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Influence of Health Beneficial Monk Fruit Sweetener on Microbial and Physicochemical Characteristics of Camel Milk Yogurt

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INFLUENCE OF HEALTH BENEFICIAL MONK FRUIT SWEETENER ON MICROBIAL COUNTS AND PHYSICOCHEMICAL CHARACTERISTICS OF CAMEL MILK YOGURT

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ABSTRACT

Camel milk contains all the essential nutrients found in cow's milk and has potentially health beneficial compounds such as anti-carcinogenic, anti-hypertensive, anti-inflammatory, and insulin and insulin-like substances. Camel milk has been reported as a treatment for diseases such as diabetes and autism. Camel milk favorably alters gut microbiota to a higher abundance of bifidobacteria.

Sweetness is one of the most desirable tastes in people's diet; however, the surplus consumption of sugar has a negative effect on human health. Monk fruit sweetener is a natural zero caloric sweetener with many health beneficial properties, namely, prevention of asthma, prevention of oxidation, liver protection, regulation of immune function, cancer prevention, lowering glucose levels, and diabetes prevention. Sweetness of monk fruit sweetener is from 100 to 250 times that of sucrose.

The objective was to study the influence of different concentrations of monk fruit sweetener on the microbiological and physicochemical properties of camel milk drinking yogurt. Monk fruit sweetener was added in the amount of 0, 0.42, 1.27, and 2.54 g/L of camel milk to manufacture camel milk drinking yogurt, which was stored for 42 days. The physicochemical and microbiological characteristics of yogurts were measured weekly. For the physicochemical characteristics, pH, titratable acidity, viscosity, and color (L*, a*, b*, C*, and h*) values were evaluated. The counts of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, coliforms, and yeast and mold were determined. Three replications were conducted. Monk fruit sweetener did not affect the growth of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, probiotic culture *Lactobacillus acidophilus* but significantly influenced pH, viscosity, and color (a*, b*, C*, and h*) values. Compared to control, the samples containing 1.27 and

2.54 g/L of monk fruit sweetener had significantly lower pH, higher viscosity, higher b*, C*, and lower h* values. Monk fruit sweetener can be used as a health beneficial sweetener in camel milk yogurts.

CHAPTER 1. INTRODUCTION

1.1. CAMEL MILK

The camel is an animal of the desert that was first domesticated more than 4,000 years ago in the southern region of Arabia. For inhabitants of the region, camels solved problems of transportation and provided a ready supply of meat and milk. Camels are well adapted to harsh desert climates and can survive without drinking water for days. Moreover, nomadic people used camel milk medicinally to relieve abdominal pain and as a main source of nutrients for centuries (Al-Zoreky & Al-Otaibi, 2015; Kaskous, 2016). Based on the most recent FAO statistics, the world population of camel is approximately 29 million, of which around 95% are dromedary (one-humped) camels (Sikkema et al., 2019).

The period of camels' lactation may vary from 9 to 18 months. The amount of obtained milk depends on many factors such as breed, animal health, stage of lactation, living conditions. Yield of camel milk is lower and unstable when compared to cow's milk, so the yield is from 735 to 10,675kg per 305 days of lactation. However, the udder structure of a camel is similar to cow's and consists of four quarters (Park & Haenlein, 2013). Therefore, enhanced feed, water and veterinary practices may increase the milk yield up to 20L per day. Camel milk plays an important role in human nutrition (Kaskous, 2016). People consume camel milk fresh, fermented, or reconstituted from powder.

1.1.1. Health benefits of camel milk

Camel milk is rich in health-beneficial substances, for example, bioactive peptides, lactoferrin, zinc, and mono and polyunsaturated fatty acids that help treat human diseases such as tuberculosis, asthma, gastrointestinal diseases, and jaundice. In addition, anti-carcinogenic, anti-hypertensive, anti-diabetic, and healing properties of camel milk significantly increase its

therapeutic potential as well as bacteriostatic activity against Gram-positive and Gram-negative cultures (Devendra et al., 2016; Kaskous, 2016).

Milk is the main source of nutrients for mammal's newborns. Milk has biologically active substances and compounds with immunological protection necessary for a healthy growth. Camel milk has many beneficial nutritional and therapeutic characteristics, anti-bacterial, anti-carcinogenic, antioxidant, anti-hypertensive, and anti-diabetic properties (Ayoub et al., 2018). Camel milk is also used to treat different human diseases because of the presence of natural bioactive components. Also, bioactive components can be produced from milk proteins by probiotic bacteria during fermentation (Devendra et al., 2016).

Camel milk can be used in curing gastrointestinal disorders. Camel milk has a good effect on the stomach and intestinal diseases because of a high level of anti-inflammatory proteins, polyunsaturated fatty acids and vitamins, which increase carbohydrate metabolism (Kaskous, 2016).

Camel milk has antibacterial and antiviral properties. These properties are explained by the presence of lysozyme, hydrogen peroxide, lactoferrin, lactoperoxidase, and immunoglobulins. All these compounds have antimicrobial functions and can oppress both gram-positive and negative bacteria, e.g. *E. coli*, *S. aureus*, and *L. monocytogenes*. The content of the antibacterial components in camel milk is higher than in cow's milk; however, the exposure of milk at 100°C for 30 min completely inactivates their beneficial properties. Moreover, whey proteins of camel milk have enhanced anti-rotaviruses functions to treat non-bacterial gastroenteritis (Devendra et al., 2016). Lactoferrin and IgG of camel milk can inhibit the hepatitis C and B viruses and prevent their replication in cells. IgG can recognize Hepatitis C virus peptides in concentrations when human IgG does not detect the presence of virus. Moreover, camel milk can heal Hepatitis B, as it

increases immune response and stops DNA replication of the virus (Kaskous, 2016). The abundance of antimicrobial components in camel milk gives it a therapeutic effect against drug-resistant tuberculosis. Thus, camel milk reliefs symptoms such as cough, breathlessness, and fever (Devendra et al., 2016).

Many food proteins, including milk proteins, contain angiotension I-converting enzyme (ACE)-inhibitory peptides in their primary structure. These peptides are also present in camel milk fermented with probiotic bacteria. Probiotic bacteria break down proteins to release peptides and amino acids essential for the bacterial growth. Bioactive peptides in fermented camel milk may have a positive effect on lowering the level of cholesterol (Devendra et al., 2016). Also, camel milk has orotic acid, which is known to decrease cholesterol level in humans (Devendra et al., 2016). Raw camel milk and fermented dairy products are a source of probiotic strains. Species of *Lactobacillus, Bifidobacterium, Enterococcus,* and *Streptococcus* were isolated from camel milk for the following implementation in the dairy industry (Shori, 2017).

Camel milk is a good source for the treatment of type-1 and type-2 diabetes due to the presence of insulin and insulin-like substances as well as immunoglobulins of a small size (Devendra et al., 2016). The level of insulin in camel milk is high and comprises about 52 units/liter (Ayoub et al., 2018). Also, these components influence the pancreas and liver, improving insulin secretion by the pancreatic b-cells, so the required dose of insulin is reduced (Kaskous, 2016). Along with the application for the diabetes treatment, camel milk reduces blood sugar, decreases insulin resistance, and improves lipid profiles (Ayoub et al., 2018).

Another possible health benefit of camel milk is its decreased allergenicity, especially among children who are allergic to cow's milk. Cow's milk causes allergy because of the high content of α-casein and low content of hypoallergenic β-casein along with the presence of β-

lactoglobulin. Especially, allergy to cow's milk is of a big concern among infants, as, in the most severe cases, cow's milk consumption can cause anaphylaxis. However, camel milk has the opposite composition of proteins: higher content of β-casein and decreased amount of α-casein and β-lactoglobulin, and this protein profile is closer to the composition of human milk. Moreover, immunoglobulins of camel milk are like in mother's milk, and this also make it safe for children to consume (Devendra et al., 2016; Izadi et al., 2019). In addition, individuals with lactose intolerance can consume camel milk safely. Camel milk has higher concentration of L-lactate compared to cow's milk rich in D-lactate, and L-lactate decreases milk allergenicity. The IgE of children allergic to cow's milk does not react with camel milk. Therefore, camel milk immunoglobulins decrease allergic symptoms (Kaskous, 2016).

Camel milk has a potential positive effect on people with autism. In the intestines of patients with this autoimmune disease, the break-down of milk's casein results in the formation of casomorphin, which is a strong opioid responsible for the brain damage. High level of β-casein content and β-lactoglobulin in cow's milk make it more likely to form opioids. On the other hand, camel milk lacking these components is a good alternative for these people. Moreover, camel milk has protective proteins (lactoferrin, lysozyme, and immunoglobulins) that may improve the development of brain (Devendra et al., 2016).

Treating of blood, lung, liver, and breast cancer is another camel milk benefit. Camel milk inhibits HepG2 and MCF7 cells proliferation as well as the stimulation of death receptors in cell lines and mechanisms caused by oxidative stress (Kaskous, 2016).

There is a correlation between camel milk and changes in the gut microbiota. Camel milk consumption helps to acquire a higher abundance of genera *Allobaculum*, *Akkermansia*, and *Bifidobacterium*. The study by Wang et al., (2018) indicates that camel milk could enhance the

abundance of *Allobaculum*, which may positively influence the physiological function of the organism. This gene produces short chain fatty acids that improve colon health, help prevent obesity, and decrease inflammations. *Akkermansia* is a mucin-degrading probiotic that is well known for its positive effects on diabetes, obesity, metabolic disorders, and inflammation (Wang et al., 2018).

1.1.2. Camel milk v/s cow's milk

Camel milk is a white liquid with a slightly salty aftertaste. The camel milk density is a bit lower than cow's milk, and the average value is 1,029 g/cm^3; the pH varies from 6.4 to 6.7; the water content changes from 87 to 90 %, and the freezing point is from -0.57 to -0.61°C. (Devendra et al., 2016). Cow's milk water content varies from 79 to 90% with the water activity about 0.993 (Chandan & Kilara, 2010). The pH of cow's milk varies from 6.4 to 6.6; density is about 1,030 g/cm^3, and the freezing point is -0.54°C. The color of cow's milk is opaque white with yellowish hue due to the presence of carotene and depends on the breed, type of feed, and fat content (NPCS Board, 2012). Cow's milk contains in average 3.6% fat, 3.0% protein, and 4.6% lactose (Srinivasan & Parkin, 2018).

The composition of camel milk is more variable than cow's milk. Camel milk composition depends on the feed, breed, stage of lactation, and age of the animal (Hadef et al., 2018). Region and season significantly change the ratio of compounds in camel milk. The most stable to the effect of these factors is the lactose content in camel milk, and it varies between 3.5 and 4.5% (Devendra et al., 2016). Lactose is the main carbohydrate in camel milk. Moreover, camel milk contains a small number of different oligosaccharides. Carbohydrates play an important role in milk. Lactose is a major energy source, and oligosaccharides protect infants against pathogens, promote the

formation of environment for *Bifidobacterium*, and help develop nervous system (Park & Haenlein, 2013).

Total protein content of camel milk varies from 2.15 to 4.90%. Camel and cow's milk have similar content of casein (α_{s1}, α_{s2}, β, and κ-casein), but they differ in the content of whey proteins. Thus, cow's milk ratio of casein to whey proteins is higher than in camel milk. This affects the firmness of coagulum, and camel milk forms softer gel than cow's milk. Casein is the main protein in camel milk, and it contributes from 52 to 87% of total proteins, while whey proteins contribute 20-25% (Devendra et al., 2016). Casein in camel milk has four fractions and accounts; the ratio of α_{s1} to α_{s2} to β to κ-casein varies significantly in camel milk and is 22 : 9.5 : 65 : 3.5 (Park & Haenlein, 2013). Camel milk has more β-casein than α-casein that is about 65 and 21%, respectively, of total casein. Cow's milk compared to camel milk has approximately equal percentage of β-casein and α-casein (36 and 38%, respectively) and higher content of κ-casein (13%), which is about four times lower in camel milk (3.47%) (Devendra et al., 2016). β-casein is more digestible and less allergic for people, as it is more sensitive to peptic hydrolysis in the gut. The higher percentage of β-casein makes camel milk beneficial for human health. Caseins micelles of camel milk have a wide range of size from 20 to 300nm diameter compared to 40-160nm in cow milk (Park & Haenlein, 2013). Overall, an average diameter of casein micelles in camel milk is larger and mineral charge is higher (Attia et al., 2001). The main whey protein of camel milk is α-lactalbumin. α-Lactalbumin obtained from camel milk is more digestible by pancreatic proteases and has higher antioxidant activity than bovine α-lactalbumin. It allows one to consider using camel milk use in infant foods (Park & Haenlein, 2013). β-lactoglobulin is lacking in camel milk, which makes camel milk less allergic, but other whey proteins such as lactoferrin and immunoglobulins are present (Devendra et al., 2016). Lactoferrin is a glycoprotein that binds two

ferric ions. Its content ranges from 0.02 to 2.1 g/L in camel milk (Park & Haenlein, 2013). Lactoferrin has anti-inflammatory, antimicrobial, antitumor, and immunomodulatory activities (Park & Haenlein, 2013). Another antimicrobial agent in milk is lysozyme, and its concentration in camel milk (150 μg/L) is higher than in cow milk (70 μg/L). Immunoglobulins are the whey proteins that play a significant role in passive immunity of neonates. IgG is the dominant immunoglobulin in camel milk. It is secreted at a concentration of around 100 g/L in colostrum but decreases rapidly during lactation to less than 10 g/L (Park & Haenlein, 2013). Differences in protein profile also affect composition of fermented camel and cow's milk. Fermented camel milk has more antioxidant peptides, probably, due to the structure of β-casein. So, β-casein in camel milk is shorter and contains more proline; its hydrolysis results in the formation of bioactive peptides and release of amino acids such as phenylalanine and tryptophan with antioxidant properties (Izadi et al., 2019).

The fat content of camel milk varies from 1.2 to 4.5% (Devendra et al., 2016). Some sources report that the content of fat in camel milk may reach up to 6.4% (Park & Haenlein, 2013). Camel milk fat profile is characterized with the presence of higher amount of unsaturated and long-chain fatty acids that helps lower the level of lipids in human serum (Park & Haenlein, 2013). The content of long-chain fatty acids is measured as 92-99%, and the percentage of unsaturated acids is 35-50% (Izadi et al., 2019). These structural differences impart "waxy texture" to the fat of camel milk. The lower content of carotene makes the color of camel milk whiter compared to cow's milk (Devendra et al., 2016).

Mineral content of camel milk is like of cow's milk, especially, in the content of Ca, Mg, P, Na, and K (Kaskous, 2016). The main distinction is in the content of Zn, Fe, Cu, and Mn, as camel milk has higher concentrations of these minerals. Increased iron concentration in camel milk

may be good for iron-deficiency anemia prevention. Also, lower citrate concentration in camel milk than in cow's milk increases lactoferrin antimicrobial activity, as it needs small levels of citrate to be beneficial (Park & Haenlein, 2013). The total mineral content of camel milk fluctuates from 0.60 to 0.90%. The salty taste of camel milk can be explained by the enhanced content of chloride obtained from the feed eaten by animals (Devendra et al., 2016). In addition, the content of ascorbic acid is higher in camel milk; therefore, it can extend the shelf-life of a product and increase its antioxidant and antiradical abilities (Izadi et al., 2019).

The concentrations of mineral salts and vitamins in camel milk depend on breed, feed, stage of lactation, and water intake. Camel milk contains higher concentration of niacin and vitamin C compared to cow's milk, but vitamin B_1, B_2, A, folic acid, and pantothenic acid are deficient in camel milk. Both camel milk and cow's milk have almost the same content of vitamins B_6 and B_{12} (Devendra et al., 2016).

Camel milk has better heat stability compared to cow's milk. The increase of camel milk temperature to 80°C caused a break-down of 32-35% whey proteins, while the increase to 90°C resulted in the denaturation of 47-53% of camel milk whey proteins (Izadi et al., 2019). The heat treatment of cow's milk at 80°C resulted in the denaturation of 70% of whey proteins, and 90°C caused the denaturation of 81% of cow's milk whey proteins (Farah, 1986).

1.2. MICROBIAL GROWTH

Strains of microorganisms belonging to the genera *Lactobacillus* and *Streptococcus* are traditionally used in yogurt production. For the yogurt production the following cultures were chosen *Streptococcus thermophilus, Lactobacillus bulgaricus,* and *Lactobacillus acidophilus* as a probiotic culture. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* have a symbiotic relationship that leads to an increased rate of lactic acid production during fermentation (Doyle &

Buchanan, 2013). At the first stage of yogurt fermentation, *S. thermophilus* dominates. This culture produces acid to decrease both redox potential and pH to 5.5 (Shah, 2000). At this pH, *L. bulgaricus* begins to multiply, and as the result of its growth, acetaldehyde and lactic acid are produced. Next, both cultures produce lactic acid, and gel forms at pH 4.5. Also, *L. bulgaricus* produces essential amino acids as growth factors for *S. thermophilus* because of its increased proteolytic properties (Shah, 2000).

The stable and expected rate of the acid formation is critical in yogurt production. Lactic acid decreases pH, and this, in turn, affects proteins' hydration and formation of matrix. The formation of bonds between protein molecules results in the development of texture and aroma of a finished product (Doyle & Buchanan, 2013). *S. thermophilus* and *L. bulgaricus* are both homofermentative bacteria that metabolize lactose, the main milk carbohydrate, into lactic acid. Some volatile compounds such as acetic acid, acetaldehyde, and diacetyl are formed from the by-products during the break-down of lactose (Routray & Mishra, 2011). To grow in milk, *S. thermophilus* and *L. bulgaricus* also require the presence of amino acids (Doyle & Buchanan, 2013). As free amino acids are lacking in milk, starter cultures have a proteolytic system containing enzymes to cleave peptides and obtain amino acids. Also, bacteriophages have influence on the process of lactic acid fermentation. If milk contains bacteriophages, starter cultures do not grow or grow slowly, and lactic acid is not produced. As a result, the finished product has defects in quality and safety (Garneau & Moineau, 2011). Moreover, this increased time of fermentation disrupts schedules of products' production. To avoid these problems, a dairy factory should have good sanitation regimes and starter rotation (Doyle & Buchanan, 2013).

Some species of *Lactobacillus* genera as well as *Bifidobacterium* are naturally found in the intestinal tract of humans. They have a positive effect on people's health and negative effect on

pathogenic bacteria (Doyle & Buchanan, 2013). These bacteria are called probiotics, and they are often added into foods to improve host's well-being (Doyle & Buchanan, 2013). *Lactobacillus acidophilus* is one of the most used probiotic strain in the dairy industry.

All three cultures must tolerate the manufacturing process and maintain cell viability during storage (Shori, 2017). In addition to the above, probiotic bacteria must survive in the gastrointestinal tract with the bacterial count no less that 10^6-10^7 CFU/g as well as tolerate low pH and bile salt (Shori, 2017). Among the factors that affect starter cultures for yogurt are common additives such as sugars, sweeteners, colorings, and flavorings.

1.2.1. Microbial growth as a result of media

Functional properties of microorganisms depend on composition of media, in which they grow, and environmental conditions. Therefore, the composition of medium impacts the rate and ability of starter cultures to grow and multiply (Roy, 2005).

The environmental conditions of dairy products influence the survival rate of probiotics (Roy, 2005). Probiotic cultures must tolerate the manufacturing process and storage of dairy products. However, they are affected by the low pH of yogurt, oxygen, and the presence ofstarter cultures (Roy, 2005). The presence of starters decreases the growth rate of *L. acidophilus*, as they produce bacteriocins, lactic and acetic acids, hydrogen peroxide, and other inhibitors that may affect probiotics (Roy, 2005). Many strains of probiotics are not acid-tolerable, but this is important in yogurt production, as pH usually decreases during product storage. Strains of *L. acidophilus* are noticeably affected by the pH below 4.0 (Shah, 2000). Also, probiotic cultures have low proteolytic activity; therefore, they grow slower than starters and undergo the lack of nutrients' availability. Therefore, the added number of probiotics into dairy products should be enough to compete with the starter cultures for the essential nutrients and be able to multiply.

Furthermore, the implementation of supplements, which can increase the growth of probiotic strains, may be beneficial. The examples of the supplements are yeast extracts, casein hydrolysates or combination of amino acids and minerals. It is important for a supplement to decrease the redox potential of the environment because low redox value is more suitable for probiotics (Roy, 2005).

Probiotic bacteria do not readily metabolize lactose, so the implementation of additional source of carbohydrate is effective to increase microbial growth. The addition of lactulose, barley glucans, galactooligosaccharides, raffinose, or inulin may enhance the growth rate of probiotics. Fibers are used to promote growth of probiotic cultures, and fruits, nuts along with grains are good sources of fibers. Thus, fortification of yogurt with pulses promotes the growth of lactobacilli cultures. However, the extra addition of these ingredients affects the physicochemical, organoleptic, and rheological properties of a finished dairy product (Zare et al., 2012).

Probiotic strains of *Lactobacillus acidophilus*, according to the study by Schär-Zammaretti et al., (2005), require carbohydrates such as sucrose and lactose. The lack of carbohydrate compounds decreases total bacterial count even though physicochemical characteristics and protein composition of the bacterial cell wall remain mostly the same. Lack of amino acids from pea protein concentrate and enzymatic digest of meat caused effects similar to microbial starvation and led to the increase of the bacteria hydrophobicity (Schär-Zammaretti et al., 2005). Hydrophobicity of the cell wall of bacteria in complete medium is low; however, increased hydrophobicity has a negative effect on the structure and physicochemical characteristics of the bacterial cell wall leading to the bacteria aggregation and prevention of growth (Schär-Zammaretti & Ubbink, 2003). Fermentation medium without a source of proteins and yeast extract has unfavorable influence on the structure and functions of bacterial cell wall (Schär-Zammaretti et al., 2005).

L. acidophilus are microerophilic microorganisms, so the presence of oxygen is important, but it can cause a problem (Shah, 2000). Oxygen is easily dissolved in the milk. Milk absorbs oxygen not only during yogurt production but also during storage, as air may come through the packaging materials. The content of oxygen is higher in plastic containers than in glass bottles; therefore, *L. acidophilus* in yogurt stored in plastic cups are more affected by air, and this decreases the bacterial count of the culture (Shah, 2000). To protect probiotics, thicker plastic materials are recommended. In addition, oxygen scavengers such as ascorbic acid can be helpful to improve growth of *L. acidophilus* (Ng et al., 2011). Another supplement that increases the survival rate of probiotics is a sulfur containing amino acid cysteine (Shah, 2000). This amino acid is both an oxygen scavenging reagent and a source of nitrogen, which is essential for bacterial growth (Shah, 2000).

1.2.2. Microbial growth as a result of different sweeteners

Sweeteners have a different influence on the growth of strains of *Lactobacillus* and *Streptococcus*. Sweeteners (acesulfame and aspartame), at the concentration normally used in fermented dairy drinks (0.03%) did not inhibit growth of lactic acid starter and probiotic bacteria (Vinderola et al., 2002). Only aspartame at the highest concentration (0.12%) was inhibitory for one strains of *S. thermophilus* (A5) and one probiotic strain *L. acidophilus* (08) (Vinderola et al., 2002). Probiotic bacteria proved to be less sensitive to the presence of sugars than lactic acid starter bacteria. However, 15% of sucrose and lactose was inhibitory for most strains of *Lactobacillus bulgaricus, Streptococcus thermophilus,* and *Lactobacillus acidophilus* (Vinderola et al., 2002).

Commercial mixtures of additives such as natural colorings, flavorings (peach, vanilla, strawberry, and banana), and flavoring–colorings (peach, vanilla, and strawberry) often may cause inhibition of bacteria growth even at the concentrations recommended by suppliers (Vinderola et

al., 2002). Flavorings of vanilla, strawberry, and banana affected some strains of yogurt bacteria (*S. thermophilus* and *L. bulgaricus*). Flavoring-coloring agents, except for the peach mixture, had inhibition effect on yogurt starter cultures. A clear bacteriostatic effect (complete growth inhibition) was observed for the strains *L. acidophilus* A3 and A9 after 24 h of cultivation with the addition of vanilla mixture (Vinderola et al., 2002).

Faria et al., (2013) studied probiotic Petit Suisse cheese with the following sweeteners: "0.024% sucralose, 0.152% stevia, 0.088% aspartame, 0.0025% neotame, or 15.2% sucrose (wt/wt)". The authors showed that the presence of sweeteners did not affect the viability of *S. thermophilus* and *L. acidophilus* in every sample of strawberry-flavored probiotic Petit Suisse cheese. Samples were kept for 28 days, and the microbial count for *S. thermophilus* lowered but remained at the level appropriate for *S. thermophilus*. Microbial count of *L. acidophilus* showed little change for 28 days (Esmerino et al., 2013).

Some studies were carried out with the buffalo's frozen yogurt. 20, 30, and 40% of sucrose were replaced with prickly pear peels sweetener (El-Fetoh & Hussein, 2011). Prickly pear peels sweetener has the mucilage's complex polysaccharides (arabinose, galactose, rhamnose, and galacturonic acid). The results showed increased survival of *S. thermophilus*, *L. bulgaricus*, and *L. acidophilus*. Also, the increased ration of the sweetener enhanced the number of cells of each culture (El-Fetoh & Hussein, 2011).

1.3. SWEETENERS

Sweet is one of the more desirable tastes in people's diet (McCain et al., 2018). Table sugar or sucrose is known as the most common source of sweetness (Carocho et al., 2017). According to the FAO and WHO consultation, daily energy intake of sugar is from 9 to 27%, and the consumption is increasing worldwide (O'Donnell & Kearsley, 2012). However, the surplus

consumption of sugar has a negative effect on humans' health. Excessive sugar intake may result in metabolic disorders, such as obesity, diabetes, and others (Carocho et al., 2017). According to the WHO investigations, these disorders are the markers of the industrialized world (Carocho et al., 2017). Sugar excess may also lead to the dental decay (O'Donnell & Kearsley, 2012). Sucrose is a good substrate of disaccharide for bacteria *Streptococcus mutans* and *S. sanguis*, which metabolize sugar to pyruvic, acetic and lactic acids. These acids dissolve the tooth enamel promoting bacterial growth (Carocho et al., 2017). Moreover, if sugar is absorbed rapidly, glycemic spike may occur followed by hormonal problems, especially in diabetic individuals. Among these disorders, cardiovascular diseases, hypertriglyceridemia, cancer, and kidney diseases are also provided by excessive sugar intake (O'Donnell & Kearsley, 2012). With the increasing number of illnesses related to sugar intake, alternative sweeteners are becoming increasingly used in foods. Their benefits are level of sweetness, the absence of harmful effects on health, economic and social profit (Carocho et al., 2017).

1.3.1. Alternative sweeteners

There are some classifications of sweeteners. The most frequently used classifications are by nutritive value, level of sweetness, and origin. Sweeteners can be synthetic or natural, nutritive or intensive.

Simple sugars, high fructose corn syrup, isomaltulose, trehalose, erythritol, isomaltitol, lactitol, maltitol, sorbitol, mannitol, and xylitol are all defined as nutritive sweeteners. Intensive sweeteners are divided into two groups: synthetic and natural. Aspartame, acesulfame K, saccharin, dulcin are the synthetic sweeteners, while thaumatin, steviol glucosides, monellin, neohesperidine dihydrochalcone, glycyrrhizin are the natural ones (Carocho et al., 2017; McCain et al., 2018). Intensive sweeteners have a big benefit for health as they are almost zero calorie

additives. Moreover, their sweetening capacity is higher than sugar, and that allows to use them in lower quantity. Intensive sweeteners are not cariogenic, and do not enhance glycemic response. So, they can be used in diets for diabetes individuals and in production of sweet foods with decreased caloric intake. Some sweeteners unlike sugar have a metabolic pathway, which does not contain insulin. These make them healthy for diabetes patients (Carocho et al., 2017; Mooradian et al., 2017).

1.3.2. Health implication of sweeteners

Carbohydrates and sugars are important part of many food products. Sugar has technological and organoleptic functions, as it adds not only sweetness but also texture, bulk, viscosity, and calories into foods (McCain et al., 2018). Moreover**,** human health depends on sugar intake. Therefore, different amounts of sugar or sugar substitutions such as sweeteners may influence people's well-being. Properly selected sweeteners can decrease the possibility of dental caries or fasten restoration of carious lesions as well as decrease caloric value and, as a result, help prevent overeating and obesity (O'Donnell & Kearsley, 2012). Also, sweeteners have a positive effect on colon, as intestinal microflora break them down to produce butyrate, which may decrease risk of colon cancer, and other compounds with increased immunological properties (O'Donnell & Kearsley, 2012).

Sweeteners can improve oral health if they substitute sucrose. Thus, oral microflora does not utilize alternative sweeteners such as stevia, aspartame, acesulfame, erythritol, and xylitol, so these substrates prevent the growth of bacteria. As a result, the formation of biofilm matrixes does not occur, and plaque becomes more porous and wholesome (Razak et al., 2017).

Sugar substitution with intensive sweeteners as, for example, aspartame or sucralose reduces glycemic response and, in turn, lowers development of diabetes, heart disease,

hypertension, stroke, and certain cancers (O'Donnell & Kearsley, 2012). Intensive sweeteners are added into foods in a small amount; hence, they do not cause the increase of sugar level in blood. Also, the break-down of such sweeteners does not result in the glucose formation due to their chemical structure. Therefore, intensive sweeteners keep the sweetness of a food product without affecting glycemic response in humans (O'Donnell & Kearsley, 2012).

Addition of non-nutritive intense sweeteners into foods is another approach towards improved health. Non-nutritive sweeteners do not have calories, so their presence may reduce energy intake of products. Thus, if sugar contributes 4 kcal/g, then the caloric value of intense sweeteners is from 1.5 to 4 time less that can provide good caloric savings. However, the composition of food plays important role in calorie reduction. For example, foods with the increased load of sugar are more desirable for calorie saving by the sugar substitution with sweeteners than foods with high-fat content, as food products with high load of fat and sugar replaced with sweeteners will have the enhanced energy density per unit weight. Also, non-nutritive sweeteners help people eat less, as they do not follow the same metabolic pathways as glucose (O'Donnell & Kearsley, 2012).

Natural sweeteners such as stevia, sugar alcohols, rare sugars, and monk fruit sweetener consist of different compounds. These compounds may cause various effects on humans and, in particular, on body weight, level of glucose in blood, fat break-down as well as antioxidant pathways (Mejia & Pearlman, 2019). In general, their implementation in food has more benefits for health than sugar. Thus, stevia may decrease levels of triglycerides, cholesterol, and blood pressure; however; it does not influence level of glucose in blood. Rare sugars such as D-Allulose (D-psicose), D-tagatose, D-sorbose, and D-allose have different effects on blood glucose and triglycerides level depending on the dose of the sweetener. Some studies show that rare sugars

either have no effect on these parameters, including blood pressure and energy intake, or decrease them. The different effect of sugar alcohols on blood glucose may be explained by the type of alcohol. For instance, xylitol and erythritol increase level of sugar in blood, while lacritose decreases this concentration of sugar in serum (Mejia & Pearlman, 2019).

1.3.3. Health benefit of monk fruit sweetener

A monk fruit is also known as Luo Han Guo or the fruits of *Siraitia grosvenorii*. *Siraitia grosvenorii* is a perennial vine of the Cucurbitaceae family. Originally, it was planted in China, the Indo-China Peninsula, and Indonesia. In China, *S. grosvenorii* fruit has been used for centuries as a natural sweetener and as a traditional medicine to cure lung congestion, colds, and sore throat. It has health beneficial properties such as cough relief, prevention of asthma, prevention of oxidation, protection of liver, regulation of immune function, prevention of cancers, lowering glucose, and diabetes prevention. (Li et al., 2014).

Monk fruit sweetener has some advantages compared to most sweeteners used. It is a zero caloric sweetener; it does not have fat. Monk fruit sweetener prevents diabetes and helps to cure cough (Baotang, 2018). Moreover, it contributes a very low influence on blood glucose and insulin. Mogrosides present in monk fruit sweetener have antioxidant effects making the sweetener beneficial. Thus, according to the FDA, monk fruit sweetener is safe to be consumed by children, pregnant women, and individuals with diabetes (Palmer, 2018). In a contrast to a stevia extract, monk fruit sweetener does not have a bitter after taste, which was reported by many consumers (Baotang, 2018). The sweetness of monk fruit sweetener is from 100 to 250 times that of sucrose (Baotang, 2018).

Cucurbitane-type triterpenoid glycosides are responsible for the sweet taste (Li et al., 2014). Mogrosides that prevail in the monk fruit have the structure of cucurbitane-type triterpenoid

glycosides. Mogrosides have the mogrolaglycone structure, [10α-cucurbit-5-ene-3β, 11α, 24(R), 25-tetraol], with two to six glucose units attached (Li et al., 2014). This is the reason for the sweet taste of *S. grosvenorii* fruit. Mogrosides are present at 1.19% in the fresh fruit and 3.82% in the dried fruit of *S. grosvenorii*. Mogroside V is the main component, with a content of 0.5-1.4% in the dried fruit of *S. grosvenorii* (Li et al., 2014). Mogroside IV, Mogroside VI, siamenoside I, and 11-oxo-mogroside V also contribute to sweetness of the fruit. Some studies of structure taste relationship showed that the number of glucose residues and the oxygen functionality at the 11-position of the aglycone moiety determine the taste sensation. Glycosides of 11α-hydroxy compounds have sweet taste, but glycosides of 11β-hydroxy compounds are tasteless (Xia et al., 2008). In addition, monk fruit contains triterpenoids, flavonoids, vitamins, proteins, saccharides, and a volatile oil (Li et al., 2014; Qing et al., 2017).

Mogroside V is responsible for the main biological effect. It was found that mogroside V has an antioxidant effect and, therefore, can prevent DNA damage and can scavenge reactive oxygen species (Zhang et al., 2011).

Monk fruit has the anti-tussive, phlegm-expelling, and dyspnea relieving activities. The water extract of mogrosides actively inhibited the mouse cough evoked by inhalation of ammonia water. It also showed a considerable phlegm-releasing action on mice and rats' models. Immunostimulatory functions of mogrosides were proved on rats. It is believed that mogrosides increase cellular and humoral immunity processes, wherein they do not have effect on the non-specific immunity (Li et al., 2014).

The ability of mogroside to suppress oxygen free radicals, red blood cells hemolysis, and lipid peroxidation showed their antioxidant functions. Studies indicated that mogroside V had the main antioxidant ability among *S. grosvenorii* fruit mogrosides. Water, ethanol, ethyl acetate, and

chloroform extracts of monk fruit had a higher level of antioxidant capacities compared to the synthetic butylatedhydroxytoluene. So, monk fruit sweetener can be used as a natural alternative antioxidant additive (Li et al., 2014).

Many in vivo studies showed a positive effect of *S. grosvenorii* fruit extract and its compounds on diabetes (Jin & Lee, 2012). It is believed, that *S. grosvenorii* fruit can decrease the level of glucose in blood after a meal and lower the fasting. This extract managed to improve the insulin response at 15 min and decreased the level of glucose in blood at 120 min. So, monk fruit sweetener may be useful in the prevention of diabetic symptoms caused by oxidative stress and hyperlipidemia (Li et al., 2014). The decrease of blood sugar showed the ability of mogrosides to prevent hyperglycemia induced by diabetes (Jin & Lee, 2012). Therefore, *S. grosvenorii* fruit extract may help alleviate the diabetic oxidative stress and kidney damage in individuals with the type 2 diabetes (Li et al., 2014). In mouse models, *S. grosvenorii* improved the insulin response and prevented the increase of levels of postprandial blood glucose after maltose consumptions (Jin & Lee, 2012).

The anti-bacterial activity of the ethanol extracts from monk fruit's leaf and stem on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Streptococcus mutans*, and *Candida albicans* was studied as well (Li et al., 2014). A work by Yemin (2008) showed that the bacteriostatic rate of the ethanol extracts of *S. grosvenorii* increased with the increasing concentration. The species of *Pseudomonas aeruginosa* were inhibited with the *S. grosvenorii* concentration 50.0 mg/ml; the level of *Staphylococcus aureus*, *Micrococcus luteus*, and *Candida albicans* inhibition was below 50% (Yemin, 2008).

Monk fruit sweetener does not enhance the level of glucose in blood during the post-consumption period of food, while sugar significantly increases this characteristic if compared to

the values of blood sugar measured before consumption. Blood sugar stability was observed during the measuring of the level of sugar in blood fasting at 15, 30, 45, 60, 90, and 120 min after ingestion of 50 g of sugar or 50 g of monk fruit. Thus, monk fruit sweetener can help control blood glucose level (Mejia & Pearlman, 2019).

The influence of monk fruit sweeteners on the growth of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and probiotic cultures of *Lactobacillus acidophilus* as well as the changes of physicochemical characteristics of camel milk drinking yogurt is not clearly understood.

1.4. HYPOTHESIS

Whether or not monk fruit sweetener influences yogurt starter culture and probiotic *L. acidophilus* counts and other characteristics of camel milk drinking yogurt.

1.5. OBJECTIVES

The objective was to study the influence of different concentrations of monk fruit sweetener on the microbiological and physicochemical properties of camel milk drinking yogurt.

CHAPTER 2. MATERIALS AND METHODS

2.1. EXPERIMENTAL DESIGN

Treatments consisted of three concentrations of monk fruit sweetener (0.42, 1.27, and 2.54 g/L) separately added into vanilla flavored camel milk drinking yogurt. The control did not have added monk fruit sweetener. Yogurts were analyzed at days 1, 7, 14, 21, 28, 35, and 42 to study the pH, titratable acidity, viscosity, color (L*, a*, b*, C*, and h*), and counts of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, coliforms, and yeast and mold of vanilla flavored camel milk. Three replications were conducted; replications were the blocks.

2.2. YOGURT PREPARATION

Vanilla flavored camel milk drinking yogurt was manufactured at the Louisiana State University Dairy Processing Plant. Dry camel milk (Drome Dairy, Centennial, CO) 500g was reconstituted with 3.785L of distilled water. Each sample had 7.57L of camel milk for each of the 4 treatments. The first sample was control (0 g/L of monk fruit sweetener (Julian Bakery Pure Monk ™, Oceanside, CA)); the second sample had 0.42 g/L; the third 1.27 g/L; and the forth 2.54 g/L. Reconstituted camel milk with monk fruit sweetener in cleaned and sanitized pails was pasteurized at 180°F for 30 min. Pasteurized reconstituted milk was tempered to 104°F; 50ml of colorless vanilla flavor (Watkins, Winona, MN) was added to each pail of 7.57L. Each sample pail of 7.57L was inoculated with 2.4ml of each of the following freshly thawed cultures *Streptococcus thermophilus* STI-06 (Chr. Hansen`s Laboratory, Copenhagen, Denmark), *Lactobacillus bulgaricus* LB-12 (Chr. Hansen's Laboratory, Copenhagen, Denmark), and *Lactobacillus acidophilus* LYO 50 (Danisco, Dairy Connection, Madison, WI). Obtained mixtures were agitated and poured into previously labeled 946.3ml and 147.9ml cups. These cups were incubated at 104°F

until the pH dropped to 4.6±0.1 measured with a calibrated pH meter (Thermo Scientific, Orion Star A111, Dawsonville, GA). Cups were then transferred to a cooler at 4±1°C until needed.

Table 1. Vanilla flavored camel milk yogurt formulations per 7.57L of yogurt.

Ingredients	Control	0.42 g/L	1.27 g/L	2.54 g/L
Camel milk powder (g)	1000	1000	1000	1000
Water (L)	7.57	7.57	7.57	7.57
Monk fruit sweetener (g)	0	3.2	9.6	19.2
Vanilla flavoring (ml)	50	50	50	50
Streptococcus thermophilus (ml)	2.4	2.4	2.4	2.4
Lactobacillus bulgaricus (ml)	2.4	2.4	2.4	2.4
Lactobacillus acidophilus (ml)	2.4	2.4	2.4	2.4

The amount of monk fruit sweetener was calculated to meet the limit of added sugar in flavored yogurt. Regular 236.6ml cup of flavored yogurt contains from 10 to 30g of added sugar. Monk fruit sweetener is 100 – 250 times sweeter than sugar. Therefore, if we consider that sweetener is only 100 times sweeter than sugar, then we need 0.3g per 236.6ml cup to meet the sweetness of 30g added sugar. Consequently, since 3.785L is comprised of 16 cups of 236.6ml, to fit the limit of 30g of added sugar, 4.8g of the sweetener per 3.785L or 9.6g per 7.57L equal to 1.27g/L is needed. However, to meet the sweetness of 10g added sugar, 1.6g of monk fruit sweetener should be added to 3.785L of camel milk or 3.2g per 7.57L equal to 0.42 g/L. The amount of 9.6g per 3.785L or 19.2g per 7.57L equal to 2.54 g/L was chosen to study the influence of excess sweetener addition on cultures' growth.

The experiment was carried out in 3 replications. Each replication was stored in the cooler for 42 days and analyzed for microbiological and physicochemical characteristics at days 1, 7, 14,

21, 28, 35, and 42. Control and treatments were randomized to minimize any possible biases during the experiment.

2.3. PHYSICOCHEMICAL CHARACTERISTICS

2.3.1. pH

The pH of the vanilla flavored camel milk yogurts was measured at days 1, 7, 14, 21, 28, 35, and 42. The pH meter (The Lab Depot, Dawsonville, GA) was calibrated with pH buffers 7.00 and 4.00 (Fischer Chemical, Pittsburgh. PA).

2.3.2. Titratable acidity

The titratable acidity of yogurt samples was measured at days 1, 7, 14, 21, 28, 35, and 42. Titratable acidity, expressed in % of lactic acid, was determined by titration of 9 ml of yogurt sample with 0.1 N sodium hydroxide solution. 5 drops of phenolphthalein were added as an indicator. The endpoint of titration was reached when solution acquired a light pink color retained for 30 seconds.

2.3.3. Viscosity

Viscosity measurements were carried out on a Brookfield DV-II viscometer (Brookfield Engineering Laboratories, Stoughton, MA) and a helipath stand. The RV-1 spindle was used at 5 rpm. Torque force from 10 to 90 was used for selection of spindle and rpm. The readings were carried out in the 946.3ml container at 6±2°C. The RV-1 spindle was inserted in the yogurt sample at a constant depth of 2cm from the surface. The helipath was set in downward motion to cut new circular layers at increasing depth. The readings were collected with the Wingather software (Brookfield Engineering Laboratories, Stoughton, MA). Average of one hundred data points were recorded per sample. Three replications were conducted.

2.3.4. Color

Color of yogurt samples was measured at days 1, 7, 14, 21, 28, 35, and 42 using a Mini Scan XE Plus colorimeter (Hunter Lab, Reston, Virginia, United States). Before measurements, the colorimeter was calibrated with black and white tiles. The following characteristics of yogurt were analyzed: L*, a*, b*, C*, and h*. Readings were taken under D 65 illumination, 10° observer, and in the reflected mode. Each sample was measured 5 times and three replications were conducted.

2.4. PREPARATION OF MEDIA

2.4.1. Peptone water

Peptone water (0.1%) was prepared by dissolving 1g of peptone powder (BactoTM Peptone Difco, Dickinson and Company, Sparks, MD) in 1L of distilled water, poured into 99ml bottles, and autoclaved at 121°C for 15 min.

2.4.2. *Streptococcus thermophilus* agar

Streptococcus thermophilus agar for *Streptococcus thermophilus* enumeration was prepared according to Dave and Shah (1996). 10g of tryptone (Becton, Dickinson and Co., Sparks, MD), 10g of sucrose (Amresco, Solon, OH), 5g of yeast extract (Becton, Dickinson and Co., Sparks, MD), and 2g of dipotassium phosphate (K_2HPO_4) (Fisher Scientific, Fair Lawn, NJ) were dissolved in 1L of distilled water. The pH was adjusted to 6.8±0.1 with solution of 1 N HCl. Then, 6ml of 0.5% bromocresol purple was added as an indicator and 12g of agar (EMD Chemicals Inc., Gibbstown, NJ) was added to the mixture as a gelling solidification agent. Then, the purple mixture was heated to boiling, poured into 200ml bottles, and autoclaved at 121°C for 15 min (Dave & Shah, 1996).

2.4.3. *Lactobacilli* MRS agar

Difco *Lactobacilli* MRS agar for *Lactobacillus bulgaricus* enumeration was prepared according to the manufacturer's directions (DifcoTM, Dickinson and Company, Sparks, MD). The procedure was carried out according to Tharmaraj and Shah (2003). 55g of MRS broth powder and 15g of agar powder (EMD Chemicals Inc., Gibbstown, NJ) were dissolved in 1L of distilled water. A solution of 1 N HCl was added to adjust the pH to 5.2±0.1. This mixture was heated to boiling, poured into 200ml bottles, and autoclaved at 121°C for 15 min (Tharmaraj & Shah, 2003).

2.4.4. MRS-sorbitol agar

MRS-sorbitol agar was used to determine *Lactobacillus acidophilus* count according to Dave and Shah (1996) and Tharmaraj and Shah (2003). To prepare MRS-sorbitol agar, MRS base medium without dextrose was made. 10g of proteose peptone #3 (United States Biological. Swampscott, MA), 10g of beef extract (Becton, Dickinsos and Co., Sparks, MD), 5g of yeast extract (Becton, Dickinsos and Co., Sparks, MD), 1g of polysorbate 80 (Sigma-Aldrich Inc., St. Louis, MO), 2g of ammonium citrate (Fisher Scientific, Fair Lawn, NJ), 5g of sodium acetate, anhydrous (EMD Chemicals Inc., Gibbstown, NJ), 0.1g of magnesium sulfate, anhydrous (EMD Chemicals Inc., Gibbstown, NJ), 0.05g of manganese sulfate, monohydrate (EMD Chemicals Inc., Gibbstown, NJ), 2g of dipotassium phosphate (K_2HPO_4) (Fisher Scientific, Fair Lawn, NJ), and 15g of agar (EMD Chemicals Inc., Gibbstown, NJ) were weight and diluted in 1 L of distilled water. The mixture was heated to boiling with continuous agitation, poured into 200ml bottles, and autoclaved at 121°C for 15 min.

Then, a 10% (w/v) sorbitol solution was prepared and filter sterilized with Nalgene Membrane Filter Units (Nalge Co., Rochester, NY). The appropriate amount of sorbitol was added

to the MRS base medium to form a 10% sorbitol solution and 90% MRS base medium mixture immediately before pouring the plates (Dave & Shah, 1996; Tharmaraj & Shah, 2003).

2.5. MICROBIAL ANALYSES

2.5.1. Culture growth

Vanilla flavored camel milk yogurt was produced using the following cultures: *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus*. The growth of the cultures was determined using the pour plate technique. Yogurt in the 147.9ml cups was agitated and 11g of yogurt was pipetted into 99ml dilution bottles with 0.1% peptone water. Serial yogurt dilutions in peptone water from 10^{-1} to 10^{-6} were prepared. Dilutions from 10^{-2} to 10^{-6} were plated on *Streptococcus thermophilus* agar, MRS-sorbitol agar, and *Lactobacilli* MRS agar in duplicate. Pour plates were incubated anaerobically at 37°C for 48h in an anaerobic jar for *Lactobacillus acidophilus,* aerobically at 37°C for 24h for *Streptococcus thermophilus*, and anaerobically at 43°C for 72h in anaerobic jars for *Lactobacillus bulgaricus*. During the growth of *S. thermophilus*, the *Streptococcus thermophilus* agar changed its color from purple to yellow because of the color change of the bromocresol purple indicator due to the decreasing pH. Quebec Darkfield colony (Leica Inc., Buffalo, NY) counter was used to count the colonies grown. The counts of cultures in each of the four samples were enumerated.

2.5.2. Coliform counts

Samples of yogurt were plated on coliforms petrifilms (3M, St. Paul, MN) with violet red bile agar. The legal standard for coliforms in pasteurized milk is 10 or fewer CFU per ml. The analysis was carried out by adding 11g of yogurt into a 99ml of 0.1% peptone water. The bottles were agitated, and 1ml of the 10^{-1} dilution was plated on the labeled petrifilms. The coliform

petrifilm were incubated in aerobic conditions for 24 h at 32 °C. After 24 h, the presence of colonies with air bubble were counted.

2.5.3. Yeast and mold counts

11g of yogurt was added to a 99ml of 0.1% peptone water. The bottles were agitated, and 1ml of the 10^{-1} dilution was plated on the labeled petrifilms (3M, St. Paul, MN) for rapid yeast and mold count. Yeast and mold petrifilms were incubated for 5 days at 18±2 °C, after which growth was recorded.

2.6. STATISTICAL ANALYSIS

Data were analyzed using Proc Mixed of the SAS 9.3 program. The *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus* counts were converted to $\log_{10}$ scale prior to analyzing by SAS. Bonferroni (Dunn) t test was used to determine significant differences at $P < 0.05$ for main effects (treatment and day) and interaction effect (treatment*day).

CHAPTER 3. RESULTS AND DISCUSSION

3.1. PHYSICOCHEMICAL CHARACTERISTICS OF YOGURT

3.1.1. pH

The pH value of vanilla flavored camel milk drinking yogurt as affected by monk fruit sweetener over 42 days storage is shown in Figure 1.

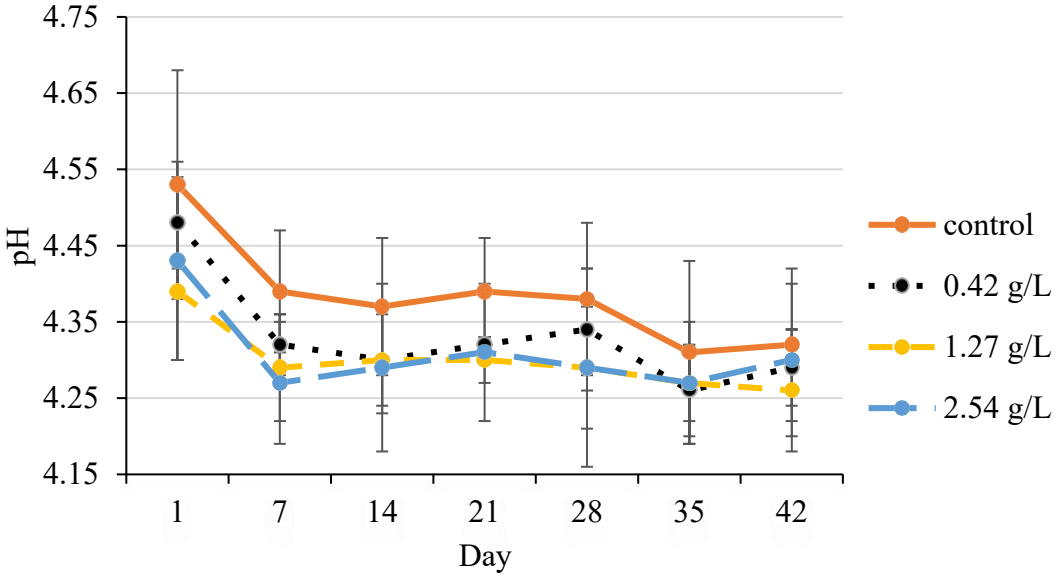

Figure 1. The pH of yogurt as affected by monk fruit sweetener over 42 days storage.

The treatment*day interaction effect was not significant ($P > 0.05$), but the treatment effect and the day effect were significant ($P < 0.05$) (Table 2). The amount of added monk fruit sweetener influenced the pH of yogurt. Control had significantly higher pH than the yogurts with 1.27, and 2.54 g/L of monk fruit sweetener, but the pH difference of samples with 1.27 and 2.54 g/L of the sweetener was not significant (Table 3). According to Kalicka et al., (2017) addition of stevia, which is like monk fruit sweetener natural nonnutritive sweetener, slightly lowered the value of yogurt pH compared to yogurt containing sucrose (Kalicka et al., 2017). Also, with the increasing dosage of stevia, the pH of the yogurt sample declined during the storage period of 21 days (Kalicka et al., 2017). The similar noticeable decrease in the pH value of yogurt with addition of

stevia extract was observed by Kim et al., (2016) during both the fermentation and storage time (Kim et al., 2016).

Table 2. Probability > F value (Pr > F) for effects of pH, titratable acidity (TA), viscosity, and color (L*, a*, b*, C*, and h*) in the yogurt containing 0, 0.42, 1.27, and 2.54 g/L of added monk fruit sweetener over storage period of 42 days.

Effect	pH	TA	Viscosity	L*	a*	b*	C*	h*
Treatment	0.0007	0.0772	0.0008	0.4451	0.0009	<0.0001	<0.0001	<0.0001
Day	<0.0001	0.6157	<0.0001	0.0033	0.0049	0.0111	0.0091	0.0198
Treatment *day	0.9983	0.9951	0.9998	0.9744	0.9846	0.4876	0.4991	0.9997

Table 3. Means as separated by the Bonferroni (Dunn) t test for pH of yogurt affected by different concentrations of monk fruit sweetener.

Monk fruit sweetener	pH
control	4.38 ± 0.10^{A}
0.42 g/L	4.33 ± 0.05^{AB}
1.27 g/L	4.30 ± 0.07^{B}
2.54 g/L	4.31 ± 0.10^{B}

^{AB}means with the same letter are not significantly different.

An overall pH decline occurred during the storage period of 42 day (Table 4). The pH value was the highest at the first day of storage, noticeably decreased by the seventh day, and remained stable during the rest period of storage (Table 4). The decrease of the pH values is explained by the continued growth of lactic acid bacteria, especially if more than one culture is used for yogurt preparation (Kailasapathy et al., 2008).

Table 4. Means as separated by the Bonferroni (Dunn) t test for pH of yogurt over 42 days.

Day	pH
1	4.46 ± 0.11^{A}
7	4.32 ± 0.07^{B}
14	4.32 ± 0.08^{B}
21	4.33 ± 0.05^{B}
28	4.33 ± 0.10^{B}
35	4.28 ± 0.08^{B}
42	4.29 ± 0.08^{B}

^{AB}means with the same letter are not significantly different.

3.1.2. TITRATABLE ACIDITY (TA)

The TA value of vanilla flavored camel milk drinking yogurt as affected by monk fruit sweetener over 42 days storage is shown in Figure 2.

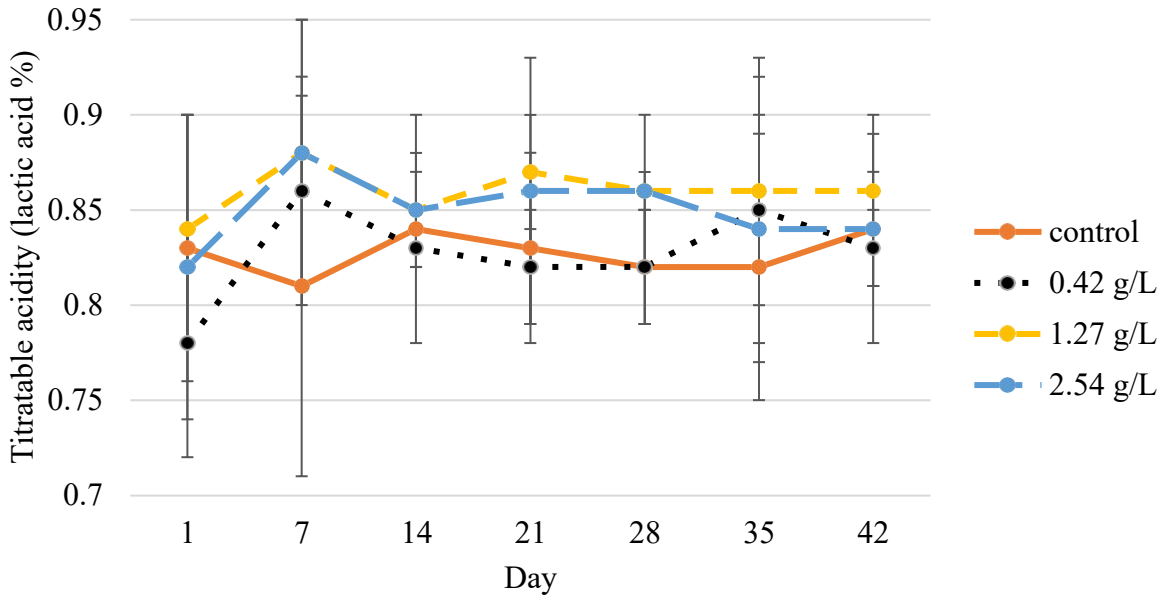

Figure 2. The titratable acidity (TA) of yogurt as affected by monk fruit sweetener over 42 days storage.

The treatment*day interaction effect, the treatment effect, and the day effect were not significant ($P > 0.05$) (Table 2). The amount of added monk fruit sweetener was not statistically significant for the overall change of yogurts' titratable acidity (Table 5). No influence on the TA was found for the nutritive sweetener erythritol (Costa et al., 2019). Erythritol like monk fruit sweetener decreased the pH of yogurt samples and did not affect the titratable acidity (Costa et al., 2019). According to these authors, erythritol inhibits the growth of lactic acid bacteria resulting in the lower amount of produced lactic acid (Costa et al., 2019).

Table 5. Means as separated by the Bonferroni (Dunn) t test for TA of yogurt affected by different concentrations of monk fruit sweetener.

Monk fruit sweetener	TA
control	0.83 ± 0.06^{A}
0.42 g/L	0.83 ± 0.04^{A}
1.27 g/L	0.86 ± 0.04^{A}
2.54 g/L	0.85 ± 0.05^{A}

^{AB}means with the same letter are not significantly different.

The TA values for all the four treatments remained stable (Table 5). The increase of the TA values is linked to the decrease of the pH values and depends on the growth rate of starter cultures (Al-Kadamany et al., 2003).

Table 6. Means as separated by the Bonferroni (Dunn) t test for pH of yogurt over 42 days.

Day	TA
1	0.82 ± 0.07^{A}
7	0.86 ± 0.08^{A}
14	0.85 ± 0.03^{A}
21	0.85 ± 0.05^{A}
28	0.84 ± 0.03^{A}
35	0.84 ± 0.07^{A}
42	0.84 ± 0.04^{A}

^{AB}means with the same letter are not significantly different.

3.1.3. VISCOSITY

The viscosity of vanilla flavored camel milk drinking yogurt as affected by monk fruit sweetener over 42 days storage is shown in Figure 3.

The treatment*day interaction effect was not significant ($P > 0.05$), but the treatment effect and the day effect were significant ($P < 0.05$) (Table 2). The viscosity of yogurts increased with the increased dosage of monk fruit sweetener. Viscosity of the control sample was significantly lower than the samples with 1.27 and 2.54 g/L of the sweetener and no different from the sample with 0.42 g/L of the sweetener (Table 7). According to Hernández-Rodriguez et al., (2017), the addition of sweeteners into yogurt may increase viscosity due to hydrophobic interactions. Thus, stevia influenced rheological parameters of yogurt (Hernández-Rodríguez et al., 2017). Similar

results with increasing viscosity, firmness, and consistency of yogurt with stevia addition were observed by Costa et al., (2019). Also, the authors noted that the interaction between stevia and milk proteins weakened by the end of storage affecting the texture of yogurt (Costa et al., 2019).

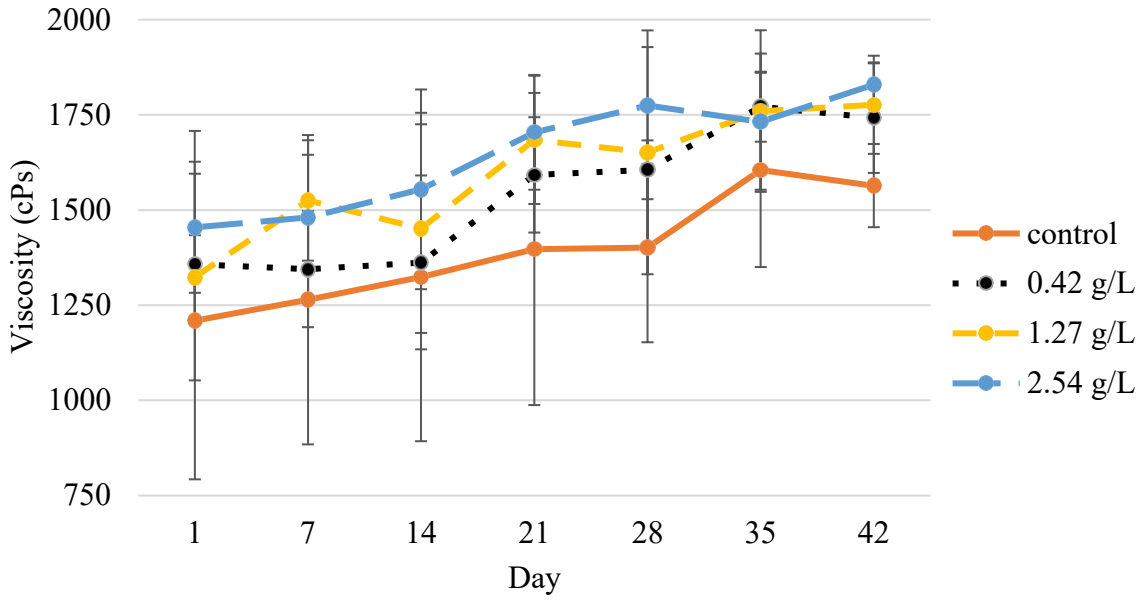

Figure 3. Viscosity of yogurt as affected by monk fruit sweetener over 42 days storage.

Table 7. Means as separated by the Bonferroni (Dunn) t test for viscosity of yogurt affected by different concentrations of monk fruit sweetener.

Monk fruit sweetener	Viscosity
control	1394.98±321.83^{B}
0.42 g/L	1539.33±131.81^{AB}
1.27 g/L	1595.78±219.20^{A}
2.54 g/L	1646.92±181.50^{A}

^{AB}means with the same letter are not significantly different.

Viscosity of all yogurt samples increased during the storage period (Table 8). As storage days increased, viscosity increased. Viscosity at the first day was not significantly different than at days 7 and 14 but was different than those at days 21, 28, 35, and 42 (Table 8). In the second week, a slight whey separation occurred, which slowly continued throughout the rest of the storage period. The phenomena of syneresis is explained with the increased association of casein and rearrangement of the protein network (Hernández-Rodríguez et al., 2017). The increase in

viscosity is explained by the process of post-acidification, which is confirmed by the pH decline during the storage. Lactic acid produced by the starter cultures increases acidification of yogurt, which leads to the strengthen of protein network and whey separation. Also, some strains of lactic acid bacteria may form exopolysaccharides that improve the viscosity of yogurt (Saint-Eve et al., 2008).

Table 8. Means as separated by the Bonferroni (Dunn) t test for viscosity of yogurt over 42 days.

Day	Viscosity
1	1336.30 ± 254.38^{C}
7	1403.54 ± 226.97^{BC}
14	1422.68 ± 299.04^{BC}
21	1594.37 ± 220.28^{AB}
28	1608.02 ± 200.00^{AB}
35	1716.95 ± 184.60^{A}
42	1727.92 ± 109.85^{A}

^{AB}means with the same letter are not significantly different.

3.1.4. COLOR

3.1.4.1. L* value (lightness – darkness)

The L* value represents the lightness-darkness and has a numerical range from 0 (black) to 100 (white). The L*, a*, and b* characteristics form a three-dimensional space where the a* and b* values represent XY axis with the L* passing through the center of the XY intersection (D. Becker, 2016). The L* characteristic of color of vanilla flavored camel milk drinking yogurt as affected by monk fruit sweetener over 42 days storage is shown in Figure 4.

The treatment*day interaction effect and the treatment effect were not significant ($P > 0.05$), but the day effect was significant ($P < 0.05$) (Table 2). The amount of the sweetener did not affect the L* values, and the control yogurts were not different from yogurts with any amount of the sweetener (Table 9). The similar results of addition of different sweeteners not affecting color of yogurts were obtained by Costa et al., (2019). Sucralose, xylitol, stevia, erythritol, erythritol with oligofructose, and erythritol with polydextrose had the similar L* values to the samples with

sucrose; however, the measurements of L* values decreased to the 28th day of storage (Costa et al., 2019).

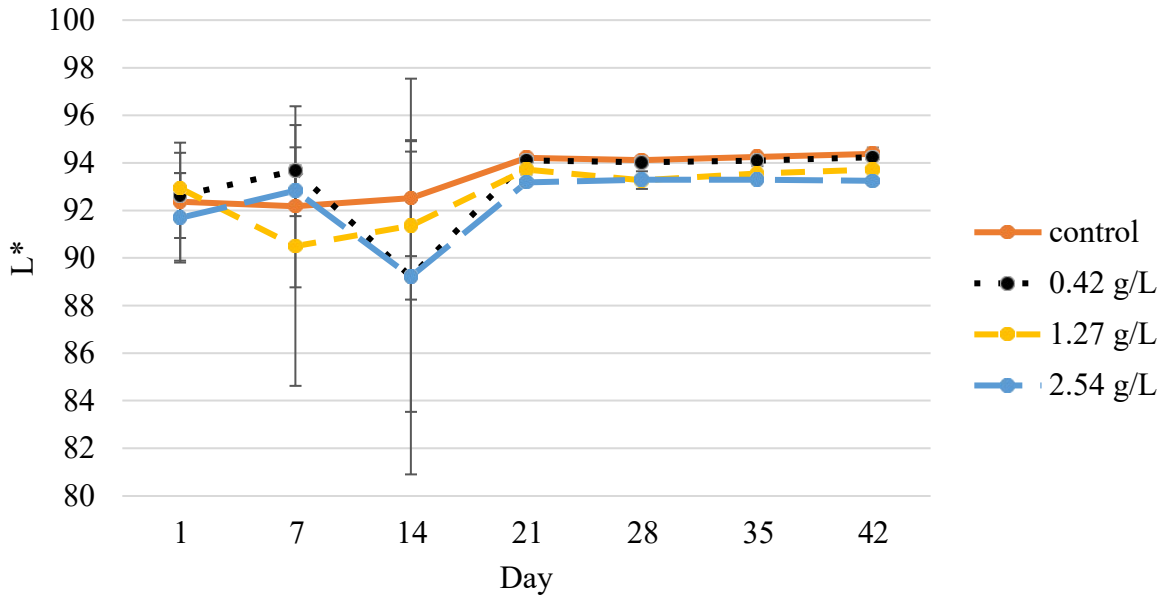

Figure 4. The L* characteristic of color of yogurt as affected by monk fruit sweetener over 42 days storage.

Table 9. Means as separated by the Bonferroni (Dunn) t test for L* characteristic of color of yogurt affected by different concentrations of monk fruit sweetener.

Monk fruit sweetener	L*
control	93.43±1.27^{A}
0.42 g/L	93.14±1.69^{A}
1.27 g/L	92.72±1.57^{A}
2.54 g/L	92.39±1.28^{A}

^{AB}means with the same letter are not significantly different.

L* values of yogurt samples at day 14 was significantly lower than the L* values at days 21, 28, 35, and 42 (Table 10). The noticeable decrease of lightness in samples on the 14th day of storage may be explained by the increased number of *L. acidophilus* in all treatments. Thus, inoculation of yogurts with 2.33g of *L. acidophilus* per 100g of yogurt significantly decreased the L* value compared to samples inoculated with 0, 0.0239, and 0,238g of *L. acidophilus* per 100g of yogurt (Olson & Aryana, 2008). Also, Costa et al., (2015) suggested that milk composition

affected the change in L* value. Thus, the L* value of goat yogurt increased during the storage compared to samples with cow's milk (Costa et al., 2015)

Table 10. Means as separated by the Bonferroni (Dunn) t test for L* characteristic of color of yogurt over 42 days.

Day	L*
1	92.41 ± 1.70^{AB}
7	92.30 ± 2.83^{AB}
14	90.58 ± 4.89^{B}
21	93.81 ± 0.13^{A}
28	93.68 ± 0.18^{A}
35	93.80 ± 0.16^{A}
42	93.90 ± 0.28^{A}

^{AB}means with the same letter are not significantly different.

3.1.4.2. a* value (red to green axis)

The a* value represents the color change from red to green. Red colors denote as positive numbers, and green colors are negative numbers. The a* characteristic of color of vanilla flavored camel milk yogurt as affected by monk fruit sweetener over 42 days storage is shown in Figure 5.

The treatment*day interaction effect was not significant ($P > 0.05$), but the day effect and the treatment effect were significant ($P < 0.05$) (Table 2). The a* values of control yogurt and yogurt with 0.42 g/L of the sweetener were significantly lower than the sample with 2.54 g/L, but they did not differ from yogurt with 1.27 g/L of monk fruit sweetener (Table 11). According to Costa et al., (2015), with the increased amount of the sweetener, the color shifted towards redness. Increase of the a* value can be explained by the addition of the plant ingredient, which tend to increase the redness of food products (Costa et al., 2015).

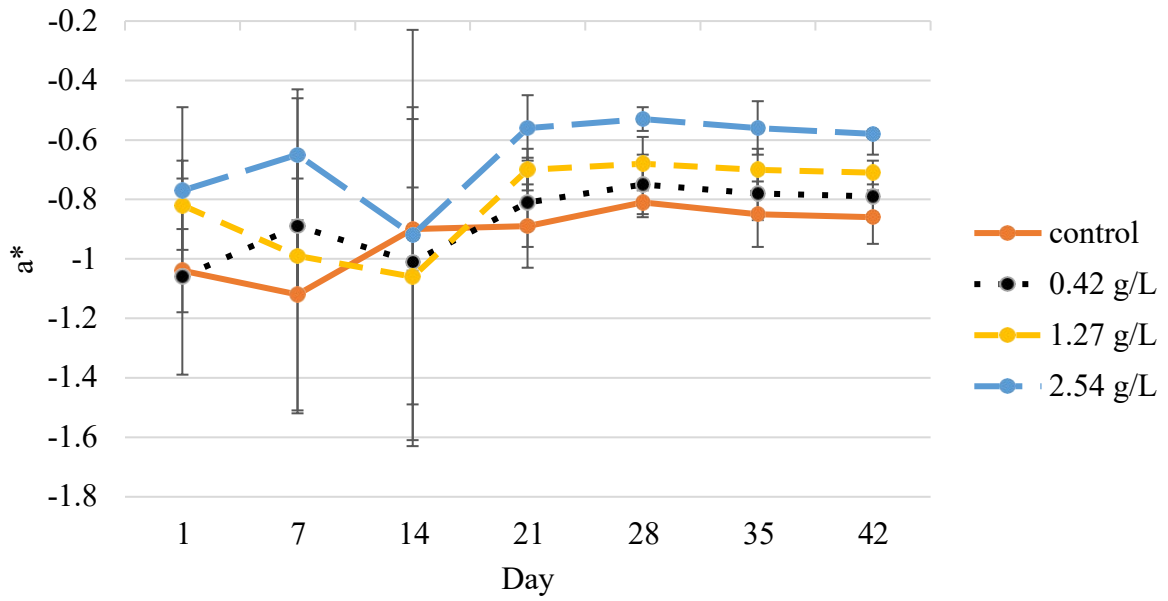

Figure 5. The a* characteristic of color of yogurt as affected by monk fruit sweetener over 42 days storage.

Table 11. Means as separated by the Bonferroni (Dunn) t test for a* characteristic of color of yogurt affected by different concentrations of monk fruit sweetener.

Monk fruit sweetener	a*
control	-0.93±0.15^{B}
0.42 g/L	-0.87±0.21^{B}
1.27 g/L	-0.81±0.22^{AB}
2.54 g/L	-0.65±0.21^{A}

^{AB}means with the same letter are not significantly different.

Only samples at the 14th and 28th days of analysis were statistically different from each other; samples at other storage days were not significantly different (Table 12). The a* value represents the color change from red to green, and, according to the results, the greenness in yogurts decreased. This phenomenon is explained by the gel stirring and pH drop during the storage. Gel stirring and decrease in pH results in the alteration of tissue structure and possible leakage of natural pigments to the yogurt matrix (Costa et al., 2015). The increase of the a* value may also be influenced by the pasteurization, as temperature treatment of milk destabilizes casein micelles, as well as by the drop of the pH, which contribute to the transfer of calcium phosphate

from the protein matrix to the whey enhancing the porosity of the casein micelles (García-Pérez et al., 2005).

Table 12. Means as separated by the Bonferroni (Dunn) t test for a* characteristic of color of yogurt over 42 days.

Day	a*
1	-0.92±0.23^{AB}
7	-0.91±0.34^{AB}
14	-0.97±0.47^{B}
21	-0.74±0.12^{AB}
28	-0.69±0.07^{A}
35	-0.72±0.09^{AB}
42	-0.73±0.07^{AB}

^{AB}means with the same letter are not significantly different.

3.1.4.3. b* value (yellow to blue axis)

The b* values relate to the range of colors from blue to yellow. Positive numbers are used to represent yellow colors, and negative numbers are for blue hues. The b* characteristic ofcolor of vanilla flavored camel milk yogurt as affected by monk fruit sweetener over 42 days storage is shown in Figure 6. The treatment*day interaction effect was not significant (P > 0.05), but the day effect and the treatment effect were significant (P < 0.05) (Table 2).

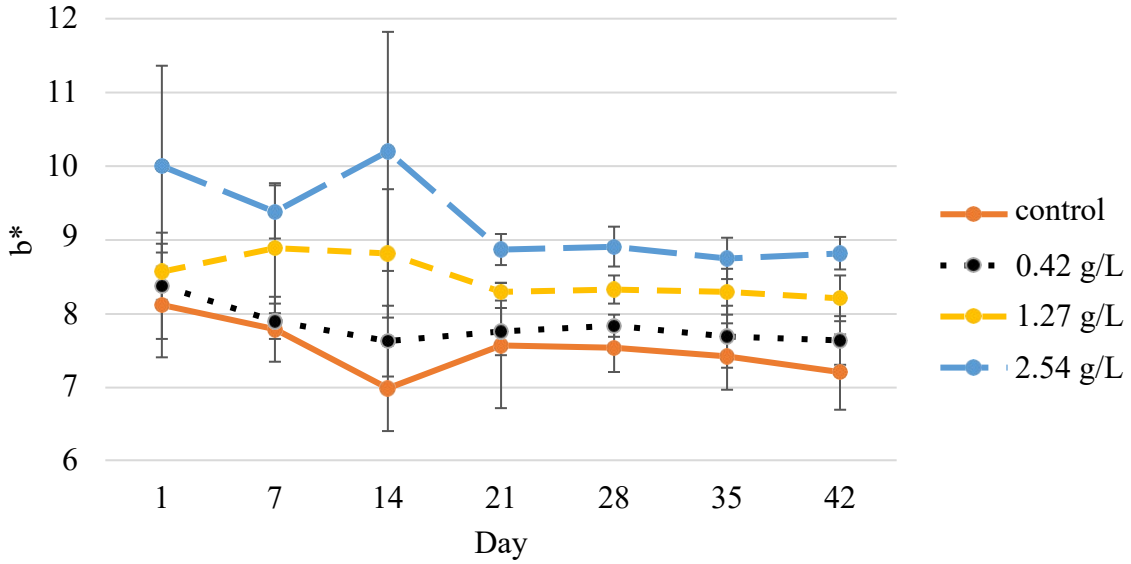

Figure 6. b* characteristic of color of yogurt as affected by monk fruit sweetener over 42 days storage.

Control and 0.42 g/L had the lowest b* value while yogurts with 2.54 g/L had the highest b* value (Table 13). The 1.27 g/L had values higher than control and 0.42 g/L but lower than 2.54 g/L (Table 13). Thus, with the increased amount of the sweetener, the color shifted towards yellowness. The decrease of the b* value is also associated with the plant source of the sweetener (Costa et al., 2015).

Table 13. Means as separated by the Bonferroni (Dunn) t test for b* characteristic of color of yogurt affected by different concentrations of monk fruit sweetener.

Monk fruit sweetener	b*
control	7.52 ± 0.55^{C}
0.42 g/L	7.83 ± 0.38^{C}
1.27 g/L	8.49 ± 0.44^{B}
2.54 g/L	9.28 ± 0.62^{A}

^{AB}means with the same letter are not significantly different.

Samples at the first day had higher b* values than the samples at the 42nd day of storage (Table 14). Comparing yogurt at day 1 and day 42, the yellowness in yogurts decreased. Costa et al., (2015) suggested that the b* parameter could decrease due to the level of carotenoids in milk and lipid oxidation.

Table 14. Means as separated by the Bonferroni (Dunn) t test for b* characteristic of color of yogurt over 42 days.

Day	b*
1	8.77 ± 0.79^{A}
7	8.49 ± 0.48^{AB}
14	8.41 ± 0.89^{AB}
21	8.12 ± 0.38^{AB}
28	8.15 ± 0.24^{AB}
35	8.04 ± 0.37^{AB}
42	7.97 ± 0.34^{B}

^{AB}means with the same letter are not significantly different.

3.1.4.4. C* value (chroma)

The C* value or chroma relates to the length from the center to the sample measurement (Nielsen, 2014). The C* characteristic of color of vanilla flavored camel milk yogurt as affected by monk fruit sweetener over 42 days storage is shown in Figure 7.

The treatment*day interaction effect was not significant (P > 0.05), but the day effect and the treatment effect were significant (P < 0.05) (Table 2). Monk fruit sweetener affected the C* value. Control and 0.42 g/L samples were similar to each other but had the lowest values followed by 1.27 g/L, which was significantly higher (Table 15). The 2.54 g/L sample had the highest C* value (Table 15). Rad et al., (2019) studied the color change in chocolate milk and reported decrease chroma values with increased amount of sweetener. The drop of the C* value was observed with substitution of sucrose with polyols such as isomalt, xylitol, and maltitol in milk chocolate (Rad et al., 2019). The authors linked the effect with different solubilities of sweeteners and light scattering effect.

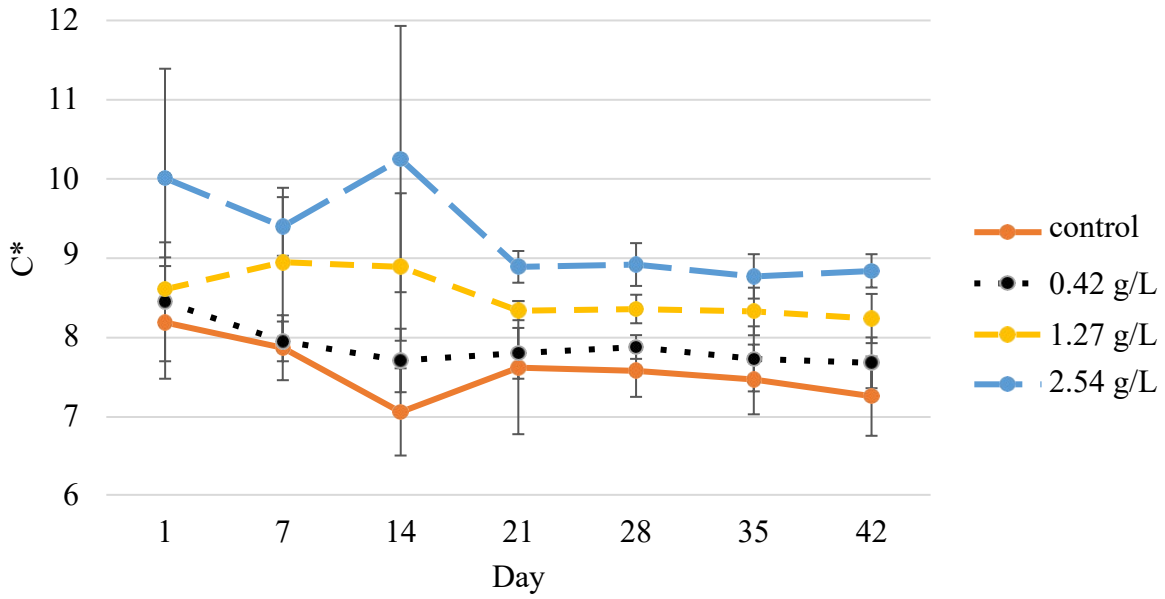

Figure 7. The C* characteristic of color of yogurt as affected by monk fruit sweetener over 42 days storage.

At the 42nd day of storage, sample were significantly lower in chroma than those the first day (Table 16). The decrease of the C* value indicates the degradation of the color intensity during the storage at 42 days. Vargas et al., (2008) suggested that the decrease in the C* value may be

due to the different levels of gel opacity. Gel opacity depends on the ratio of caseins in milk and the degree of their aggregation. (Vargas et al., 2008).

Table 15. Means as separated by the Bonferroni (Dunn) t test for C* characteristic of color of yogurt affected by different concentrations of monk fruit sweetener.

Monk fruit sweetener	C*
control	7.58 ± 0.54^{C}
0.42 g/L	7.89 ± 0.37^{C}
1.27 g/L	8.53 ± 0.45^{B}
2.54 g/L	9.30 ± 0.63^{A}

^{AB}means with the same letter are not significantly different.

Table 16. Means as separated by the Bonferroni (Dunn) t test for C* characteristic of color of yogurt over 42 days.

Day	C*
1	8.82 ± 0.81^{A}
7	8.55 ± 0.49^{AB}
14	8.48 ± 0.89^{AB}
21	8.16 ± 0.37^{AB}
28	8.19 ± 0.23^{AB}
35	8.08 ± 0.36^{AB}
42	8.01 ± 0.34^{B}

^{AB}means with the same letter are not significantly different.

3.1.4.5. h* value (hue angle)

The h* value is hue angle, and it represents the angle from the start of the positive a* value to the sample measurement (Nielsen, 2014). The h* characteristic of color of vanilla flavored camel milk yogurt as affected by monk fruit sweetener over 42 days storage is shown in Figure 8.

The treatment*day interaction effect was not significant ($P > 0.05$), but the day effect and the treatment effect were significant ($P < 0.05$) (Table 2). The incorporation of monk fruit sweetener affected the h* value of yogurts. Control yogurts had significantly higher h* value than 1.27 and 2.54 g/L (Table 17). Samples with 1.27 g/L had significantly higher h* value than 2.54 g/L, which had significantly the lowest value (Table 17). The h* value represents the ratio of b* to a*; therefore, with decrease of the b* value and increase of the a* value, the overall h* value of the samples decreased.

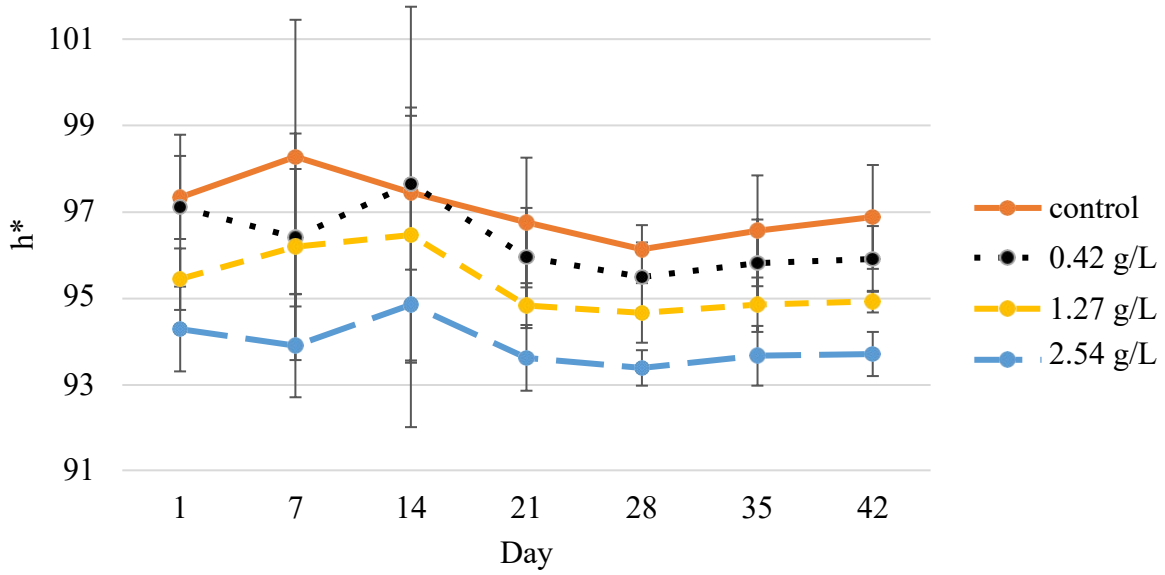

Figure 8. The h* characteristic of color of yogurt as affected by monk fruit sweetener over 42 days storage.

Table 17. Means as separated by the Bonferroni (Dunn) t test for h* characteristic of color of yogurt affected by different concentrations of monk fruit sweetener.

Monk fruit sweetener	h*
control	97.06±1.49^{A}
0.42 g/L	96.34±1.58^{AB}
1.27 g/L	95.35±1.20^{B}
2.54 g/L	93.93±1.05^{C}

^{AB}means with the same letter are not significantly different.

The h* value of all yogurt samples did not change significantly during the storage period (Table 18). Only samples at day 14 were significantly higher than samples at day 28 (Table 18). The results could be attributed to the whey separation of yogurts.

Table 18. Means as separated by the Bonferroni (Dunn) t test for h* characteristic of color of yogurt over 42 days.

Day	h*
1	96.05±1.08^{AB}
7	96.20±2.14^{AB}
14	96.61±2.91^{A}
21	95.30±0.98^{AB}
28	94.93±0.62^{B}
35	95.23±0.90^{AB}
42	95.37±0.68^{AB}

^{AB}means with the same letter are not significantly different.

3.2. BACTERIAL GROWTH

3.2.1. *Streptococcus thermophilus*

The growth of *Streptococcus thermophilus* as affected by the addition of monk fruit sweetener during the storage is shown in Figure 9.

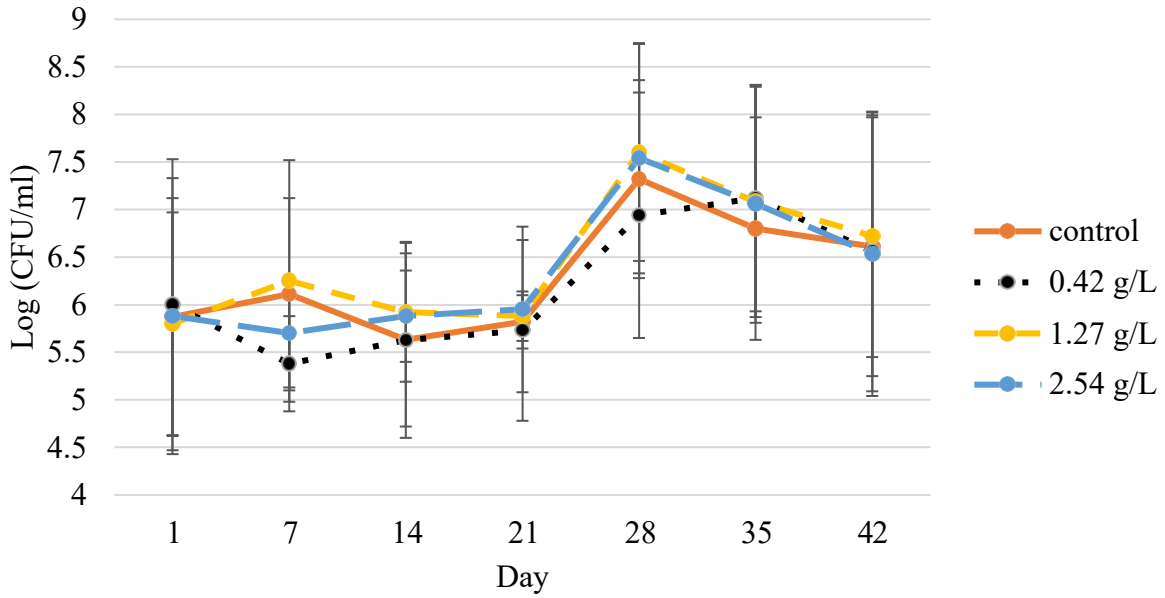

Figure 9. Growth of *Streptococcus thermophilus* in yogurt as affected by monk fruit sweetener over 42 days storage.

The treatment*day interaction effect and the treatment effect were not significant (P > 0.05), but the day effect was significant (P < 0.05) (Table 19).

Table 19. Probability > F value (Pr > F) for effects of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus* in the yogurt containing 0, 0.42, 1.27, and 2.54 g/L of added monk fruit sweetener over storage period of 42 days.

Effect	*Streptococcus thermophilus*	*Lactobacillus bulgaricus*	*Lactobacillus acidophilus*
Treatment	0.8568	0.9188	0.9034
Day	0.0005	<0.0001	<0.0001
Treatment*day	1.0000	0.9809	0.9996

Overall, the addition of monk fruit sweetener did not have an influence on the growth of *Streptococcus thermophilus* (Tables 19 and 20). Cultures of *Streptococcus thermophilus* show a high level of survival in the presence of different concentrations of totals sugars (Vinderola et al., 2002). These authors also showed that incorporation of acesulfame and aspartame at the dosages normally used in the dairy industry did not interfere with the growth of *Streptococcus thermophilus* (Vinderola et al., 2002). In the study conducted by Abdel-Hamid et al., (2020) the effect of water extract of *Siraitia grosvenorii* (monk fruit) fruits on the growth of starter cutlers in the buffalo yogurt was shown. The authors did not obtain the influence of the monk fruit water extract on the count of *Streptococcus thermophilus* (Abdel-Hamid et al., 2020).

Table 20. Means as separated by the Bonferroni (Dunn) t test for the counts of *Streptococcus thermophilus* in yogurt affected by different concentrations of monk fruit sweetener.

Monk fruit sweetener	*S. thermophilus*
control	6.31 ± 1.00^{A}
0.42 g/L	6.19 ± 1.14^{A}
1.27 g/L	6.47 ± 1.01^{A}
2.54 g/L	6.36 ± 1.05^{A}

^{AB}means with the same letter are not significantly different.

Log_{10} counts at day 1, 7, 14, and 21 were not significantly different from each other but were significantly lower compared to log_{10} counts at day 28 (Table 21). After peaking at day 28 log_{10} counts remained the same until end of storage at 42 days (Table 21).

Species of *Streptococcus thermophilus* usually predominates over other starter cultures in yogurt (Beal et al., 1999). The increase in the count of *Streptococcus thermophilus* at day 28 compared to the day 1 can be explained by the symbiotic growth with *Lactobacillus bulgaricus,* which is more proteolytic (Shihata & Shah, 2002). Birollo et al., (2000) reported that the use of the dominant *Streptococcus thermophilus* species resulted in the total increase of the viable cell counts at the end of storage. The decrease in *Streptococcus thermophilus* log_{10} on the 42^{nd} compared to the 28^{th} day was possibly due to the continuous pH decrease (Birollo et al., 2000).

Shori (2013) reported increase in the count of *Streptococcus thermophilus* by the 14th day of storage that decreased to the 21st day of storage but was higher than initial bacterial count. The author attributed the decrease in the count of *Streptococcus thermophilus* between 14th and 21st days with increase in pH and accumulation of organic acids (Shori, 2013). Also, *Streptococcus thermophilus* strains are sensitive to the presence of acetaldehyde and diacetyl. These compounds are produced during the lactic acid fermentation and may be the reason for the reduction in the bacterial count (Vinderola et al., 2002).

Table 21. Means as separated by the Bonferroni (Dunn) t test for the counts of *Streptococcus thermophilus* in yogurt over 42 days.

Day	*S. thermophilus*
1	5.89 ± 1.35^{B}
7	5.86 ± 0.84^{B}
14	5.77 ± 0.79^{B}
21	5.85 ± 0.59^{B}
28	7.35 ± 1.17^{A}
35	7.01 ± 1.21^{AB}
42	6.61 ± 1.40^{AB}

^{AB}means with the same letter are not significantly different.

3.2.2. *Lactobacillus bulgaricus*

The growth of *Lactobacillus bulgaricus* in vanilla flavored camel milk yogurt as affected by monk fruit sweetener over 42 days storage is shown in Figure 10.

The treatment*day interaction effect and the treatment effect were not significant ($P > 0.05$), but the day effect was significant ($P < 0.05$) (Table 19). Overall, the addition of monk fruit sweetener did not have an influence on the growth of *Lactobacillus bulgaricus* (Table 22). Some sweeteners may decrease the growth of *Lactobacillus bulgaricus*. Saccharose and lactose at concentrations 15 and 20% were inhibitory to some strains of *Lactobacillus bulgaricus*, but aspartame even at increased concentration (0.12%) did not affect the growth of the bacteria (Vinderola et al., 2002). The decrease of *Lactobacillus bulgaricus* viability in the presence of

sweeteners can be explained by the increased osmotic pressure produced by the sweeteners (Birollo et al., 2000). Apparently, it is not the case for monk fruit sweetener. Manca de Nadra et al., (2007), also showed that *Lactobacillus bulgaricus* strains can grow in the presence of artificial noncaloric sweeteners such as cyclamate and aspartame at concentrations normally present in yogurt; however, saccharin had an inhibition effect on the bacteria (Manca de Nadra et al., 2007). Artificial sweeteners were utilized as sources of energy and carbon when the amount of glucose was not sufficient for growth (Manca de Nadra et al., 2007).

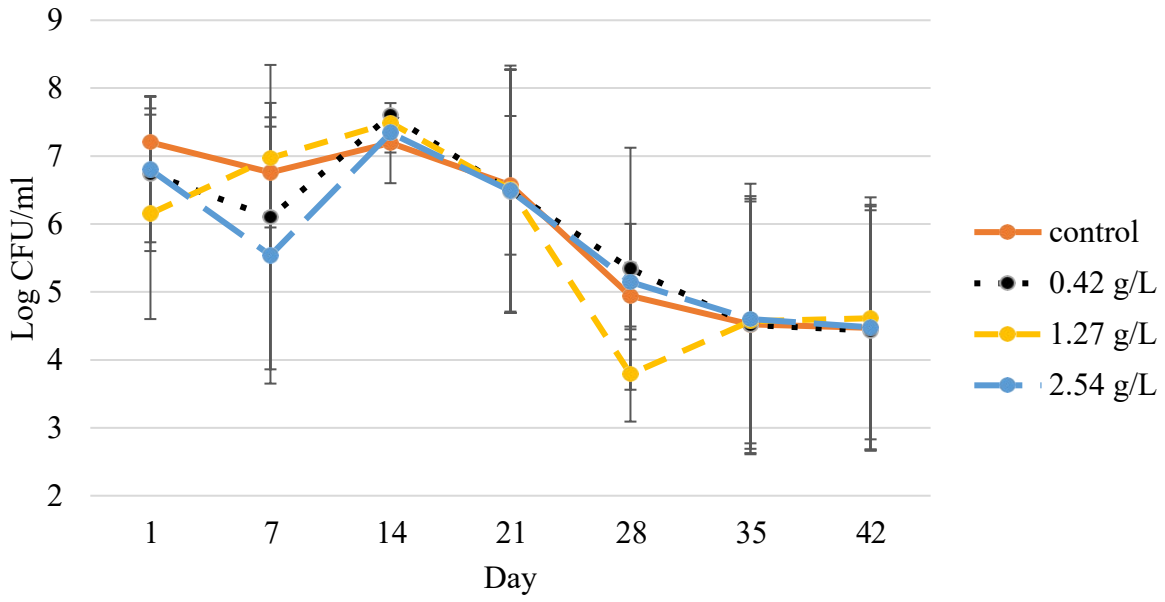

Figure 10. Growth of *Lactobacillus bulgaricus* in yogurt as affected by monk fruit sweetener over 42 days storage.

Table 22. Means as separated by the Bonferroni (Dunn) t test for the counts of *Lactobacillus bulgaricus* in yogurt affected by different concentrations of monk fruit sweetener.

Monk fruit sweetener	*L. bulgaricus*
control	5.95±1.00^{A}
0.42 g/L	5.89±1.51^{A}
1.27 g/L	5.73±1.23^{A}
2.54 g/L	5.77±1.38^{A}

^{AB}means with the same letter are not significantly different.

Overall, the $\log_{10}$ of the bacteria decreased by the end of storage compared to the first day. The $\log_{10}$ counts were similar for the first, 7th, 14th, and 21st days, which were significantly higher than at days 28, 35, and 42 (Table 23). According to Attia et al., (2001), camel milk is a less favorable medium for the growth of the *Lactobacillus* species. The authors reported that the growth rate of lactic acid bacteria was lower in camel milk than in cow's milk; therefore, the final count of the bacteria at the end of fermentation was lower. The noticeable decline in the bacterial growth during fermentation was explained by the presence of inhibitors in camel milk such as lactoferrin, IgG, and lysozyme (Attia et al., 2001). The decrease in the number of viable cells during storage is explained by the post-acidification, increase in the amount of hydrogen peroxide, and the presence of active antibacterial compounds in camel milk (Shori, 2013).

Table 23. Means as separated by the Bonferroni (Dunn) t test for the counts of *Lactobacillus bulgaricus* over 42 days.

Day	*L. bulgaricus*
1	6.72 ± 1.04^{A}
7	6.34 ± 1.44^{A}
14	7.41 ± 0.26^{A}
21	6.51 ± 1.60^{A}
28	4.80 ± 0.96^{B}
35	4.55 ± 1.88^{B}
42	4.50 ± 1.79^{B}

^{AB}means with the same letter are not significantly different.

3.2.3. *Lactobacillus acidophilus*

The growth of *Lactobacillus acidophilus* in vanilla flavored camel milk yogurt as affected by monk fruit sweetener over 42 days storage is shown in Figure 11.

The treatment*day interaction effect and the treatment effect were not significant ($P > 0.05$), but the day effect was significant ($P < 0.05$) (Table 19). Overall, the addition of monk fruit sweetener did not have an influence on the growth of *Lactobacillus acidophilus* (Tables 19 and 24). Probiotic bacteria are less sensitive to the presence of sugars (Vinderola et al., 2002). Thus,

the addition of honey to goat milk increased *Lactobacillus acidophilus* counts by 1 $\log_{10}$ CFU/g, and the bacterial count was above 6 $\log_{10}$ CFU/g during the storage for 28 days (Machado et al., 2017). In the study by Esmerino et al., (2013), 0.088% aspartame, 0.024% sucralose, 0.152% stevia, 0.0025% neotame, and 15.2% sucrose were added to the fermented Suisse cheese, and the growth of probiotic *Lactobacillus acidophilus* was determined. No effect on the growth rate of the bacteria was obtained during the storage for 28 days (Esmerino et al., 2013). Davoodi et al., (2016) studied the influence of glucose, sucrose, stevia leaf and stevioside in concentrations of 20, 10, 5, and 2.5 g/l on the growth of probiotic species from the Lactobacillus genera. The results showed that cultures could grow with the presence of these sweeteners, emphasizing that natural zero caloric sweeteners like stevia and stevioside were utilized by the microorganisms (Davoodi et al., 2016).

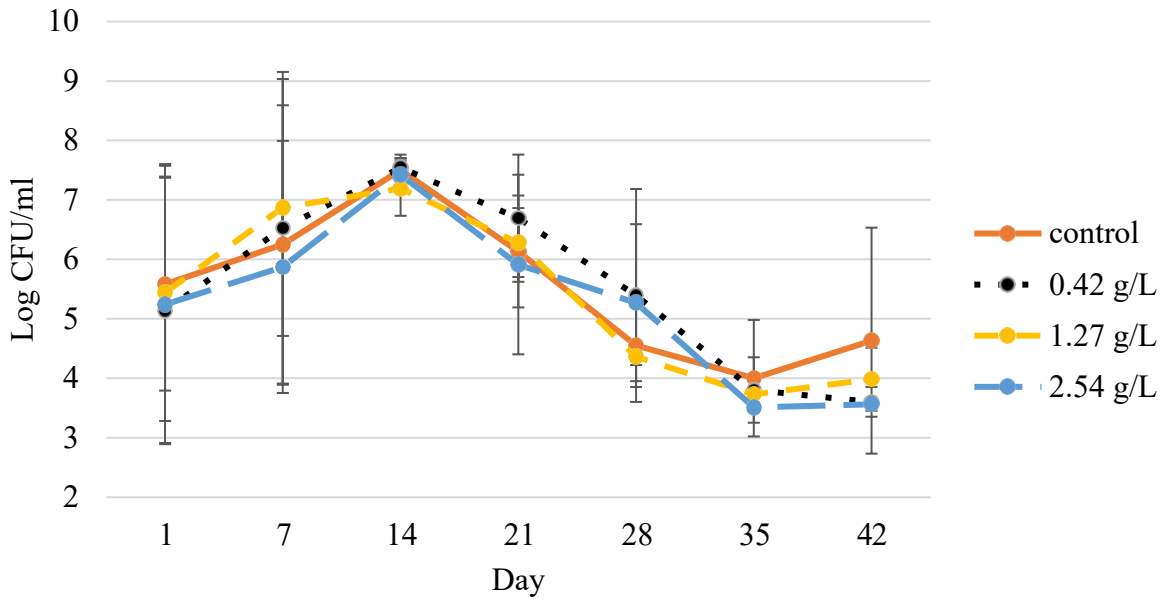

Figure 11. Growth of *Lactobacillus acidophilus* in yogurt as affected by monk fruit sweetener over 42 days storage.

Table 24. Means as separated by the Bonferroni (Dunn) t test for the counts of *Lactobacillus acidophilus* in yogurt affected by different concentrations of monk fruit sweetener.

Monk fruit sweetener	*L. acidophilus*
control	5.52 ± 1.27^{A}
0.42 g/L	5.53 ± 1.24^{A}
1.27 g/L	5.41 ± 0.87^{A}
2.54 g/L	5.25 ± 1.10^{A}

^{AB}means with the same letter are not significantly different.

The counts increased at day 14 compared to day 1 and then decreased at days 28, 35, and 42 of storage (Table 25). The dramatic decrease in the count of probiotic culture *Lactobacillus acidophilus* is mainly explained by the presence of inhibitory factors in camel milk such as lysozyme, lactoferrin, lactoperoxidase, IgG, and IgA (Attia et al., 2001). The decrease in pH during storage probably was not the main factor in the decrease of viable cells. According to Donkor et al., (2007), *Lactobacillus acidophilus* are more acid tolerant than *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. However, acid tolerance of *Lactobacillus* species is strain dependent (Donkor et al., 2007). Also, *Lactobacillus bulgaricus* may cause antagonistic activity on the probiotic growth due to the formation of hydrogen peroxide that can disrupt the cell walls of bacteria (Mani-López et al., 2014).

Table 25. Means as separated by the Bonferroni (Dunn) t test for the counts of *Lactobacillus acidophilus* in yogurt over 42 days.

Day	*L. acidophilus*
1	5.35 ± 2.13^{BC}
7	6.38 ± 2.31^{AB}
14	7.41 ± 0.29^{A}
21	6.25 ± 1.03^{AB}
28	4.89 ± 0.99^{BC}
35	3.76 ± 0.42^{C}
42	3.94 ± 0.69^{C}

^{AB}means with the same letter are not significantly different.

3.2.4. Coliform, yeast, and mold

During the storage for 42 day, coliforms, yeast, and mold were not obtained in any of the yogurt sample. This suggests that the heat treatment was carried out properly, and the storage conditions prevented the growth of yeast and mold.

CHAPTER 4. CONCLUSION

During the yogurt storage, the presence of monk fruit sweetener significantly decreased the pH and increased the viscosity of yogurts. The color characteristics a*, b*, C*, and h* were also significantly affected by the addition of the sweetener. The sweetener did not influence the titratable acidity and the L* value of yogurt samples. Increase in the amount of monk fruit sweetener did not significantly influence counts of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus*. Increase in storage days significantly influenced counts of cultures. Counts of *Streptococcus thermophilus* reached maximum at day 28 and then were not significantly different. The counts of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* significantly decreased over the storage days. Monk fruit sweetener can be used as a zero caloric health beneficial sweetener in the camel milk yogurt production.

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