt with 15% of aloe Vera, YI = yogurt with inulin, YIA5 = yogurt with inulin and 5% of aloe vera, YIA15 = yogurt with inulin and 15% of aloe vera.

Table 2. Ingredients information

Name	Description	Company
Milk	Reduced fat 2% fat milk	Great Value ® ((Walmart, Bentonville, AK)
Non-fat Dry Milk	Extra Grade Spray Process Pasteurized	Associate Milk Producers Inc (New Ulm, MN)
Aloe Vera Juice	Organic Aloe Vera Juice with Micro Pulp, Unflavored	Dynamic Health (Brooklyn, NY)
Inulin	Chicory root inulin powder (FOS), soluble organic inulin fiber prebiotic supplement, unflavored	Jetsu™ Health and Beauty (Valley Cottage, NY)
Starter Culture	FD-DVS ABY-3 Probio-Tec: Thermophilic lactic acid culture. Contains the documented probiotic strains BB-12® and LA-5®.	Chr. Hansen ® (New Berlin, WI)

3.3 Quantification of inulin content using HPLC

Inulin content was analyzed at day 1,7,14 and 30 after yogurt manufacture. The extraction of inulin was done for the treatments following this procedure: 10g of yogurt were weighed in an Erlenmeyer flask, 30 ml of distilled water were added using a graduated cylinder and both were boiled for 10 min. Proteins were precipitated by addition of 5 ml Carrez I reagent ($K_4Fe(CN)_6 \times 3H_2O$, using 15g/100 ml) (Sigma-Aldrich, St. Louis, MO) and 5 ml Carrez II reagent (Zn $(CH_3COO)_2 \times 2H_2O$, using 30 g/ 100 ml) (Sigma-Aldrich, St. Louis, MO). The sample was filtered through a 0.45µm membrane filter (Pall Corporation, New York), and transferred to a 50 ml volumetric flask and made up to the mark with deionized water. Each sample were passed through a cellulose acetate filter with pore size 0.2 (VWR international, Radnor, PA) using a 60 ml syringe (Becton, Dickinson and Co., Sparks, MD), before injection into the HPLC column. For inulin quantification, a HPLC-RID method was performed with some modifications (Petkova and Denev, 2013). The HPLC separation was carried out on a Shodex® Sugar SP0810 (Phenomenex Company, Torrance, CA), with Pb^{2+} a guard column (50 × 92 mm i.d) (Sigma-Aldrich, St. Louis, MO), and (300 mm × 8.0 mm i.d.) analytical column at 30 ºC. The mobile phase was Milli-Q water.

3.4 Titratable acidity

The titratable acidity was analyzed at days 1,7,14 and 30. It was determined using 9 g of yogurt with 9 ml of distilled water and 0.5 ml of phenolphthalein. Samples were titrated with 0.1 N NaOH until to get a slight pink for 30 seconds and, the volume of 0.1 N NaOH used was recorded.

3.5 pH

The pH was measured at days 1,7,14 and 30 by using an Thermo Orion 3 Star pH Benchtop Meter (Fisher Scientific, Instruments, Pittsburgh, PA), it was calibrated using pH

4.00 and 7.00 buffer solutions (Thermo Fisher Scientific, Beverly, MA) before reading the samples.

3.6 Syneresis

10 g of each sample was weighted and then centrifuged in an AccuSpin™ 400 (Fisher Scientific, Instruments, Pittsburgh, PA) at 5,000 rpm for 20 min. The clear supernatant was weighted (Guinee *et al.* 1995). The ratio was expressed following this formula:

(Weight of the clear supernatant / Initial weight of the sample) × 100 = Syneresis (%)

3.7 Color

The L*a*b* values were determined using a Minolta CM 508d hand-held spectrophotometer (Minolta Co., Ltd, Japan). It was standardized with white and black tiles. On average, five values were taken per replication. Total color differences (ΔE^*) were calculated as follows:

$$\Delta E^* = (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2$$

Where $\Delta L^* = L^*_{treatment} - L^*_{references,}$ $\Delta a^{*}\, {*} = a^*_{treatment} - a^*_{references}$, and $\Delta b^{*}\, {*} = b^*_{treatment} - b^*_{references}$. (references = day 0 values)

3.8 Viscosity

Viscosity was measured at day 1, 7, 14, and 30 by using a Brookfield DV-II viscometer (Brookfield Engineering Lab Inc., Stoughton, MA) Samples were measured at 5 ± 2 ºC, with a helipath stand mounted with a T-C spindle was used at 30 rpm. The data were acquired using Wingather® software (Brookfield Engineering Lab Inc., Stoughton, MA). One hundred points were averaged per sample per replication (Aryana 2003).

3.9 Phosphate and magnesium chloride dilution water

1.25 ml of stock phosphate solution and 5 ml of stock magnesium chloride solution were added to 1 L of distilled water. This solution was dispensed in 99 ml dilution bottles and autoclaved at 121 ºC for 15 minutes.

3.10 Enumeration of *Streptococcus thermophilus*

Streptococcus thermophilus agar was prepared using: to 1 L of distilled water the following ingredients were weighted: 10 g of sucrose (Amresco, Solon, OH), 2 g of K_2HPO_4 (Fisher Scientific, Fair Lawn, NJ), 5 g of Bacto yeast extract and 10 g of Bacto Tryptone (Becton, Dickinson and Co., Sparks, MD) using individual plastic weighing boats. Distilled water was transferred from the graduated cylinder to a 2L Erlenmeyer flask. The mix was stirred to dissolve the ingredients. To reduce the pH to 6.8 ± 0.1, 1 N HCl was added. Then, 12 g of agar (Fisher Scientific, Fair Lawn, NJ) were added to the medium and 6 ml of 0.5% bromocresol purple were added (Fisher Scientific, Fair Lawn, NJ). The media was heated to boiling and it was autoclaved at 121 ºC for 15 minutes (Dave and Shah 1996). Growth of *Streptococcus thermophilus* was measured at days 1,7,14 and 30. 11 g of yogurt sample were diluted with 99 ml of sterilized $MgCl_2$ KOH, getting different dilution. Then, 1 ml of each diluted sample was pipetted into petri dishes and then the media was aseptically poured into the petri dish. Petri dishes were aerobically incubated at 37 ºC for 24 h. To enumerate the colonies a Quebec Darkfield Colony Counter (Leica Inc., Buffalo, NY) was used.

3.11 Enumeration of *Lactobacillus bulgaricus* (including *Lactobacillus acidophilus*)

The *Lactobacillus bulgaricus* agar was prepared using: for 1 liter of distilled water use 15 g of agar (Fisher Scientific, Fair Lawn, NJ) and 55 g of Lactobacilli MRS broth powder (Becton, Dickinson and Co., Sparks, MD). The pH was adjusted to 5.2 ± 0.1 using 1 N HCl. The media was heated to boiling with agitation. Then, the media was autoclaved at 121 ºC for 15 minutes (Tharmaraj and Shah 2003). Growth of *Lactobacillus bulgaricus*, (Including *Lactobacillus acidophilus*) was measured at days 1,7,14 and 30. 11 g of yogurt sample were diluted to serial dilutions with 99 ml of sterilized $MgCl_2$ KOH. 1 ml of each diluted sample was pipetted into petri dishes and then the media was aseptically poured into the petri dish. Petri dishes were placed in a BBL GasPaks (BBL, Becton, Dickinson and Co., Cockeysville, MD) and then they were incubated anaerobically at 43 ºC for 72 h. to enumerate the colonies a Quebec Darkfield Colony Counter (Leica Inc., Buffalo, NY) was used.

3.12 Sensory evaluation

Sensory evaluation was conducted by the School of Nutrition and Food Science with 160 random participants, including faculty, students and staff at Louisiana State University. The research protocol for use of human subjects for this study was approved (IRB # HE18-3) by the Louisiana State University Agricultural Center Institutional Review Board (Appendix A). Six treatments were evaluated (PY, YA5, YA15, YI, YIA5, YIA15). They were packed after fermentation in 2 oz. clear plastic cups. After three fermentation days of the yogurts, three treatments were given to participants following a randomized incomplete block design. Different codes with 3-digit random numbers for each treatment were used (631 for PY, 542 for YI, 893 for YA5, 104 for YIA5, 735 for YA15 and 326 for YIA15).

All sample evaluation was performed in partitioned sensory booths. Participants were instructed do not talk to others during the evaluation and instructed to evaluate the product and check the space that best reflects their feeling about the product. Between samples, they were required to rinse their palate with water and a non-salted cracker. Approximately 1 oz. per treatment was given to each participant. Different spoons were used to evaluate each sample. The evaluation forms consisted of a 9-point rating scale, with 1 = dislike extremely and 9 = like extremely, to evaluate aroma, overall appearance/color, sourness, mouthfeel (viscosity), overall taste, overall liking. Sourness and mouthfeel viscosity were evaluated using a 3-point Just-About-Right (JAR) scale (not enough, just about right and Too much) (Stone and Sidel 1993). Other questions were used to evaluate purchase intent of the products and acceptability (Appendix B).

3.13 Statistical analysis

Experiment was replicated three times. Means and standard deviations were reported. Data were analyzed as a factorial arrangement with repeated measures over time using Proc Mixed of SAS (Version 9.4 SAS® Institute Inc., Cary, NC) software. Differences of least square means were used to determine significant differences at $P < 0.05$ for main effects (inulin, aloe vera and day) and interaction effect (inulin*aloe vera, inulin*aloe vera*day) withTukey's adjustment to evaluate significant differences ($P < 0.05$). The results for sensory evaluation was subjected to ANOVA using the SAS® 9.4 software. Significant differences between means were determined at $\alpha = 0.05$ using Tukey's adjustment. The McNemar's test (Fleiss and Everitt 1971; Stone and Sidel 1993; Sae-Eaw *et al.*, 2007) was used to determine the significance differences in purchase intent before and after.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 pH

pH is an indicator of the presence of lactic acid and organic acids, it is an important factor to assure the quality of yogurt (Azari-Anpar 2016). The results of this study listed in Table 4 show that there was no significant difference (P < 0.05) in the interaction of inulin*aloe*day. This means that treatments were not affected by time. However, there was a significant difference (P < 0.05) in the main effects inulin, aloe vera, day and the interaction of inulin and aloe. The pH of the functional plain yogurt as influenced by the addition of inulin and different concentrations of aloe vera is shown in Figure 3.

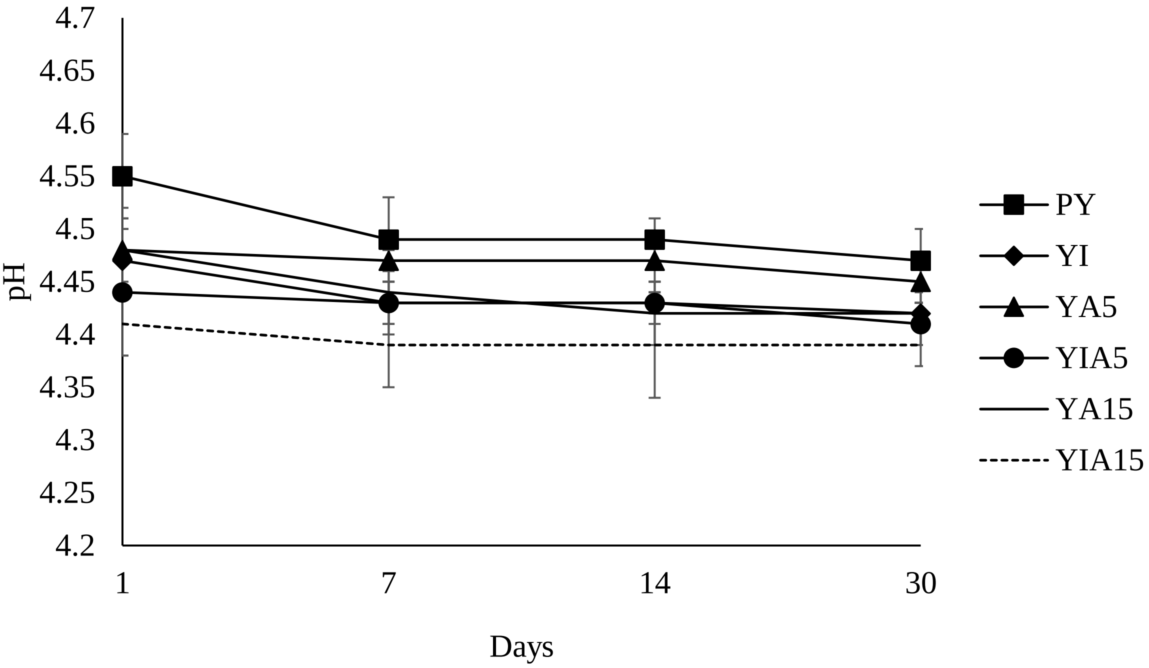

Figure 3. pH of yogurt for 30 days storage

Table 3. Probability > F (Pr > F) of main effects for pH in yogurt

Effect	Pr > F
Inulin	**<.0001**
Aloe	**<.0001**
Inulin*Aloe (Treatment)	**0.0007**
Day	**<.0001**
Inulin*Aloe*Day	0.4799

Table 4 shows that there was an effect on pH over time. Recent studies found that pH decreases during the shelf life of yogurt due the activity of lactic acid bacteria, producing lactic acid from lactose. Also, others organic acids are formed from the residual enzymes produced by starters during fermentation (Damin *et al*., 2009; Christopher *et al*., 2009). In addition, Table 4 shows that the concentration of inulin and aloe vera significantly reduces the pH. The addition of inulin in yogurt decreases the pH. Bacteria degrade inulin and stimulate the metabolism of the probiotic bacteria thus accelerating the fermentation in the yogurt. The interaction between prebiotic and probiotic was positive (Aryana *et al*., 2007; Donkor *et al*., 2007; Guven *et al*., 2005). Also, it was found that the addition of different concentration of aloe vera juice in fermented dairy products decreases pH. This could be due to the presence of the prebiotic polysaccharides and other nutrients that might sustain the metabolic activity of lactic acid bacteria or by the natural acidity of the aloe vera juice, it was 4.21 (Basannavar *et al*., 2014; Hussain *et al*., 2016; Boghani *et al*., 2012; Hussain *et al*., 2015).

Table 4. Means for pH in yogurt as influenced by concentration of inulin, aloe vera, treatment and day.

Effect	Description	Value
Inulin	0 %	4.47 ± 0.05^{A}
	1.5 %	4.42 ± 0.04^{B}
Aloe Vera	0 %	4.47 ± 0.05^{A}
	5 %	4.45 ± 0.03^{B}
	15%	4.42 ± 0.04^{C}
Inulin*Aloe (Treatment)	PY	4.50 ± 0.05^{A}
	YI	4.44 ± 0.03^{C}
	YA5	4.47 ± 0.03^{B}
	YIA5	4.43 ± 0.04^{C}
	YA15	4.44 ± 0.04^{C}
	YIA15	4.40 ± 0.04^{D}
Day	1	4.47 ± 0.06^{A}
	7	4.44 ± 0.04^{B}
	14	4.43 ± 0.04^{BC}
	30	4.42 ± 0.03^{C}

^{ABCD} Means ± Standard Deviation with different letter within the table according the main effect are significantly different (P<0.05). PY = plain yogurt, YA5 = yogurt with 5% of aloe vera, YA15 = yogurt with 15% of aloe Vera, YI = yogurt with inulin, YIA5 = yogurt with inulin and 5% of aloe vera, YIA15 = yogurt with inulin and 15% of aloe vera.

4.2 Titratable acidity

Titratable acidity values are due to lactose fermentation by yogurt lactic acid bacteria (Paseephol *et al.*, 2008). The effect of the addition of inulin and aloe vera in plain yogurt is shown in figure 4. The effects of inulin, aloe, day and the interaction of inulin*aloe were significantly different (P<0.05), while the interaction between inulin*aloe*day was not significant different (P>0.05) (Table 5).

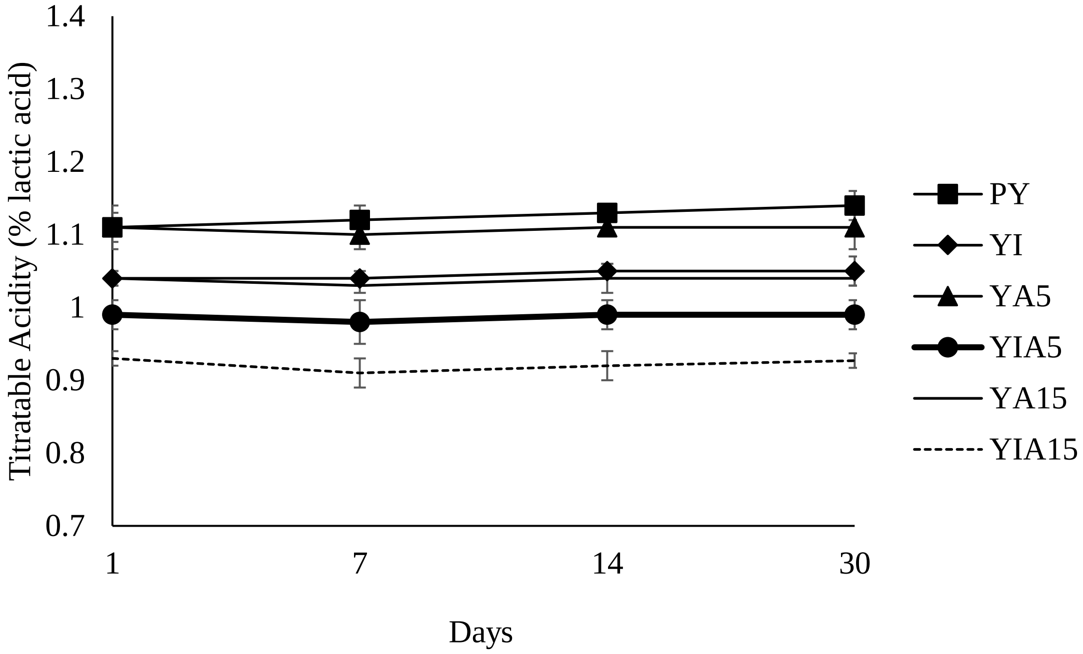

Figure 4. Titratable acidity of yogurt for 30 days storage

Table 5. Probability > F (Pr > F) of main effects for titratable acidity in yogurt

Effect	Pr > F
Inulin	**<.0001**
Aloe	**<.0001**
Inulin*Aloe (Treatment)	**<.0001**
Day	**0.0028**
Inulin*Aloe*Day	0.7867

Table 6 shows that titratable acidity increased significantly over time. There was significant difference (P<0.05) among days 7, 14 and 30. Some studies have found the highest value in titratable acidity after day 15. This could be attributed to the utilization of residual carbohydrates by viable microorganisms and the production of lactic acid (Guven *et al*., 2005; Panesar and Shinde 2012). The addition of inulin significantly decreased ($P<0.05$) titratable acidity, due to the proportional rate of non-fat dry milk powder and inulin in yogurt. Low concentration of non-fat dry milk may decrease nutrients available for bacteria (Ramchandran and shah 2008; Kavas and Bakirci 2014). Increased levels of aloe vera juice in yogurt significantly decreased ($P<0.05$) the percent lactic acid. These results are in agreement with Azari-Anpar *et al*., 2016. Increasing levels of aloe vera may decrease lactose levels and milk proteins available for lactic acid bacteria to produce lactic acid. For these reasons, the interaction of inulin with aloe vera decreases titratable acidity. The decrease is lower than the other treatments when the concentration of aloe vera mixed with inulin is increased.

Table 6. Means for titratable acidity in yogurt as influenced by concentration of inulin, aloe vera, treatment and day.

Effect	Description	Value
Inulin	0 %	1.09 ± 0.04^{A}
	1.5 %	0.98 ± 0.05^{B}
Aloe Vera	0 %	1.09 ± 0.04^{A}
	5 %	1.05 ± 0.06^{B}
	15%	0.98 ± 0.05^{C}
Inulin*Aloe (Treatment)	PY	1.13 ± 0.02^{A}
	YI	1.04 ± 0.01^{C}
	YA5	1.11 ± 0.02^{B}
	YIA5	0.98 ± 0.02^{D}
	YA15	1.04 ± 0.01^{C}
	YIA15	0.93 ± 0.02^{E}
Day	1	1.03 ± 0.06^{B}
	7	1.03 ± 0.07^{B}
	14	1.04 ± 0.07^{A}
	30	1.04 ± 0.07^{A}

^{ABC} Means ± Standard Deviation with different letter within the table according the main effect are significantly different ($P<0.05$). PY = plain yogurt, YA5 = yogurt with 5% of aloe vera, YA15 = yogurt with 15% of aloe Vera, YI = yogurt with inulin, YIA5 = yogurt with inulin and 5% of aloe vera, YIA15 = yogurt with inulin and 15% of aloe vera.

4.3 Color

The effect of the addition of inulin and aloe vera in yogurt after 30 days of storage on L* values (Lightness- darkness) is shown in Figure 4. The main effects of aloe, day and the interaction of inulin*aloe were significantly different (P<0.05). The effect of inulin and the interaction between inulin*aloe*day was not significantly different (P>0.05) (Table 7), demonstrating L* values of treatments were not affected by time.

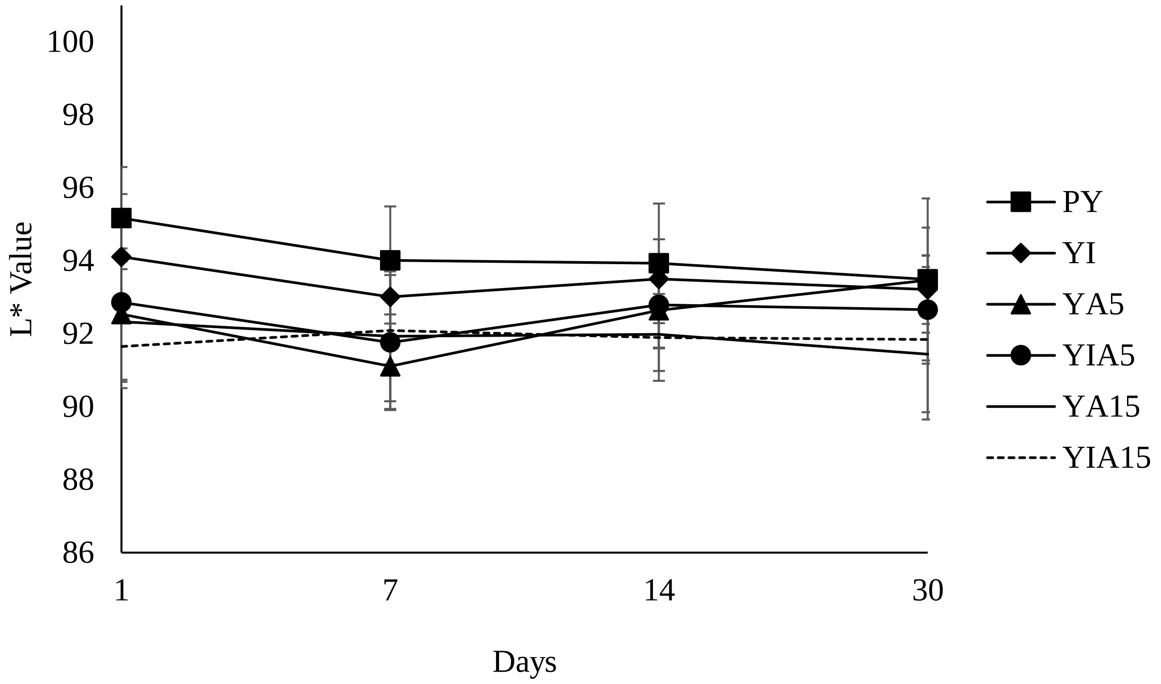

Figure 5. L* in yogurt storage by 30 days

Table 7. Probability > F (Pr > F) of main effects for color (L*, a*, b*) in yogurt

Effect	Pr > F		
	L*	a*	b*
Inulin	0.2504	**<.0001**	0.9492
Aloe	**<.0001**	**<.0001**	**<.0001**
Inulin*Aloe (Treatment)	**0.0218**	**<.0001**	**<.0001**
Day	**0.0357**	**<.0001**	**<.0001**
Inulin*Aloe*Day	0.6099	**0.0017**	**0.0001**

Table 8 shows that the addition of aloe vera juice significantly decreased (P<0.05) L* values. Lower L* values in treatments with 5 and 15% aloe vera, and the interaction of aloe with inulin had a loss of lightness compared to the control. This may be due the color of aloe vera juice (Hussain *et al.*, 2015). L* values significantly decreased at day 7. L* values were stable at days 14 and 30 (Table 8).

a* values are shown in figure 6. Inulin an aloe vera affected a* (red-green axis) values after 30 days of storage. The main effects of inulin, aloe, day, the interaction of inulin*aloe and the interaction between inulin*aloe*day were significantly different (P<0.05) (Table 7). Table 9 shows the interaction of inulin and aloe vera significantly decreased (P<0.05) a* values. Treatments with 5% of aloe vera juice with inulin, and 15% aloe vera juice with inulin obtained lower values which decreased over time. Values were negative, estimating that a* values were in the green color space. Those changes may be attributed to the pale green color of aloe vera juice (Aryana *et al*., 2007; Hussain *et al*., 2015; Paseephol *et al*., 2008).

Yogurt enhanced with inulin and aloe vera after 30 days of storage had an effect in b* (yellow-blue) values as shown in Figure 7. For b* values, the main effects of aloe, day, the interaction of inulin*aloe and the interaction between inulin*aloe*day were significantly different (P<0.05). The effect inulin was not significant (Table 7). Table 10 shows the interaction of inulin with 15% of aloe vera juice was significant (P<0.05). Values increased over time. All the b* values were positive therefore they were found in the yellow color space.

Table 8. Means for L* in yogurt as influenced by concentration of inulin, aloe vera, treatment and day.

Effect	Description	Value
Aloe Vera	0 %	93.80 ± 1.57^{A}
	5 %	92.47 ± 1.60^{B}
	15%	91.90 ± 1.43^{C}
Inulin*Aloe (Treatment)	PY	94.15 ± 1.76^{A}
	YI	93.46 ± 1.29^{AB}
	YA5	92.44 ± 1.58^{C}
	YIA5	92.51 ± 1.65^{BC}
	YA15	91.92 ± 1.55^{C}
	YIA15	91.87 ± 1.32^{C}
Day	1	93.11 ± 1.96^{A}
	7	92.31 ± 1.67^{B}
	14	92.78 ± 1.37^{AB}
	30	92.68 ± 1.80^{AB}

^{ABC} Means ± Standard Deviation with different letter within the table according the main effect are significantly different (P<0.05). PY = plain yogurt, YA5 = yogurt with 5% of aloe vera, YA15 = yogurt with 15% of aloe Vera, YI = yogurt with inulin, YIA5 = yogurt with inulin and 5% of aloe vera, YIA15 = yogurt with inulin and 15% of aloe vera.

Table 9. Means for a* in yogurt

Treatment	Days			
	1	7	14	30
PY	-1.62 ± 0.05^{AB}	-1.57 ± 0.04^{A}	-1.61 ± 0.03^{AB}	-1.61 ± 0.05^{AB}
YI	-1.54 ± 0.31^{A}	-1.66 ± 0.05^{ABC}	-1.70 ± 0.04^{BCD}	-1.71 ± 0.04^{BCD}
YA5	-1.60 ± 0.10^{AB}	-1.56 ± 0.07^{A}	-1.63 ± 0.11^{AB}	-1.74 ± 0.06^{CD}
YIA5	-1.67 ± 0.09^{ABC}	-1.66 ± 0.06^{ABC}	-1.72 ± 0.08^{CD}	-1.93 ± 0.27^{D}
YA15	-1.55 ± 0.07^{A}	-1.62 ± 0.07^{AB}	-1.76 ± 0.09^{ABCD}	-1.69 ± 0.11^{ABCD}
YIA15	-1.84 ± 0.18^{CD}	-1.89 ± 0.12^{CD}	-2.20 ± 0.19^{E}	-2.53 ± 0.49^{F}

^{ABCDEF} Means ± Standard Deviation with different letter within the table are significantly different (P<0.05). PY = plain yogurt, YA5 = yogurt with 5% of aloe vera, YA15 = yogurt with 15% of aloe Vera, YI = yogurt with inulin, YIA5 = yogurt with inulin and 5% of aloe vera, YIA15 = yogurt with inulin and 15% of aloe vera.

Table 10. Means for b* in yogurt

Treatment	Day			
	1	7	14	30
PY	11.31 ± 0.25^{CD}	11.22 ± 0.16^{CD}	11.20 ± 0.20^{CD}	11.18 ± 0.19^{CD}
YI	10.99 ± 0.26^{CD}	10.92 ± 0.20^{D}	10.98 ± 0.29^{CD}	10.96 ± 0.22^{CD}
YA5	11.23 ± 0.28^{CD}	11.11 ± 0.19^{CD}	11.22 ± 0.12^{CD}	11.59 ± 0.23^{BC}
YIA5	10.83 ± 0.28^{D}	10.80 ± 0.21^{D}	10.88 ± 0.36^{D}	11.35 ± 0.72^{CD}
YA15	11.03 ± 0.22^{CD}	11.19 ± 0.22^{CD}	11.33 ± 0.32^{CD}	11.12 ± 0.43^{CD}
YIA15	11.29 ± 0.37^{CD}	11.27 ± 0.35^{CD}	11.76 ± 0.44^{B}	12.74 ± 1.13^{A}

^{ABCD} Means ± Standard Deviation with different letter within the table are significantly different (P<0.05). PY = plain yogurt, YA5 = yogurt with 5% of aloe vera, YA15 = yogurt with 15% of aloe Vera, YI = yogurt with inulin, YIA5 = yogurt with inulin and 5% of aloe vera, YIA15 = yogurt with inulin and 15% of aloe vera.

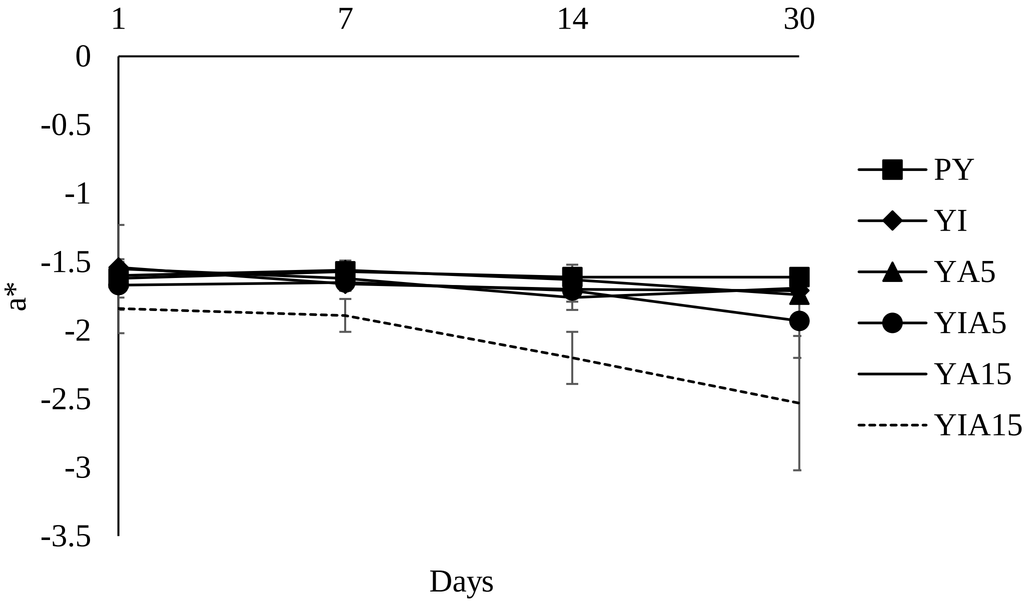

Figure 6. a* in yogurt storage by 30 days

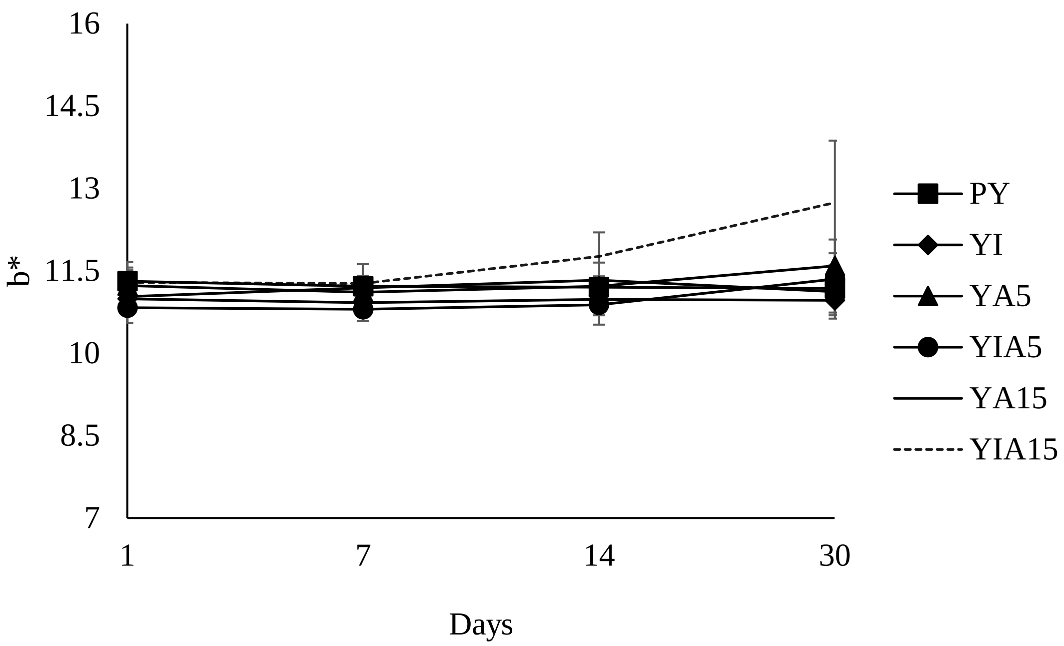

Figure 7. b* in yogurt storage by 30 days

The ΔE* values increased during storage time, but significant changes were not detected. ΔE* values in the treatment with 15% aloe vera and inulin increased at day 30. The slight increase in ΔE* for YI15A may be due to an increase in b* values and decrease in a* values. These changes may be due to the whey release in yogurt over time (Estrada 2011). All the ΔE* values were less than 3.0 (Table 11); therefore, they cannot be easily detected by the human eye (Caner, 2005).

Table 11. Total color differences of yogurts during 30 days of storage

Treatment	Storage time		
	Day 1 - Day 7	Day 7 -Day 14	Day 14 - Day 30
PY	1.49 ± 1.13^{AB}	1.58 ± 1.29^{AB}	2.20 ± 1.65^{AB}
YI	1.43 ± 0.79^{AB}	1.11 ± 0.59^{B}	1.19 ± 0.52^{AB}
YA5	1.68 ± 0.76^{AB}	0.84 ± 0.53^{AB}	1.30 ± 1.16^{AB}
YIA5	1.75 ± 1.18^{AB}	0.97 ± 0.70^{AB}	1.51 ± 0.79^{AB}
YA15	1.58 ± 0.78^{AB}	0.96 ± 0.60^{AB}	1.70 ± 1.04^{AB}
YIA15	0.90 ± 0.55^{B}	1.29 ± 0.51^{AB}	2.71 ± 0.37^{A}

^{AB} Means ± Standard Deviation across the same row are significantly different (P<0.05). PY = plain yogurt, YA5 = yogurt with 5% of aloe vera, YA15 = yogurt with 15% of aloe Vera, YI = yogurt with inulin, YIA5 = yogurt with inulin and 5% of aloe vera, YIA15 = yogurt with inulin and 15% of aloe vera.

4.4 Syneresis

Syneresis is the serum liberated by the product. Whey separation is a very important defect in yogurt (Guven *et al*., 2005). In some cases, syneresis has a direct relationship with pH and titratable acidity; the decrease of pH and an increase of titratable acidity results in an increase of whey separation (Azari-Anpar *et al*., 2017). Syneresis in yogurt enhanced with inulin and aloe vera storage by day 30 is shown in Figure 8. The main effects of inulin, aloe, day and the interaction of inulin*aloe were significantly different ($P<0.05$), while the interaction between inulin*aloe*day was not significant ($P>0.05$) meaning the treatments were not affected by time (Table 12).

Table 12. Probability > F (Pr > F) of main effects for syneresis in yogurt

Effect	Pr > F
Inulin	**<.0001**
Aloe	**<.0001**
Inulin*Aloe (Treatment)	**0.0158**
Time	**<.0001**
Inulin*Aloe*Time	0.7048

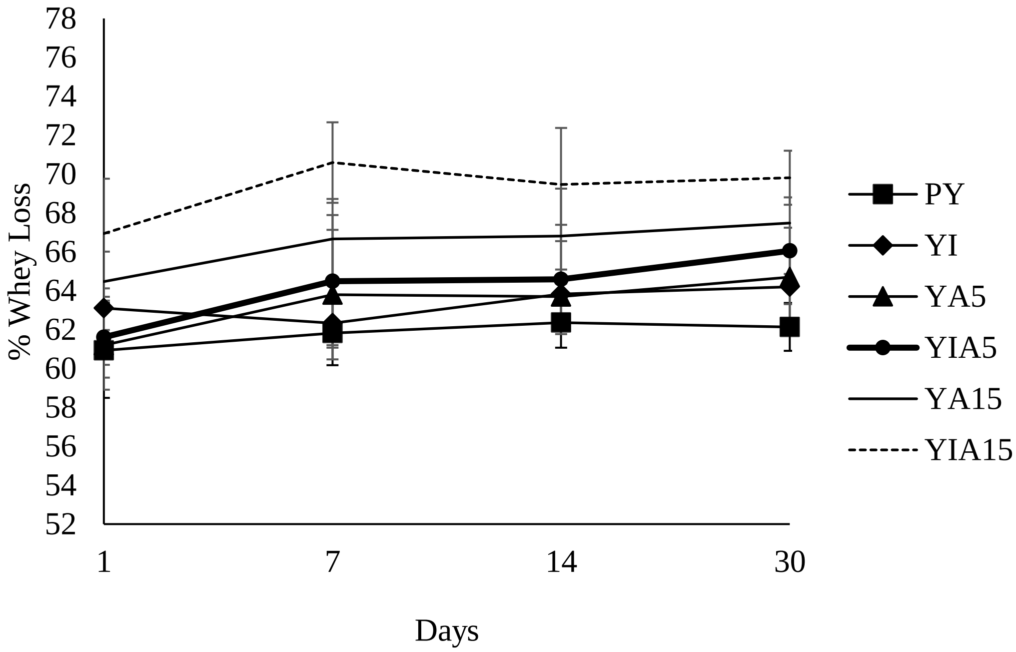

Figure 8. Syneresis of yogurt for 30 days storage

Table 13 shows a significant effect (P<0.05) on syneresis over time. Syneresis increased from day 1 to day 7 and stayed constant until day 30. The increase of whey loss is due to starter culture growth and release of proteolytic enzymes in fermented milk, resulting in breakdown the protein network. (Shihata and Shah, 2002; Adriana and McGrew, 2007). The addition of inulin had a significant effect (P<0.05) on syneresis causing it to increase. Inulin could have interfered with the development of a 3-dimensional structure of casein resulting in a weaker gel decreasing the capacity of retaining water (Paseephol *et al*., 2008). Increasing the levels of aloe vera juice in yogurt significantly increased (P<0.05) syneresis. This may be due to a direct relationship between the low pH (Table 4) and syneresis in yogurt. Low pH decreases colloidal stability of casein micelles and increases yogurt syneresis (Fox *et al*., 2000; Guven *et al*., 2005). The high-water content in aloe vera juice, could also play a factor. The interaction of 15% of aloe vera and inulin had the highest whey loss.

Table 13. Means for syneresis in yogurt as influenced by concentration of inulin, aloe vera, treatment and day

Effect	Description	Value
Inulin	0 %	63.84 ± 2.89^{B}
	1.5 %	65.58 ± 3.62^{A}
Aloe Vera	0 %	62.59 ± 2.01^{C}
	5 %	63.77 ± 2.71^{B}
	15%	67.78 ± 2.87^{A}
Inulin*Aloe (Treatment)	PY	61.81 ± 1.73^{D}
	YI	63.37 ± 1.99^{C}
	YA5	63.36 ± 2.48^{C}
	YIA5	64.18 ± 2.90^{C}
	YA15	66.36 ± 2.33^{B}
	YIA15	69.20 ± 2.66^{A}
Day	1	63.05 ± 3.22^{B}
	7	64.95 ± 3.78^{A}
	14	65.13 ± 3.11^{A}
	30	65.73 ± 2.82^{A}

^{ABCD} Means ± Standard Deviation with different letter within the table according the main effect are significantly different (P<0.05). PY = plain yogurt, YA5 = yogurt with 5% of aloe vera, YA15 = yogurt with 15% of aloe Vera, YI = yogurt with inulin, YIA5 = yogurt with inulin and 5% of aloe vera, YIA15 = yogurt with inulin and 15% of aloe vera.

4.5 Viscosity

The curd stability of yogurt is one of the most important physical properties. There are different factors which have an influence on curd stability. These can be: acidity of yogurt, total solid and protein content, homogenization, activity of starter culture and storage temperature (Guven *et al*., 2005). The effect of inulin and aloe vera on viscosity after 30 days of storage is shown in figure 9. The main effects inulin, aloe, day and the interaction of inulin*aloe were significantly different ($P<0.05$). The interaction between inulin*aloe*day was not significant different ($P>0.05$) (Table 14).

Table 14. Probability > F (Pr > F) of main effects for viscosity in yogurt

Effect	Pr > F
Inulin	**<.0001**
Aloe	**<.0001**
Inulin*Aloe (Treatment)	**0.0005**
Day	**0.0203**
Inulin*Aloe*Day	0.9083

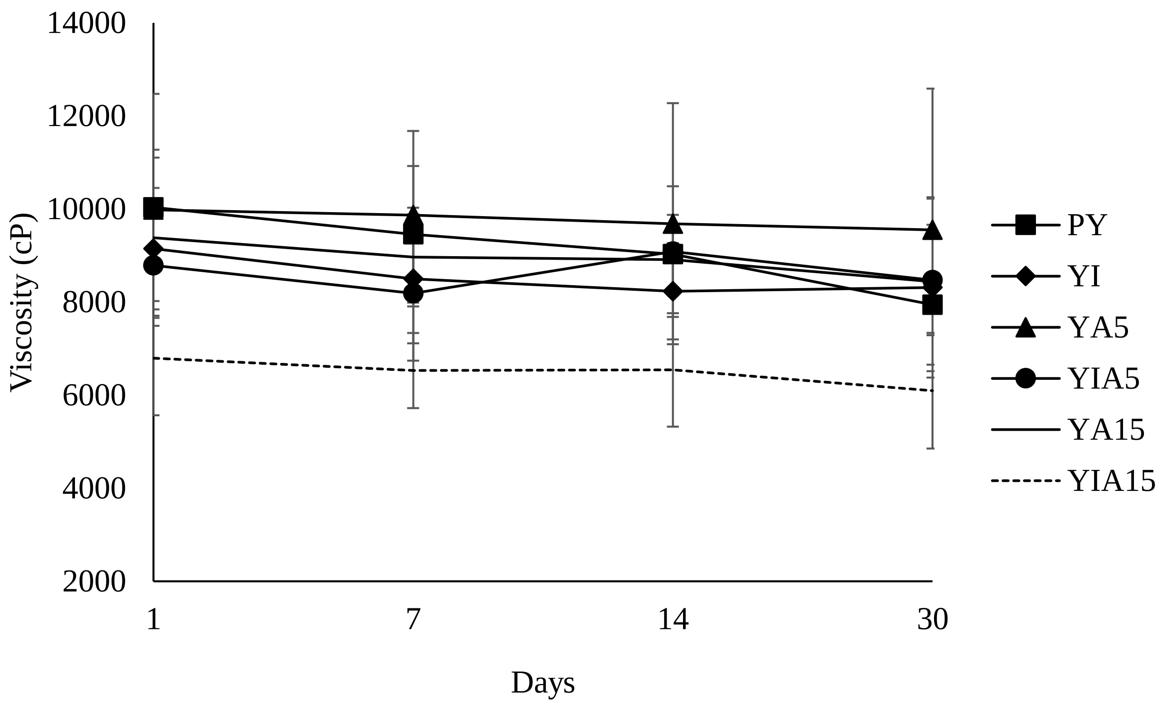

Figure 9. Viscosity of yogurt for 30 days storage

Table 15 shows viscosity to decrease significantly (P<0.05) over time. The decrease in pH in yogurt could have influenced the decreasing viscosity. As pH decreases, the distance from the isoelectric point of casein (4.6) increases; therefore, reducing stability of this protein and reducing curd stability (Bylund 1995).

Addition of inulin significantly decreased (P<0.05) viscosity, due to inulin being dispersed within casein micelles, interfering with the formation of protein matrix and resulting in a weak gel (Passephol *et al*., 2008). In addition, there was a decrease in viscosity when 15% of aloe vera juice was added to the yogurt. The aloe vera juice has a high moisture content and makes up a fragile coagulum in yogurt (Singh *et al*., 2012; Azari-Anpar *et al*., 2017). Therefore, the interaction of inulin and 15% aloe vera juice decreases viscosity significantly (P<0.05).

Table 15. Means for viscosity (cPs) in yogurt as influenced by concentration of inulin, aloe vera, treatment and day

Effect	Description	Value
Inulin	0 %	9270.84 ± 1795.63^{A}
	1.5 %	7890.87 ± 1615.56^{B}
Aloe Vera	0 %	8832.67 ± 1392.55^{A}
	5 %	9202.77 ± 2002.53^{A}
	15%	7707.12 ± 1749.55^{B}
Inulin*Aloe (Treatment)	PY	9116.69 ± 1305.52^{AB}
	YI	8548.65 ± 1436.47^{B}
	YA5	9771.66 ± 2415.60^{A}
	YIA5	8633.89 ± 1278.25^{B}
	YA15	8924.17 ± 1382.60^{AB}
	YIA15	6490.08 ± 1118.34^{C}
Day	1	9021.58 ± 1866.84^{A}
	7	8584.76 ± 1694.87^{AB}
	14	8580.53 ± 1709.00^{AB}
	30	8136.55 ± 2010.37^{B}

^{AB} Means ± Standard Deviation with different letter within the table according the main effect are significantly different (P<0.05). PY = plain yogurt, YA5 = yogurt with 5% of aloe vera, YA15 = yogurt with 15% of aloe Vera, YI = yogurt with inulin, YIA5 = yogurt with inulin and 5% of aloe vera, YIA15 = yogurt with inulin and 15% of aloe vera.

4.6 *Streptococcus thermophilus* and *Lactobacillus bulgaricus*

Streptococcus thermophilus and *Lactobacillus bulgaricus* counts in yogurt enhanced with inulin and aloe vera after 30 days of storage are shown in Figure 10 and Figure 11 respectively. The main effect of day in both bacteria was significantly different ($P<0.05$). The effects inulin, aloe vera and the interaction between inulin*aloe and inulin*aloe*day were not significant different ($P>0.05$) in *S. thermophilus* and *L. bulgaricus* (Table 16).

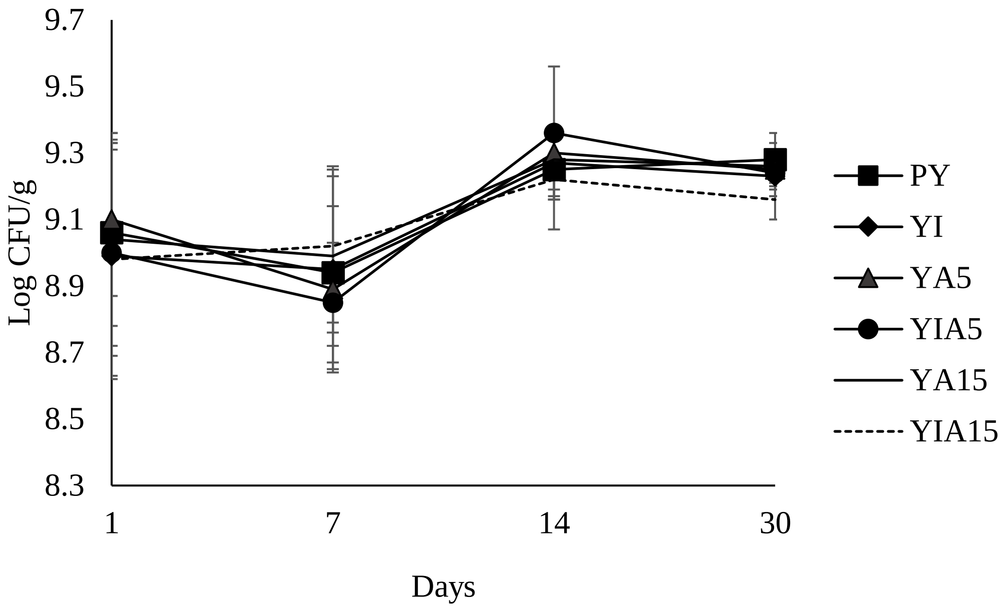

Figure 10. *Streptococcus thermophilus* counts in yogurt for 30 days storage

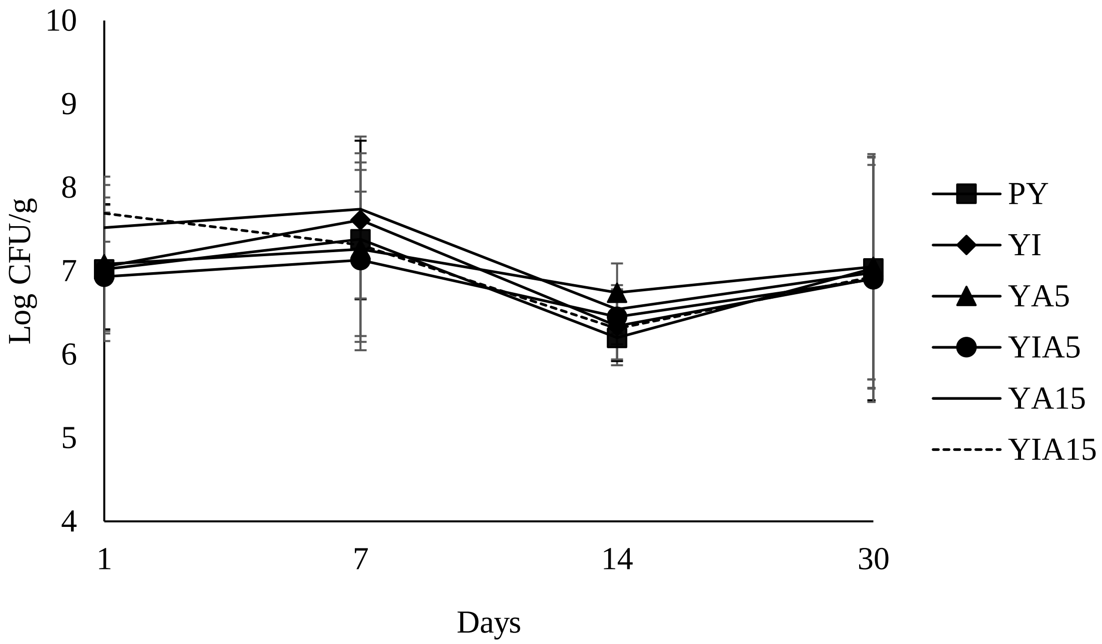

Figure 11. *Lactobacillus bulgaricus* counts in yogurt for 30 days storage

Table 16. Probability > F (Pr > F) of main effects for the growth of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in yogurt

Effect	Pr > F	
	S. thermophilus	*L. bulgaricus*
Inulin	0.2269	0.5389
Aloe	0.9877	0.4429
Inulin*Aloe (Treatment)	0.8851	0.7332
Day	**<.0001**	**<.0001**
Inulin*Aloe*Day	0.9453	0.9827

Table 17 shows, *Streptococcus thermophilus* count in yogurt enhanced with inulin and aloe vera increased over time. Counts were stable at day 1 and 7, but there were significant increases (P<0.05) at days 14 and 30. These results correlate with Srisuvor *et al*., 2013, who found *Streptococcus thermophilus* to increase in number over time. *Lactobacillus bulgaricus* counts had a significant effect over time (P<0.05). Counts were stable at days 1 and 7 but then decreased significantly (P<0.05) at day 14 and increased at day 30 (P<0.05). Therefore, counts of this probiotic bacteria was unstable. The viability of *Streptococcus thermophilus* was higher than *Lactobacillus bulgaricus* (Figure 10 and Figure 11) (Passephol and sherkat, 2009). Several researches have reported that low numbers of *Lactobacillus bulgaricus* could help to improve the viability of probiotics bacteria due to *L. bulgaricus* reducing post acidification (Holcomb & Frank, 1991; Shah, 1995). These probiotics bacteria should be present in the food product in minimal amounts of 10^6 CFU /g / day (Figure 10 and Figure 11). High dosages are required to compensate the loss of cells during the passage through the gastro intestinal tract (Granato *et al*., 2010). The addition of inulin did not have a significant effect (P>0.05) on improving the viability of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Table 16) due to *L. bulgaricus* inability to degrade inulin. In addition, inulin was no shown to influence *S. thermophilus* and *L. bulgaricus* during cold storage (Paseephol *et al*., 2008; Karimi *et al*., 2015; Kanjan and Hongpattarakere, 2017).

Table 17. Means for the growth of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in yogurt as influenced by day

Day	*S. thermophilus* (Log CFU/g)	*L. bulgaricus* (Log CFU/g)
1	9.03 ± 0.30^{B}	7.21 ± 0.70^{A}
7	8.94 ± 0.23^{B}	7.40 ± 0.91^{A}
14	9.28 ± 0.12^{A}	6.43 ± 0.37^{B}
30	9.24 ± 0.07^{A}	6.97 ± 1.28^{A}

^{AB} Means ± Standard Deviation with different letter within the table are significantly different ($P<0.05$)

4.6 Inulin quantification

Inulin has a variety of uses such as a replacement for sugar, fat, texturizing agent, low-calorie bulking agent. It is used as a soluble and fermentable dietary fiber, and in dairy products for its prebiotic properties. Inulin improves the survival and activity during the storage of other probiotics such as *Lactobacillus acidophilus* and *Bifidobacterium spp.* (Alkalin *et al*., 2004; Tungland and Meyer, 2002; Kaplan and Hutkins, 2003). Quantification of inulin was conducted after yogurt manufacture using a standard curve of inulin (Figure 12), which was prepared by injecting different volumes (5, 25 and 50 µl) of the inulin standard and running a lineal regression in excel. Obtaining the equation: y=4976.4x – 1058.9. The standard curve showed a good fit by linear regression ($R^2 = 0.9992$).

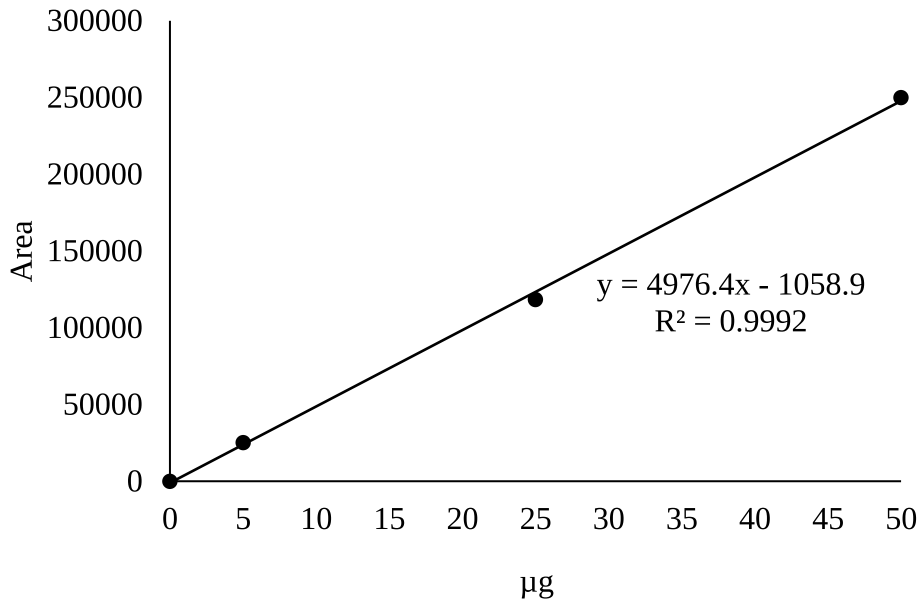

Figure 12. Standard curve for inulin

Inulin content in yogurt was analysed for 30 days. The content of inulin in yogurts enhanced with this prebiotic is shown in Figure 13. The main effects of day and treatment were significantly different (P<0.05), while the interaction treatment*day was not significantly different (P>0.05) (Table 18).

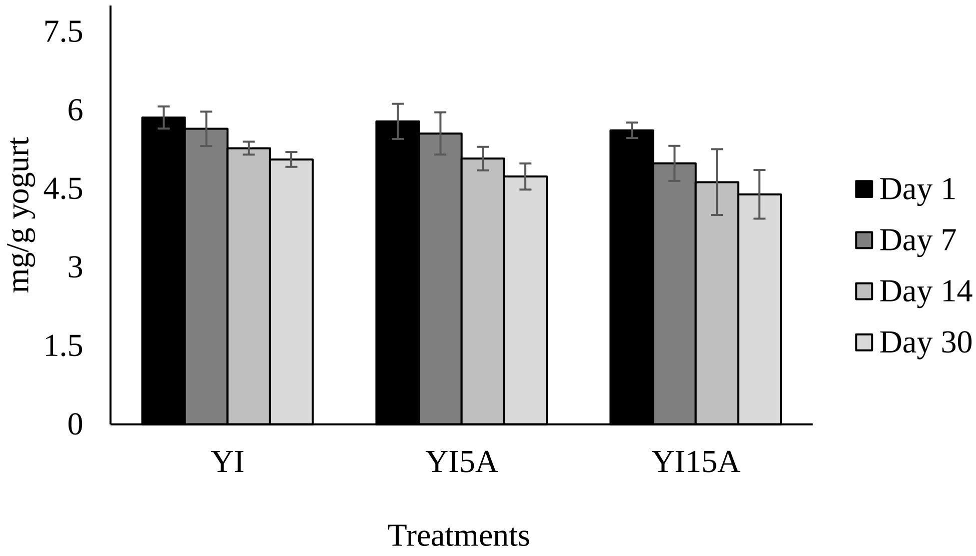

Figure 13. Inulin content in yogurt storage by 30 days

Table 18. Probability > F (Pr > F) of main effects for inulin content in yogurt

Effect	Pr > F
Treatment	**0.0010**
Day	**<.0001**
Inulin*Aloe*Day	0.8668

Inulin content decreased significantly (P<0.05) over time, with the lowest concentration at day 30 (Table 19). This may be due this prebiotic providing additional nutrients to stimulate culture growth. Concentrations of 1.5% are enough to stimulate the viability of probiotic microorganisms. Inulin also helps to protect probiotics cells from acid injury (Makras *et al.*, 2005; Ariana *et al.*, 2007; Desai *et al.*, 2004). The microorganism able to ferment inulin is *Bifidobacterium.* It has demonstrated the highest ability to degrade inulin (Rossi *et al.*, 2005). Inulin is an excellent subtract to promote and stimulate the viability of this bacteria (Akalin *et al.*, 2004, Bruno *et al.*, 2002, Shin *et al.*, 2000). Wiele *et al.*, 2007, found inulin to help increase the survival of *Lactobacillus acidophilus* during storage. The significant decrease of inulin content (P<0.05) in yogurts enhanced with 15% aloe vera may be attributed to the low pH. pH is an important factor in inulin hydrolysis (Blecker *et al*., 2002). The range of inulin content in yogurt without aloe vera was 5.86 to 5.06 mg/g yogurt. Yogurt with 5% of aloe vera juice had an inulin range from 5.79 to 4.73 mg/g yogurt. Yogurt with 15% aloe vera juice had inulin ranges from 5.61 to 4.39 mg/g yogurt (Figure 13). The most effective inulin level to reduce cholesterol, serum triglyceride and LDL-cholesterol in the blood was found to be 8-10 g per day (Abrams *et al*., 2005; Canzi *et al*., 1995; Williams, 1999). 15-20 g per day was found to be effective in relieving constipation (Gibson *et al*., 1995). Inulin behaves as dietary fiber, and according U.S. FDA, 2013, the daily intake of total dietary fiber should be 25 g/day.

Table 19. Means for inulin content in yogurt

Effect	Description	Value
Treatment	YI	5.46 ± 0.38^{A}
	YIA5	5.28 ± 0.51^{A}
	YIA15	4.91 ± 0.62^{B}
Day	1	5.75 ± 0.25^{A}
	7	5.40 ± 0.45^{AB}
	14	4.99 ± 0.46^{BC}
	30	4.73 ± 0.41^{C}

^{AB} Means ± Standard Deviation with different letter within the table according the main effect are significantly different (P<0.05). YI = yogurt with 1.5% inulin, YIA5 = yogurt with 1.5% inulin and 5% of aloe vera, YIA15 = yogurt with 1.5% inulin and 15% of aloe vera.

4.7 Sensory evaluation

A consumer acceptability study was conducted using a 9-point hedonic scale. Table 20 shows scores for each parameter evaluated by the consumer. The acceptability of overall appearance/color in the treatments was reduced significantly (P<0.05) by the incorporation of 15% aloe vera juice with inulin. The significant changes may be due to the pale green color of aloe vera juice and high-water content resulting in a soft gel (Hussain *et al*., 2015). Sensory scores for aroma and overall taste in treatments without aloe vera juices were significantly higher (P<0.05) than treatments with aloe vera. There was a significant difference in the scores of aroma and overall taste (P<0.05) in samples with 5% aloe vera and samples with 15% aloe vera and inulin. All the aroma scores were higher than 5. While scores for overall taste were lower than 5, representing dislike from the consumers.

For mouthfeel (viscosity), sensory scores decreased significantly (P<0.05) when the concentration of aloe vera increased. The lowest score for mouthfeel (viscosity) (P<0.05) was for samples with 15% aloe vera juice. Consumers were able to detect the effect of aloe and inulin in yogurt on decreasing values of viscosity (Table 15). Hence, results indicated that higher levels of addition of aloe vera juice decreased sensory scores for overall taste, aroma and mouthfeel (viscosity) (Singh *et al*., 2012; Guven, 2005; Panesar and Shinde 2011; and Hussain *et al*., 2012). Consumers were able to perceive the acidity and low pH in the treatment with 15% of aloe vera juice and inulin (Table 4), significantly (P<0.05) decreasing the sensory score for sourness in this treatment. Sensory scores for overall liking have a direct effect on the acceptability of the samples (Figure 14).

There was a significant difference (P<0.05) in samples with and without aloe vera. Increasing the concentration of aloe vera juice in yogurt significantly decreased the sensory scores of overall liking in the treatments and reduced the acceptability by the consumers. Samples without aloe vera juice had a higher acceptability by the consumers (Figure 14).

Table 20. Consumer acceptability scores[£] of functional plain yogurt enhanced with inulin and aloe vera

TRT	Overall Appareance / Color	Aroma	Overall Taste	Sourness	Mouthfeel (Viscosity)	Overall Liking
PY	6.57 ± 1.70^{A}	6.44 ± 1.95^{A}	4.48 ± 2.34^{A}	4.84 ± 2.14^{A}	6.33 ± 1.99^{A}	4.80 ± 2.28^{A}
YI	6.50 ± 1.46^{A}	6.44 ± 1.53^{A}	4.90 ± 2.10^{A}	$4.97 \pm 1.88\ ^{A}$	5.68 ± 1.91^{AB}	4.95 ± 2.11^{A}
YA5	6.36 ± 1.61^{A}	5.82 ± 1.78^{B}	3.71 ± 1.98^{B}	4.38 ± 1.96^{AB}	5.24 ± 1.91^{B}	3.81 ± 1.99^{B}
YIA5	6.46 ± 1.46^{A}	5.68 ± 1.64^{BC}	3.69 ± 1.97^{BC}	4.54 ± 1.91^{AB}	5.29 ± 1.87^{B}	3.92 ± 1.98^{B}
YA15	6.31 ± 1.52^{A}	5.52 ± 1.56^{BC}	3.28 ± 1.88^{BC}	4.11 ± 1.86^{BC}	3.93 ± 1.99^{C}	3.42 ± 1.86^{BC}
YIA15	5.70 ± 1.91^{B}	5.18 ± 1.72^{C}	2.95 ± 1.86^{C}	3.92 ± 1.75^{C}	3.43 ± 1.71^{C}	3.04 ± 1.76^{C}

[£] Mean from responses based on 9 -point hedonic scale. [ABC] Means ± Standard Deviation values with different letter in the same column are significantly different (P>0.05). TRT = Treatment. PY = plain yogurt, YA5 = yogurt with 5% of aloe vera, YA15 = yogurt with 15% of aloe Vera, YI = yogurt with inulin, YIA5 = yogurt with inulin and 5% of aloe vera, YIA15 = yogurt with inulin and 15% of aloe vera.

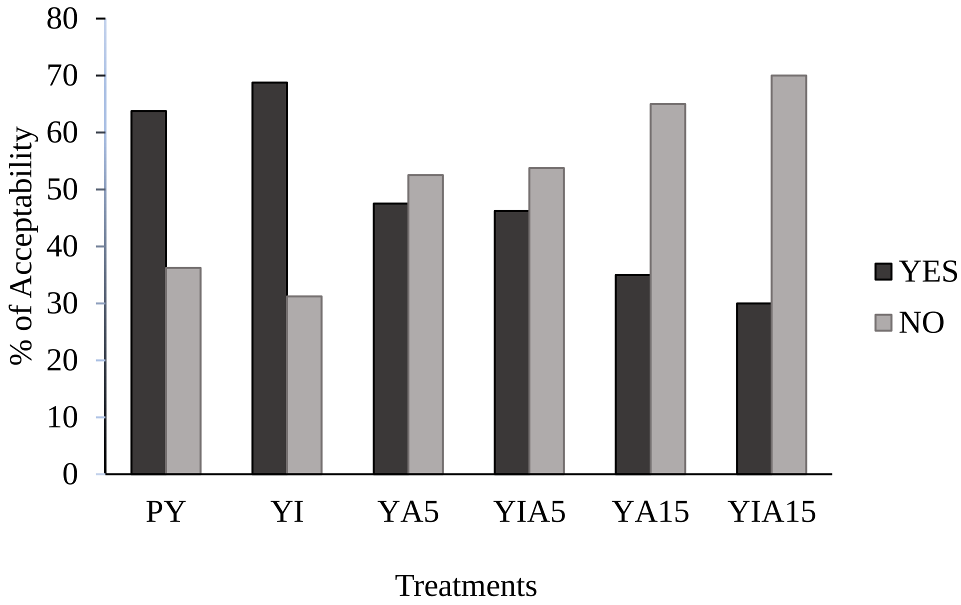

Figure 14. Frequency for acceptability of yogurt

Table 21. Purchase intent before and after of functional plain yogurt enhanced with inulin and aloe vera

Treatment	PIb (%)[€]	PIa (%)[€]
PY	28.75	**60**
YI	32.50	**66.25**
YA5	18.75	**43.75**
YIA5	20	**41.25**
YA15	15	**36.25**
YIA15	10	**26.25**

[€] Statistically significant p-values in bold print (P<0.05) based on McNemar Exact probability from 80 consumers responses. PY = plain yogurt, YA5 = yogurt with 5% of aloe vera, YA15 = yogurt with 15% of aloe Vera, YI = yogurt with inulin, YIA5 = yogurt with inulin and 5% of aloe vera, YIA15 = yogurt with inulin and 15% of aloe vera. PIb = Purchase intent before, Pia = Purchase intent after.

The purchase intent of yogurt samples more than doubled across all treatments when consumers were made aware that (Table 21 the yogurts were enhanced with inulin and/or aloe vera and contained beneficial bacterial that provides gut health benefits. According to a study conducted by Allgeyer *et al*., 2010, when the consumers have knowledge about health benefits of probiotics/prebiotic yogurts, the preference to consume this kind of products increases. Approximately 37% of consumers reported consuming yogurts with prebiotics several times a month, while 42% reported consuming probiotic yogurt. Stanton *et al*., 2001 reported that one trend for people is to consume functional foods because of increasing awareness of Americans about the benefits of functional ingredients such as probiotics and prebiotics. The market demand for prebiotics was $2.3 billion in 2012. It is estimated to reach $4.5 billion in the year 2018 (Transparency Market Research, 2015).

CHAPTER 5. CONCLUSION

This study aimed to develop a functional stirred plain yogurt enhanced with inulin and aloe vera juice (AVJ) and to evaluate its characteristics while measuring the growth of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* during 30 days of refrigerated storage. A consumer acceptability study also conducted. The results from this study show that pH was affected by the interaction of inulin and AVJ. Inulin and concentrations of AVJ decreased pH. Titratable acidity was reduced with the addition of inulin and the increase of AVJ. The interaction of those compounds decreases titratable acidity compared to the control. Therefore, treatments with 15% AVJ and inulin had the lowest pH and titratable acidity compared to the other treatments. pH decreased, and titratable acidity increased over time. Syneresis increased with inulin addition and with the increase in aloe vera juice. The interaction of inulin and aloe vera increases whey loss in yogurt. Viscosity showed the lowest value in treatment with 15% of AVJ and inulin. Viscosity and synereses were affected over time. Syneresis increased and viscosity decreased. Addition of aloe vera decreased L*. Treatment with 15% AVJ and inulin decreased a* values and increased b* values. Counts of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* increased over time, showing addition of inulin and aloe vera do not affect its viability. All the treatments were higher than 10^9 and 10^6 CFU/g respectively. Both bacteria were present with higher counts than the minimal amounts 10^6 CFU/g / day required to provide health benefits. Inulin content in yogurt decreased over time. The lowest amount of inulin was detected in treatment with 15% AVJ and inulin. Syneresis, viscosity, pH, titratable acidity, inulin content, L*, and counts of *S. thermophilus* and *L. bulgaricus* did not have significant differences in the interaction between treatment and day, meaning they were not affected by time. For consumer acceptability, the sensory scores for aroma, overall taste and overall liking were higher in treatments without AVJ. Increasing concentration of aloe vera juice decreased sensory scores. Treatments with aloe vera at 15%

and with inulin received the lowest scores in overall appearance/color, sourness, mouthfeel (viscosity) and overall liking. Purchase intent was found to increase after consumers were aware of the health benefits of aloe vera, probiotics and inulin.

Further studies are needed to improve the flavor. Also, use a natural gum to improve viscosity and reduce syneresis in treatments with high percentage of aloe vera juice should be done. Enumeration of probiotic bacteria *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *Lactis*, using its specific media should be conducted. Quantification of acemannan in yogurt with added aloe vera should be also investigated.

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APPENDIX

APPENDIX A. Research consent form

APPROVED BY
LSU AG CENTER
IRB AS HE18-3
ON 2-10-2018

Participant #________________ **Gender: Male** ________**Female** ________

RESEARCH CONSENT FORM

I, ______________________, agree to participate in the research entitled "Functional Plain Yogurt Enhanced with Inulin and Aloe Vera.", which is being conducted by Dr. Charles Boeneke, School of Nutrition and Food Sciences at Louisiana State University Agricultural Center, (225)578-4383.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed.160 consumers will participate in this research. For this particular research, about 5-10-minute participation will be required for each consumer.

The following points have been explained to me:

1. In any case, it is my responsibility to report to the investigator prior to my participation any food allergies I may have.

2. The reason for the research is to gather information on consumer perception of **Functional plain yogurt enhanced with Inulin and Aloe Vera**. The benefit that I may expect from it is a satisfaction that I have contributed to quality improvement of this product.

3. The procedures are as follows: three coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.

4. Participation entails minimal risk: The only risk which can be envisioned is an allergic reaction to milk inulin and aloe vera. However, because it is known to me beforehand what type of food to be tested, the situation can normally be avoided.

5. The results of this study will not be released in any individual identifiable form without my prior consent unless required by law.

6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions regarding the study should be directed to the investigator listed above. In addition, I understand the research at Louisiana State University AgCenter which involves human participation is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. Michael J. Keenan, Chair, LSU AgCenter Institutional Review Board at 578-1708. I agree with the terms above.

________________________ ________________________
Signature of Investigator Signature of Participant

Witness: ________________ Date: ____________________

APPENDIX B. Hedonic scale for consumer acceptance

Sample # ____________ **Gender:** [ ] Male [ ] Female

Please evaluate the product and check the space that best reflects your feeling about the product. Between samples, you are required to rinse your palate with water and unsalted cracker.

1. How would you rate the **OVERALL APPEARANCE/COLOR** of this product?

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

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

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

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

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

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

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

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

Not Sour Enough	Just about right	Too Sour
[ ]	[ ]	[ ]

6. How would you rate the **MOUTHFEEL (VISCOSITY)** of this product?

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

7. How would you rate the **MOUTHFEEL (VISCOSITY)** of this product?

Not Viscous Enough	Just about right	Too Viscous
[ ]	[ ]	[ ]

8. How would you rate the **OVERALL LIKING** of this product?

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

9. Is this product **ACCEPTABLE**? YES [ ] NO [ ]

10. Would you **BUY** this product if it were commercially available? YES [ ] NO [ ]

11. Would you **BUY** this product if you know it contained beneficial bacterial which provide gut health benefits and aloe vera which help to supports immune health, and even aids digestion? YES [ ] NO [ ]

VITA

Janny Melissa Mendoza Mencia was born in April 1990 in Tegucigalpa, Honduras. She received her bachelor’s degree of Science in Food Agroindustry in Zamorano Pan-American Agricultural School, Honduras, in December 2014. She decided to enter in the Department of Animal Science at Louisiana State University in August 2015 where she is currently a candidate for a master’s degree in Animal, Dairy and Poultry Science with a concentration in dairy sciences. She will receive her degree in May 2018.