10-27-2022

Influence of Hesperidin on the Physico-Chemical, Microbiological, and Sensory Characteristics of Frozen Yogurt

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INFLUENCE OF HESPERIDIN ON THE PHYSICO-CHEMICAL, MICROBIOLOGICAL, AND SENSORY CHARACTERISTICS OF FROZEN YOGURT

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Nutrition and Food Sciences

by
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B.S., Escuela Agrícola Panamericana, Zamorano, 2016
December 2022

ACKNOWLEDGMENTS

Firstly, I would like to thank God and the Good Mother who gave me the opportunity to start my master´s program at Louisiana State University. Then, my parents who deserve my total admiration and gratitude for their continued support in my decisions and sharing of their advice with a true honesty.

I want to express my gratitude to the AgCenter and Dr. William Richardson, who was leading the college of Agriculture in 2020, for their financial support and the opportunity to demonstrate my potential and skills in the Graduate School. I want to express my admiration to Dr. Kayanush Aryana who is my main advisor and the research associate Dr. Doug Olson for his advice. Additionally, I want to thank my committee members Dr. Witoon Prinyawiwatkul and Dr. Charles Boeneke.

My sincere gratitude to my Zamorano´s friends members of Zamorano Agricultural Society at LSU (ZAS), especially Jorge Ortega and Nicolas Álvarez. Moreover, I would like to thank all the lab mates I had during my program for their support and advice who were: Luis Medina, David Paz, Ashly Castro, Miguel Tábora, Fátima Gutierrez, Ryan Page, and Ricardo Santos. Moreover, an especial thanks to Nur Orak for her advice and unconditional support.

Finally, I would like to thank all those who are not mentioned above who helped me in my master's program and research project. Thanks to all the ones that believed in me: this degree is in your honor.

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ABSTRACT

Frozen yogurts contain yogurt culture bacteria which might impart health benefits to its consumers. Global frozen yogurt market sales are expected to grow 4.8% by 2028 which represents an important opportunity for the industry, consumers, and researchers. Polyphenols are metabolites found in plants which have antioxidant and anti-inflammatory properties and might prevent chronical diseases such as cancer, diabetes, and cardiovascular diseases. The objective of this study was to elucidate the effect of the polyphenol hesperidin on the physico-chemical, microbiological, and sensory characteristics of frozen yogurts. Hesperidin was incorporated in frozen yogurt at three concentrations (500, 250 and 125 mg/90g of product). Yogurt with no hesperidin was used as control. Viscosity and overrun of the frozen yogurt were analyzed as randomized block design at day 0. Hardness, pH, color, enumeration of *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* counts were determined as randomized block design over days (0, 30, and 60). A factorial arrangement with three factors (hesperidin concentration, day, and minutes/hours) in a randomized block design over days (0, 30, and 60) was done for melting rate at minutes 60 and 90, bile tolerance of *Streptococcus thermophilus*, bile tolerance of *Lactobacillus bulgaricus* (0, 4, and 8 hours), acid tolerance of *Streptococcus thermophilus* (0, 1 and 2 hours), and acid tolerance of *Lactobacillus bulgaricus* (0, 15, and 30 minutes) were performed at days 7 and 60. A hedonic scale of 9 points was used to measure sensory attributes. Sensory data were analyzed as a completely randomized design. Data were analyzed at $\alpha = 0.05$ using PROC GLM and ANOVA with Tukey adjustment. McNemar's test was used to analyze purchase intent. Hesperidin did not influence pH, overrun, and microbial characteristics. Polyphenol addition decreased melting rate and increased hardness and bile tolerance of *L. bulgaricus*, L*, and b*. Sensory characteristics were not influenced by the lowest concentration of hesperidin being statistically the same

compared to the control. Moreover, consumers were interested in purchasing frozen yogurt with hesperidin added after a health claim. This study serves as an important tool to develop a healthier frozen yogurt in an increasingly demanding market.

CHAPTER 1. INTRODUCTION

1.1. Frozen Dairy Desserts

Milk-based frozen desserts are very popular in the human diet. These types of desserts have had a very important role in human society since there are records of their consumption since the 11th century. Moreover, around the years 1600's their consumption was considered a luxurious product for the European royal courts. However, it was not until the 19th century that the production of ice cream on a big scale started thanks to the invention of the first freezers in the industry (Gösta, 2003).

There are many different types of frozen dairy desserts. According to Goff (2018), ice cream is the most popular and the most regulated one. Nevertheless, there are also other types of products such as gelato, frozen custards, sherbets, smoothies, and frozen yogurts. Additionally, the US consumption per capita in 2012 frozen dairy desserts was 23.9 pounds (USDA, 2022). Consequently, frozen dairy desserts have a very important market value. For instance, the global ice cream market in 2017 was valued at 57 billion U.S. dollars and is expected to grow to 75 billion by 2024, representing an increase of 31% (Coppola, 2020). On the other hand, the market of frozen yogurt represents an important increase and it is expected to grow by 4.8% from 2021 to 2028, achieving a market value of 9.2 billion (DBMS, 2021).

1.2. Frozen Yogurt

Frozen yogurt is a dairy based product that is getting popularity among consumers. This product is characterized by an acidic taste keeping the refreshing and cold feature of an ice cream. It can

be served soft, hard or in a mousse way (Tamime and Robinson, 2007). Another important characteristic is that it contains probiotic bacteria (Behare *et al.*, 2016).

According to the Standard of North Carolina (02 NCAC 09K .0214 2000), frozen yogurt is defined as "a food that is prepared by freezing while stirring a pasteurized mix of the ingredients provided for in ice cream and which may contain other ingredients permitted under the Federal Food, Drug, and Cosmetic Act (21 USC 321 et seq.). All dairy ingredients in frozen yogurt shall be cultured after pasteurization by one or more strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and shall contain not less than 3.25 percent milk fat and not less than 8.25 percent non-fat milk solids, except that when bulky characterizing ingredients are used the percentage of milk fat shall not be less than 2.5 percent. The finished frozen yogurt shall weigh not less than five pounds per gallon. The titratable acidity of frozen yogurt shall not be less than 0.5 percent, calculated as lactic acid, unless the frozen yogurt primary flavor is a non-fruit characterizing ingredient. This characteristic acidity is developed by the bacterial activity and no heat or bacteriostatic treatment, other than refrigeration, which may result in destruction or partial destruction of the organisms which shall be applied to the product after culturing. The product, when in a package form, shall be labeled according to applicable sections of 21 CFR Part 101, incorporated by reference in 02 NCAC 09B .0116(o)(41)."

There are two ways to produce frozen yogurt. The most common one is made by mixing 10-20% of yogurt into 80-90% of ice cream mix. On the other hand, the alternative way to produce it is by inoculating the culture bacteria into the frozen yogurt mix and letting them to ferment (Chandan and Kilara, 2013).

1.3. Probiotics and their Impact on Human's Health

One of the most important benefits of yogurt (frozen yogurt) is its probiotic bacteria content. If the product is heat treated after fermentation, many bacteria might die (Puhan, 1979). Probiotic bacteria are defined according to Degnan (2008) as "living microorganisms that, when consumed, have the potential to confer a beneficial health." At the same time, the World Health Organization (FAO-WHO) defined probiotics as "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host". Safety, functionality, and technological usability are some important characteristics that bacteria should fulfill (Markowiak and Slizewska, 2017).

Yogurt showed to be associated with a better overall quality of their diets and healthier metabolic profile (Wang *et al.,* 2013*)*. The most representative probiotic bacteria are *Bifidobacterium*, *Lactococcus, Lactobacillus, Bacillus, Leuconostoc* and *Pediococcus* (Fijan, 2003). Moreover, they have been studied due to their capability to prevent some illnesses provoked by pathogenic bacteria in the intestinal track healthy and contributing to the microbiota complexity (Tridip *et al*., 2022). For these reasons, there is an important trend in the industry, market, researchers, and consumers regarding the interest of probiotic foods and supplements (Sanders, 2018). Another example of the positive impact of probiotics is the negative effects on *Helicobacter pylori* that *Bifidobacterium* spp. have, by the release of inhibitory components (bacteriocins) and competitive colonization (Wang *et al*., 2013).

The required bacteria to produce yogurt are *Streptococcus thermophilus* and *Lactobacillus bulgaricus* according to the regulations of the US. However, they are not limited to use only these types of bacteria (21CFR131.200). These bacteria are representative probiotics that might help in

the intestinal track by maintaining the systemic homeostasis and gut barrier function due to its influence on the intestinal microbiome and its metabolites (Yuki *et al*., 2018).

S. Thermophilus is a gram-positive bacterium that has an ovoid shape, lactic acid homofermentative and facultative aerobe. It is non-motile and has a diameter between 0.7 and 0.9 µm and some of the sugars that it can ferment are, lactose, sugar, fructose, and glucose. A very important characteristic of this bacteria is that it occurs in pairs and chains that often are long. It is important to highlight that it is a catalase-negative bacteria and its optimum growth temperature is between 40 and 45°C (Zirnstein and Hutkins, 1999). This species is not just used in yogurt due to its ability to provide a thick consistency to the food matrix but also is used to elaborate Italian cheese (Callanan and Ross, 2004) and probiotic supplements (Martinović *et al*., 2020).

On the other hand, *Lactobacillus delbrueckii* subsp. *bulgaricus* is a gram-positive that has a rod shape. Its size varies from 0.5 to 0.8 µm, it is found in small chains composed by three or four cells. It is a homofermentative producing lactic acid. Nevertheless, it also can produce acetone, acetoin, and diacetyl in low concentrations that might change flavor profiles (Robinson, 2012).

In yogurt there exists a synergistic relationship between *L. bulgaricus* and *S. Thermophilus.* In the incubation *L. Bulgaricus* stimulates the growth of *S. Thermophilus* due to the release of peptides and amino acids (Mchiouer, 2017). At the same time, *S. thermophilus* produces formic acid which promotes the growth of *L. bulgaricus* (Doelle *et al.,* 2009). Finally, pH is lowered, flavors developed, and yogurt is formed (Horiuchi and Sasaki, 2012).

1.4. Polyphenols and Oxidative Stress

Polyphenols comprises one of the most numerous and widely distributed groups of substances in the plant kingdom mainly found in fruits and vegetables. These compounds are the secondary metabolism of plants that carries one or more hydroxyl groups (Bravo, 1998). Food polyphenols are categorized according to their structure in phenolic acids, flavonoids, stilbenes and lignans (Amawi *et al*., 2017). Moreover, these molecules have antioxidant properties and the ability to regulate enzymatic activities (Quiñones *et al*., 2011). On the other hand, reactive oxygen species are defined as molecules which have oxygen in their structure which might have charge (positive or negative) and possess the ability to oxidize other molecules (Ray *et al*., 2012). Related to this, the oxidative stress is understood as the unbalance that exists between the oxidant agents such as reactive oxygen species (ROS) and antioxidants such as superoxide dismutase (SOD) or polyphenols (Venereo, 2002).

The result of this unbalance can lead to damage in the organism cells since these agents might attack lipids and DNA (Betteridge, 2000). Furthermore, according to Pandley and Rizvi (2009) "Epidemiological studies and associated meta-analyses strongly suggest that a long-term consumption of diets rich in plant polyphenols offer protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases." It is why, in the recent years, more studies have been done since the polyphenols have antioxidant and free radical-scavenging abilities, associated to a possible positive impact in human health by preventing chronical diseases such as cancer, osteoporosis, neurodegenerative and cardiovascular diseases (Scalbert *et al*., 2005). Therefore, a diet rich in food containing polyphenols is recommended.

This is an area that the consumers and the industry are very interested in. For instance, “The global polyphenols market size was valued at USD 1.6 billion in 2021 and is anticipated to grow at a compound annual growth rate of 7.4% from 2022 to 2030” (Grand Research View).

1.5. Hesperidin

Hesperidin is defined as “a flavanone glycoside consisting of the flavone hesperitin bound to the disaccharide rutinose” (Suzuki *et al.,* 2014*)*. This compound is the major polyphenol (flavonoid) found in citrus fruits such as lemons and oranges, containing up to 41 and 60 mg of hesperidin per 100 mL of juice, respectively (Meneguzzo *et al*., 2020). However, it can be found in other types of fruits. This compound shows the ability to be antihypertensive, antidiabetic and cardioprotective due to the antioxidant action by reducing the production of inflammatory markers such as cytokine (Mas-Capdevilla *et al*., 2020). Moreover, this polyphenol demonstrated antimicrobial activity against pathogenic bacteria (Hassan *et al*., 2018).

An important characteristic of this polyphenols is that it is stable up to 80°C for ten minutes which means that it is heat stable for most of the pasteurization treatments (Zhang *et al.*, 2019). On the other hand, in relation to the stability of this compound at different levels of pH, hesperidin showed to be stable from pH 1 to 7 (Majumdar and Srirangam, 2008).

The objectives were to:

- To determine the effect of added hesperidin on the physico-chemical characteristics in frozen yogurt.

- To evaluate the acid tolerance of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in frozen yogurt with hesperidin added.
- To evaluate the bile tolerance of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in frozen yogurt with hesperidin added.
- To study the effect of hesperidin on sensory attributes in frozen yogurt and the purchase intent before and after a health statement.

CHAPTER 2. MATERIALS AND METHODS

2.1. Location

The present research was conducted at Louisiana State University and Agricultural & Mechanical College. The LSU AgCenter laboratories used were the dairy microbiology laboratory, the dairy physicochemical laboratory, and the dairy food processing plant, all of them located at South Stadium Dr, Baton Rouge, LA 70808. Sensory analysis was done in the sensory laboratory located at Forestry Ln, Baton Rouge, LA 70803.

2.2. Experimental Design

Treatments were the hesperidin concentrations. Three concentrations of the polyphenol hesperidin were incorporated to frozen yogurt manufacture at 500, 250 and 125 mg per 90 g of serving (Table 2.1). A negative control had no polyphenol added. All experiments were replicated three times. Two experimental designs were used. First, viscosity and overrun of the frozen yogurt mix at day 0 were conducted using a randomized block design where the blocks were the replications. To enumerate *Streptococcus thermophilus*, *Lactobacillus bulgaricus* counts, pH, hardness, and color, data were analyzed as randomized block design with measurements over days 0, 30, and 60 where the replications were the blocks. A factorial arrangement with three factors (hesperidin concentration, day, and minute/hour) in a randomized block design over days was done for melting rate at minute 60 and 90 (days 0, 30, and 60), bile tolerance of *Streptococwere thermophilus,* bile tolerance of *Lactobacillus bulgaricus* (0, 4, and 8 hours), acid tolerance of *Streptococcus thermophilus* (0, 1 and 2 hours), and acid tolerance of *Lactobacillus bulgaricus* (0, 15, and 30 minutes) were performed at days 7 and 60. Sensory attributes of the frozen yogurt on day 60 was

analyzed using a completely randomized design using 103 consumers, where each consumer was a replication.

Table 2.1. Treatments of frozen yogurts with hesperidin added.

Compounds	**Concentration of the polyphenol (mg) per 90 g of frozen yogurt**		
	500	**250**	**125**
Hesperidin	FY500	FY250	FY125
Negative Control		FY0	

FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

2.3. Frozen Yogurt Manufacture

Frozen yogurt mixes were prepared as described in Table 2.2 (Alfaro, 2012). Before manufacturing the frozen yogurt treatments, all the equipment and utensils used for its production were cleaned with detergent and water solution to subsequently being sanitized with Cholorinzer Plus (Afco Industries Inc, Alexandria, LA) with a solution at a concentration of 200 ppm of chlorine. First, dry ingredients were weighed and mixed according to the different proportions based on the concentrations of hesperidin. Pasteurized whole milk at 3.25% fat content (Great Value™, Bentonville, AR) acquired from a local supermarket was poured into 8 L stainless-steel containers. Ingredients mixed (milk was pre heated to 70° ± 2°C) were sugar (Domino Foods, Inc., Yonkers, NY), maltodextrin (Bulksuplements.com®, Henderson, NV), grade A nonfat dry milk (Dairy America, Fresno, CA), Corn Starch (Argo®, Oakbrook Terrace, Il) from a local supermarket, and hesperidin (Bulksuplements.com®, Henderson, NV) according to each formulation using a stainless-steel manual mixer until all the ingredients were properly integrated.

Table 1.2. Formulations of treatments of frozen yogurts with hesperidin added.

Compounds	Treatments			
	FY500	**FY250**	**FY125**	**FY0**
Milk with 3.25% fat (Kg)	7.56	7.56	7.56	7.56
Sucrose (Kg)	1.36	1.36	1.36	1.36
Nonfat dry milk (g)	399.52	399.52	399.52	399.52
Maltodextrin (g)	363.20	363.20	363.20	363.20
Corn Starch (g)	20.00	20.00	20.00	20.00
Lactobacillus bulgaricus (g)	3.00	3.00	3.00	3.00
Streptococcus thermophilus (g)	3.00	3.00	3.00	3.00
Hesperidin (g)	59.66	29.74	14.85	-

FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

Afterwards, the mixes were pasteurized at 75 ± 1°C for 30 minutes. Mixes were tempered to 43 ± 1°C to inoculate them with *Lactobacillus bulgaricus* (LB- 12) and *Streptococcus thermophilus* (ST-06) (Chr. Hansen's Laboratory, Milwaukee, WI) in a ratio of 1:1. Mixes were fermented in an incubator (model 815 Thermo Scientific, Two Rivers, WI) at 43 ± 1°C until pH reached 4.7 ± 0.1. After that, yogurts were stored at 4 ± 1°C to age the mix for twenty-four hours. Later, the mixes were stirred manually for 60 seconds to breakdown the coagulum until no clumping was observed. The mixes were poured into a batch freezer (Emery Thompson 20NW, Brooksville, FL) and frozen

for nine minutes and 30 seconds. Finally, the frozen yogurts were packed in plastic containers (polypropylene) with lids of 2, 5.5, 12, 16, and 32 fl oz for different measurements over time and stored at -25 ± 2°C until its use.

2.4. Overrun

Overrun was measured according to Muse and Hartel (2004). The percentage of overrun (%) was measured by weighing a known amount of volume of the frozen yogurt mix before freezing it in the batch freezer and the weight of the same volume of the finished frozen yogurt using the following equation:

$$\text{Overrun (\%)} = \frac{\text{Weight of frozen yogurt mix-Weight of frozen yogurt}}{\text{Weight of frozen yogurt}} \times 100$$

2.5. Viscosity

The viscosity of the frozen yogurt mix was analyzed according to Aryana (2003) with slight modifications. At day 0, yogurt mixes were stirred manually for 60 seconds at 4 ± 1°C in containers of 32 fl oz with dimensions of 13.97 cm height, top diameter of 11.43 cm, and 8.89 cm of bottom diameter (Pantry Value, Converse, TX) to measure the viscosity. Viscometer Brookfield model SV-22 (Brookfield Engineering Lab Inc.) as well as the software Wingather® 32 (Brookfield Engineering Lab Inc.) and a spindle RV 5 at 20 rpm was implemented in the measurement. An average of 20 data points taken per treatment per repetition and its average was used for the analysis purposes.

2.6. Hardness

Before hardness of the frozen yogurt was measured, the samples were tempered to -18 ± 1°C for twenty-four hours to simulate the hardness of the product at a consumption temperature in cups of 12 oz capacity with a height of 6.35 cm, top diameter of 11.43 cm, and bottom diameter of 8.89 cm. A texture analyzer (TA. XT Plus Connect, Texture Technology Corp., Hamilton, MA), connected to a 50 Kg load cell according to the manufacturer was used. The probe TA-43R was used with a settled pre-test speed of 2.0 mm/s, test speed at 3 mm/s, and a post-test speed at 10.0 mm/s. The hardness was measured as the peak force (N) needed to penetrate the product by 3.5 cm at the geometrical center of the container. The measurements for hardness were done at days 0,30 and 60 of storage.

2.7. pH

The samples used for pH were completely defrosted before its analysis and analyzed at 25 ± 1 °C. using an Orion Star™ A111 (Thermo Fischer Scientific Inc., Waltham MA) pH-meter. The measurements for pH were done at days 0, 30 and 60. Before its use, the instrument was calibrated using reference buffer solutions of pH 4 and 7.

2.8. Color

Color was measured using a handheld colorimeter (Hunterlab MiniScan XE Plus) The instrument was calibrated with a black and white standard references. L*, a*, and b* values were taken as the average of three consecutive measurements per replication. The measurements for color were done at days 0, 30 and 60 of storage.

2.9. Melting Rate

Melting rate was conducted according to Januário *et al*. (2016). When frozen yogurts were manufactured, 90 grams of sample in semi soft consistency was placed into containers of 5.5 fl oz capacity with a height of 6.03 cm, top diameter of 7.30 cm, and bottom diameter of 4.76 cm. Frozen yogurt samples in containers were tempered at 18 ±1°C for 24 hours prior test. Samples were placed without its container over a stainless-steel net of 1 mesh and left to melt at a controlled temperature of 25 ± 1°C. The product was collected using a funnel over a graduated cylinder and the volume of melted product was recorded after 60 and 90 minutes. The measurements were done at days 0, 30 and 60 after production.

2.10. Enumeration of *Streptococcus thermophilus*

S. thermophilus agar was prepared according to Dave and Shah (1996). One liter of distilled water was mixed with 10 g of sucrose (Amresco, solon, OH), 5 g of yeast extract (Becton Disckinson and CO., Sparks, MD), 2 g of dipotassium phosphate (K_2HPO_4) (Fischer Scientific, Fair Lawn, NJ), and 10 g of bacto tryptone (Becton Disckinson and Co., Sparks, MD) in a 2 L Erlenmeyer flask. All the reagents were mixed until they were dissolved completely. The pH was adjusted to 6.8 ± 0.1 with 1 N HCl. 12 g of agar and 30 mg of Bromocresol purple (Fisher Scientific, Fair Lawn, NJ) were added to the media. The mix was heated to boil while stirring, autoclaved at 121°C for 15 minutes, and stored into water bath at 60 ±1 °C until its use.

For the enumeration procedure, all dilution bottles and petri dishes were properly labeled with date, dilution factor, identification of the sample and initials. Frozen yogurt samples were completely thawed before use. 11 g of sample was tenfold serially diluted using peptone water at

0.1% (Becton Disckinson and CO., Sparks, MD.) Afterwards, aseptically, 1 mL of the diluted solution was pipetted into a Petri dish. The media at an approximated temperature of 45 ± 1°C was poured into the plate and gently shaken to homogenize the mixture. The Petri dishes were cooled at room temperature until the mixture was solidified and was incubated aerobically for 24 h at 37 ± 1 °C. The colony forming units per gram were counted using a colony counter (Quebec Darkfield, Leica Inc., Buffalo, NY). Enumeration of *Streptococcus thermophilus* was done at day 0, 30 and 60. This procedure was done in triplicate.

2.11. Enumeration of *Lactobacillus bulgaricus*

L. bulgaricus agar was prepared according to Nwadiuto and Shah (2017) with slight modifications. One liter of distilled water was mixed with 55 g lactobacilli MRS broth powder (Becton Disckinson and CO., Sparks, MD) and 15 g of agar (Fisher Scientific, Fair Lawn NJ) in a 2L Erlenmeyer flask until the broth powder dissolved completely. The pH was adjusted to 5.2 ± 0.1 using 1 N HCl. The mix was heated to boil while stirring, autoclaved at 121°C for 15 minutes, and stored into a water bath at 60 ± 1 °C until its use.

For the enumeration procedure, all dilution bottles and Petri dishes were properly labeled with date, dilution factor, identification of the sample, and initials. The frozen yogurt sample was completely thawed before use. Then, 11 g of sample was tenfold serially diluted using 0.1% peptone water (Becton Disckinson and CO., Sparks, MD). Aseptically, 1 mL of the desired sample was pipetted into a petri dish and then the agar at 45 ± 1°C was also poured into the Petri dish and gently shaken to homogenize the mixture. The Petri dishes were cooled down at room temperature until the mixture was solidified and were incubated anaerobically for 72 h at 43 ± 1°C. The colony

forming units per gram were counted using a colony counter (Quebec Darkfield (Leica Inc., Buffalo, NY). Enumeration of *Lactobacillus bulgaricus* was done at day 0,30 and 60. This procedure was done in triplicate.

2.12. Bile Tolerance of *S. thermophilus* and *L. bulgaricus*

The agar of *S. thermophilus* and *L. bulgaricus* previously described were used to pour plates. The preparation of the broths and procedures were done according to Pereira and Gibson (2002) with some modifications.

MRS-THIO broth was used to enumerate of *L. bulgaricus*. 55 g lactobacilli broth powder (Becton Disckinson and CO., Sparks, MD) was mixed with 1 L of distilled water and supplemented with 0.3% of oxgall (bile salts) (US Biological, Swampscott, MA) and 0.2 % sodium thioglycolate (Acros Organics, Fair Lawn, NJ) which worked as an oxygen scavenger. Then, the broth was autoclaved at 121°C for 15 minutes and stored at 43 ± 1 °C until used. Before its use, the broth was supplemented with 0.5% of lactose. 11 g of the sample was tenfold diluted into the MRS-THIO broth previously prepared and serially diluted using 0.1% peptone water (Becton Disckinson and CO., Sparks, MD). Aseptically, 1 mL of the desired sample was pipetted into a Petri dish and the MRS agar at 45 ± 1°C was poured. The plates were gently shaken to homogenize the mixture. The plates were cooled to room temperature until the mixture was solidified and incubated anaerobically for 72 h at 43 ± 1°C. This enumeration was done using the same diluted sample in the MRS-THIO broth and enumerated at hours 0, 4 and 8. The colony forming units per gram were counted using a colony counter (Quebec Darkfield, Leica Inc., Buffalo, NY). Acid tolerance of *L. bulgaricus* was done at day 7 and 60. The enumeration was done in triplicate.

M17 broth was used for the enumeration of *S. thermophilus*, 37.25 g of M17 broth powder (Becton Disckinson and CO., Sparks, MD) were mixed with 950 mL of distilled water and supplemented with 0.3% of oxgall (bile salts) (US Biological, Swampscott, MA). Broth was autoclaved at 121°C for 15 minutes and stored at 37 ± 1 °C until used. The broth was supplemented with 0.5% of lactose. 11 g of the sample was tenfold diluted into the M17 broth previously prepared and serially diluted using 0.1% peptone water (Becton Disckinson and CO., Sparks, MD). One milliliter of the desired sample was pipetted into a Petri dish and the agar at 45 ± 1°C was poured. The plates were gently shaken to homogenize the mixture. Petri dishes were cooled to room temperature until the mixture was solidified and incubated aerobically for 24 h at 37 ± 1°C. This enumeration was done using the same diluted sample in the M17 broth and enumerated at hours 0, 4 and 8. The colony forming units per gram were counted using a colony counter (Quebec Darkfield, Leica Inc., Buffalo, NY). Acid tolerance of *S. thermophilus* was done at days 7 and 60. The enumeration was done in triplicate.

2.13. Acid Tolerance of *S. thermophilus* and *L. bulgaricus*

For acid tolerance, the agar of *S. thermophilus* and *L. bulgaricus* previously described were used to pour plates. The preparation of the broths and procedures were done following the method used by Pereira and Gibson (2002) with some modifications.

For the enumeration of *L. bulgaricus*, 55 g lactobacilli MRS broth powder (Becton Disckinson and CO., Sparks, MD) was mixed with 1 L of distilled water. The broth was acidified using HCl while stirring. The broth was autoclaved at 121°C for 15 minutes and stored at 43 ± 1 °C until used. The broth was supplemented with 0.5% of lactose. 11 g of the sample was tenfold diluted into the MRS

broth acidified previously prepared and serially diluted using 0.1% peptone water (Becton Disckinson and CO., Sparks, MD). Aseptically, 1 mL of the desired dilution was pipetted into a Petri dish and the agar at a temperature of 45 ± 1°C was poured. The plates were gently shaken to homogenize, cooled to room temperature until the mixture was solidified, and incubated anaerobically for 72 h at 43 ± 1°C. This enumeration was done using the same diluted sample in the acidified broth and enumerated at minutes 0, 15 and 30. The colony forming units per gram were counted using the colony counter (Quebec Darkfield, Leica Inc., Buffalo, NY). Acid tolerance of *L. bulgaricus* was done at days 7 and 60. The enumeration was done in triplicate.

The acidified broth of M17 was produced for *S. thermophilus* acid tolerance. 37.25 g of M17 broth powder (Becton Disckinson and CO., Sparks, MD) were mixed with 950 mL of distilled water. The broth was acidified using HCl while stirring, autoclaved at 121°C for 15 minutes, and stored at 37 ± 1 °C until its use. The broth was supplemented with 0.5% of lactose. 11 g of the sample was tenfold diluted into the acidified M17 broth previously prepared and serially diluted using 0.1% peptone water (Becton Disckinson and CO., Sparks, MD). 1 mL of the desired sample was pipetted into a Petri dish and the agar at a temperature of 45 ± 1°C was poured. The plates were gently shaken to homogenize the mixture., cooled to room temperature until the mixture was solidified, and incubated aerobically for 24 h at 37 ± 1°C. This enumeration was done using the same diluted sample in the acidified broth and enumerated at hours 0, 1 and 2. The colony forming units per gram were counted using the colony counter (Quebec Darkfield, Leica Inc., Buffalo, NY). Acid tolerance of *S. thermophilus* was done at days 7 and 60. The enumeration was done in duplicate per repetition.

2.14. Sensory Analysis

Sensory study was exempted from the oversight by the LSU institutional review board with the IRB exception number IRBAG-22-0076. Four treatments of frozen yogurt were served with three different concentrations of hesperidin (500, 250 and 125 mg per 90 g of serving) along with a negative control. For this study, 103 random participants tried the four treatments. The serving containers were made of polypropylene of 2 oz with lids. To participate in the study, every person in this study accepted an informed consent where the potential risks were explained. A hedonic scale of 9 points was used in this study where 9 meant like extremely, 1 meant dislike extremely, and 5 meant neither like nor dislike. Appearance, color, aroma, texture, iciness/sandiness, flavor, sourness, and overall liking were evaluated by participants in this sensory evaluation. Additionally, purchase intent was asked before and after a health statement regarding the polyphenol presence in the product.

2.15. Statistical Analysis

The means and standard deviations were reported in triplicate. All data were analyzed using Statistical Analysis System (SAS® version 9.4). For physicochemical and microbial properties, PROC GLM was used with Tukey adjustment. Differences of least square means were used to determine the statistical differences ($P < 0.05$) for main effects (treatments, day, and hour/minute) and their interactions. For sensory attributes ANOVA was performed with Tukey adjustment. Significant differences in purchase intent before and after a health claim were determined using a McNemar's test.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Viscosity

Viscosity represents an important feature for quality assurance purposes. Viscosity is understood as the resistance to flow of a certain substance. This means that if a substance has a low viscosity, it will be easy to deform and modify its shape (Zhang, 2016). Fenelon *et al*., (2000) established that a yogurt viscosity is related to the amount of protein, fat, and the proportions of casein and whey protein. The viscosity of the frozen yogurt mix (Figure 3.1) was measured on the frozen yogurt mix at day 0. No statistical differences were found between treatments ($P < 0.05$). An explanation of this results might be the standardized process that all frozen yogurt mixes experienced before being poured into batch freezer which was 60 seconds of stirring to break the coagulum formed in the fermentation. Another reason could be due to the small amount that was added of hesperidin which ranged from 59.66 to 14.85 g. Consequently, the addition of hesperidin did not affect the viscosity of the frozen yogurt mixes when compared to a plain frozen yogurt mix.

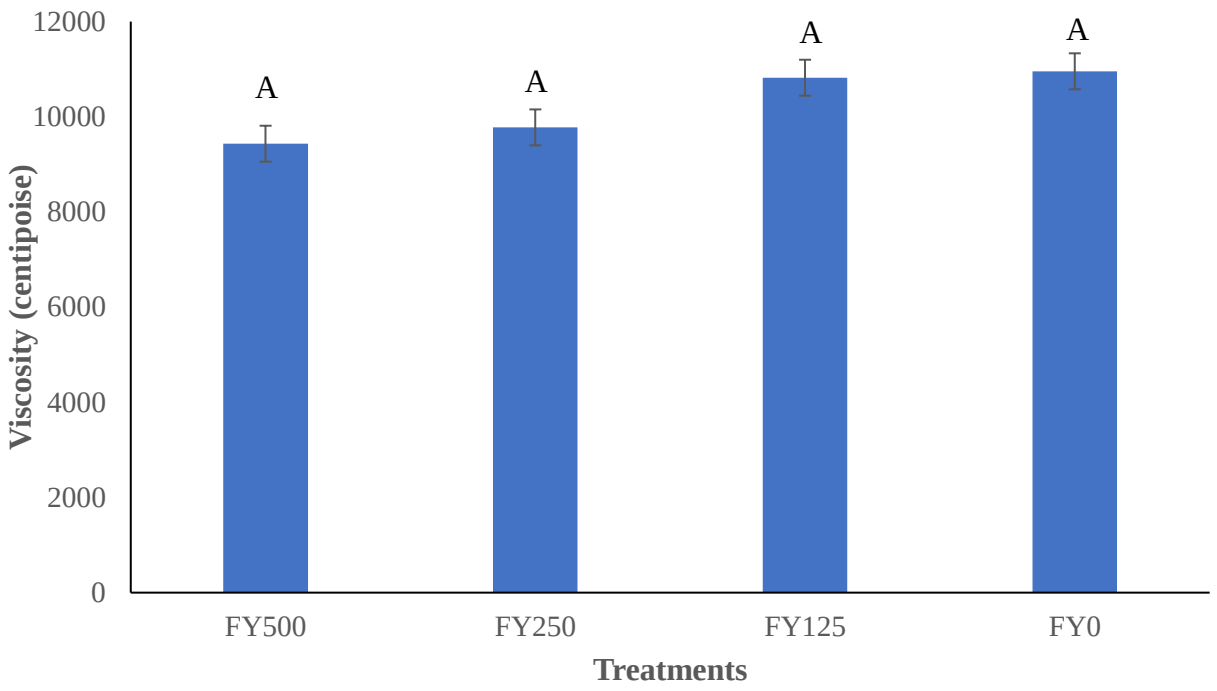

Figure 3.1. Viscosity of the frozen yogurt mixes with hesperidin added before processing in the batch freezer. [A] Different letters are significantly different. FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin. Standard deviations ranged from 10.15 to 5.71%.

3.2. Overrun

Overrun characteristic as influenced of hesperidin are shown in Figure 3.2. Overrun is one of the most important features in the frozen dairy dessert industry, representing the percentage of air that a product contains in its matrix. This feature might influence other characteristics such as hardness, melting rate, and mouthfeel (Marshall *et al.*, 2012). No statistical differences ($P < 0.05$) were found among treatments in this essay. These results can be explained since the procedure that was followed in the frozen yogurt manufacture was standardized to nine minutes and 30 seconds in the batch freezer machine. Another factor that might affected is that the hesperidin is poor aqueous solubility (Anwer *et al.*, 2014.) the foaming capacity of hesperidin is limited since a good foaming

agent should decrease the surface tension between air and water (Huppertz 2010) like proteins do (Mauer, 2013). In other words, the addition of hesperidin did not enhance the overrun.

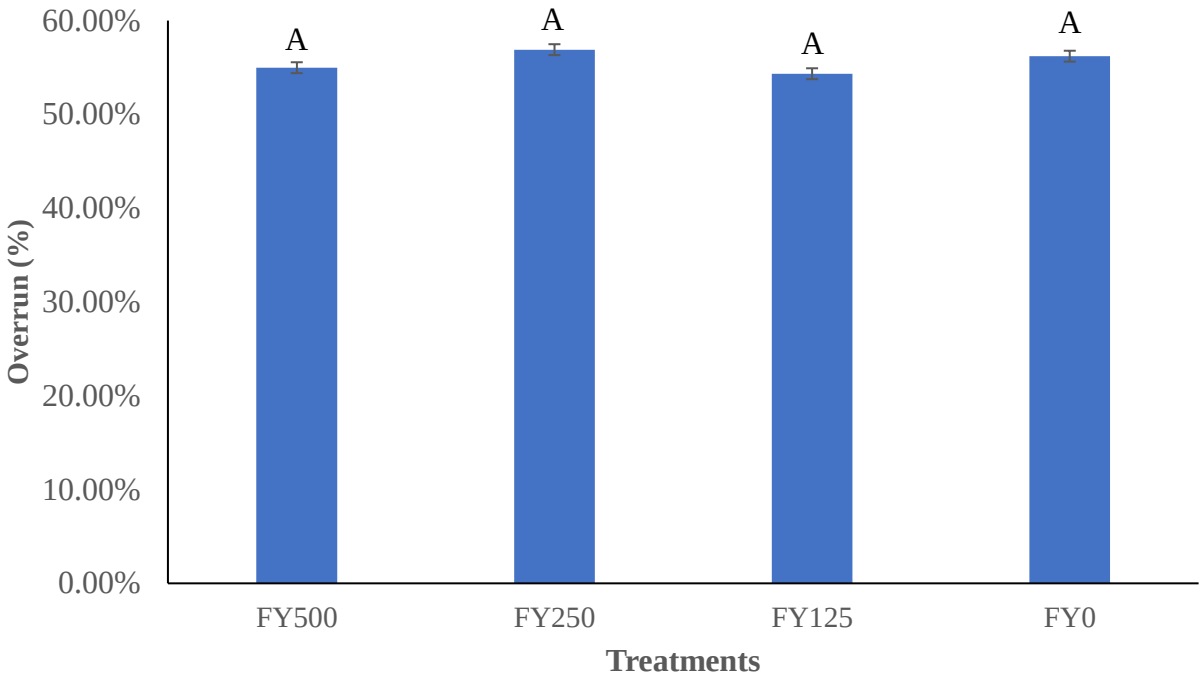

Figure 3.2. Overrun measurements in frozen yogurt with hesperidin added. [A] Different letters are significantly different. FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

3.3. Enumeration of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*

Main effects of enumeration of *S. thermophilus* and *L. bulgaricus* counts in frozen yogurt over 60 days of storage are shown in Table 3.1. The main effects that influenced the bacterial counts of *S. thermophilus* were treatment and day ($P < 0.05$). Table 3.2 shows that the lowest bacterial counts were found in treatment FY125. Similar results are shown in the Table 3.3 where statistical ($P < 0.05$) reductions were observed at day 30 and 60 when compared to day 0. Even though statistical

differences ($P < 0.05$) were found in both cases, the reductions were less than half of one log CFU/g which can be considered that there is no practical significance between treatments and days. These results are like the ones found by Atallah *et al.* (2022), where they discovered that frozen yogurt with different types of sweeteners reduced the counts of *S. thermophilus* on a period of 60 days of storage. Freezing process might damage bacteria by mechanical damage on their cell walls, increasing damaging solutes in the extracellular medium, and dehydration (Gill, 2014) which explains the slight reductions in this essay.

Table 2.1. Probability (Pr > F) of treatment, day, and its interaction in the enumeration of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in frozen yogurt with hesperidin added.

Effect	*S. thermophilus*	*L. bulgaricus*
	Pr > F	Pr > F
Treatment	0.0079	0.1986
Day	0.0013	0.3250
Treatment*Day	0.9202	0.7180

Table 3.2. Means and standard deviations as influenced by treatment for bacterial count of *S. thermophilus* in frozen yogurt.

Treatment	*S. thermophilus* (Log CFU/g)
FY500	8.74 ± 0.18^{A}
FY250	$8.77 \pm 0.13A^{A}$
FY125	8.56 ± 0.15^{B}
FY0	8.77 ± 0.24^{A}

^{AB} Means ± standard deviations with different letters are significantly different ($P < 0.05$). FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

Table 3.3. Means and standard deviations as influenced by day for bacterial count of *S. thermophilus* in frozen yogurt with hesperidin added.

Day	*S. thermophilus* (Log CFU/g)
0	8.84 ± 0.14^{A}
30	8.70 ± 0.17^{B}
60	8.61 ± 0.20^{B}

^{AB} Means ± standard deviations with different letters are significantly different ($P < 0.05$).

For *L. bulgaricus* counts (Figure 3.3), no statistical differences ($P < 0.05$) were found in the main effects of treatment, day, and treatment*day. This stability in *L. bulgaricus* counts in frozen environment was also found by Davidson *et al*. (2000) where the survival of *L. bulgaricus* showed no statistical differences over a storage of 11 weeks in frozen yogurt. In the same way, Alfaro *et al*. (2015) found that probiotic bacteria count in frozen yogurt with purple rice bran oil remained stable throughout time of storage for 6 weeks. Furthermore, Olson *et al*. (2021) did not find decreases in microbial counts of *L. bulgaricus* after 8 weeks of storage in frozen yogurt. For both bacteria, the counts of lactic acid bacteria (LAB) remained stable through the storage period. It is important to highlight that in this study both bacteria achieved 10^6 CFU/g which is the concentration needed in probiotic foods (Terpou *et al*., 2019).

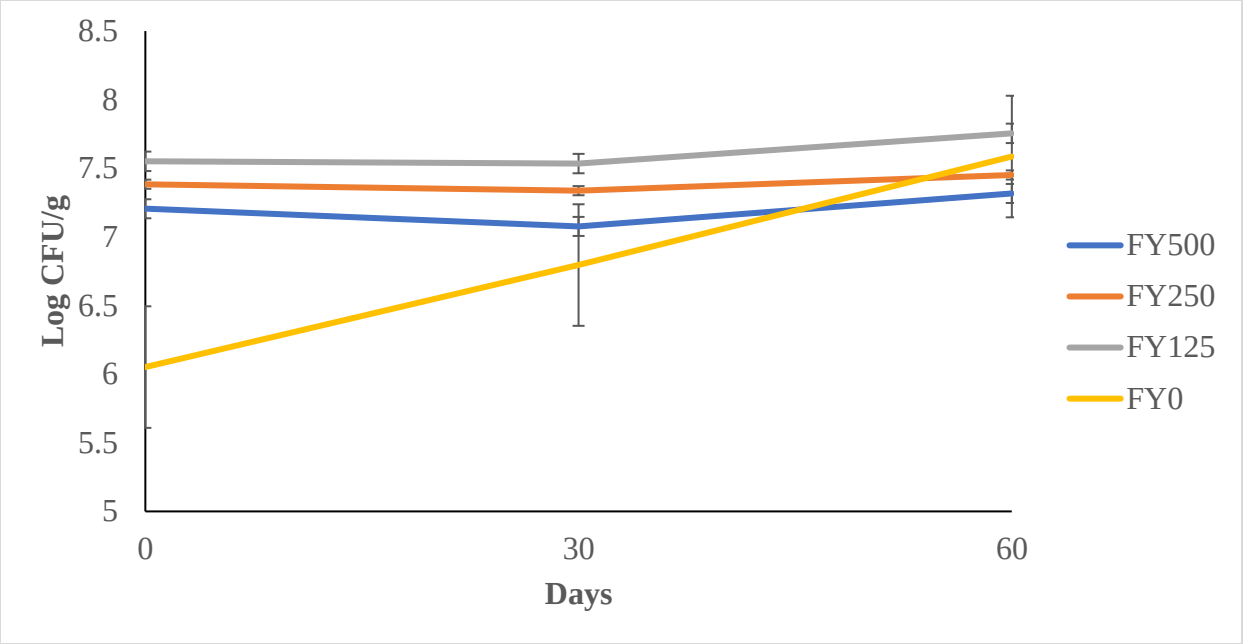

Figure 1.3 *L. bulgaricus* counts in frozen yogurt with hesperidin added over 60 days of storage. FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

3.4. pH

pH is a measurement on how acidic or alkaline is a product in aqueous solution and it is measured on a scale from 0 to 14 (Prichard and Lawn, 2003). In the dairy industry, pH measurement is a very important parameter since acidic components directly affects the stability, flavor, and shelf life of the dairy products (Burke *et al*., 2018). The results obtained in this study are shown in Figure 3.4. Values ranged from 4.54 and 4.72 showing a relative stability in pH. Table 3.4 shows that no statistical differences ($P < 0.05$) were found between treatments, days, and interaction treatment*day.

Table 3.4. Probability (Pr > F) of treatment, day, and its interaction of pH measurements in frozen yogurt with hesperidin added.

Effect	Pr > F
Treatment	0.2287
Day	0.0503
Treatment*Day	0.8562

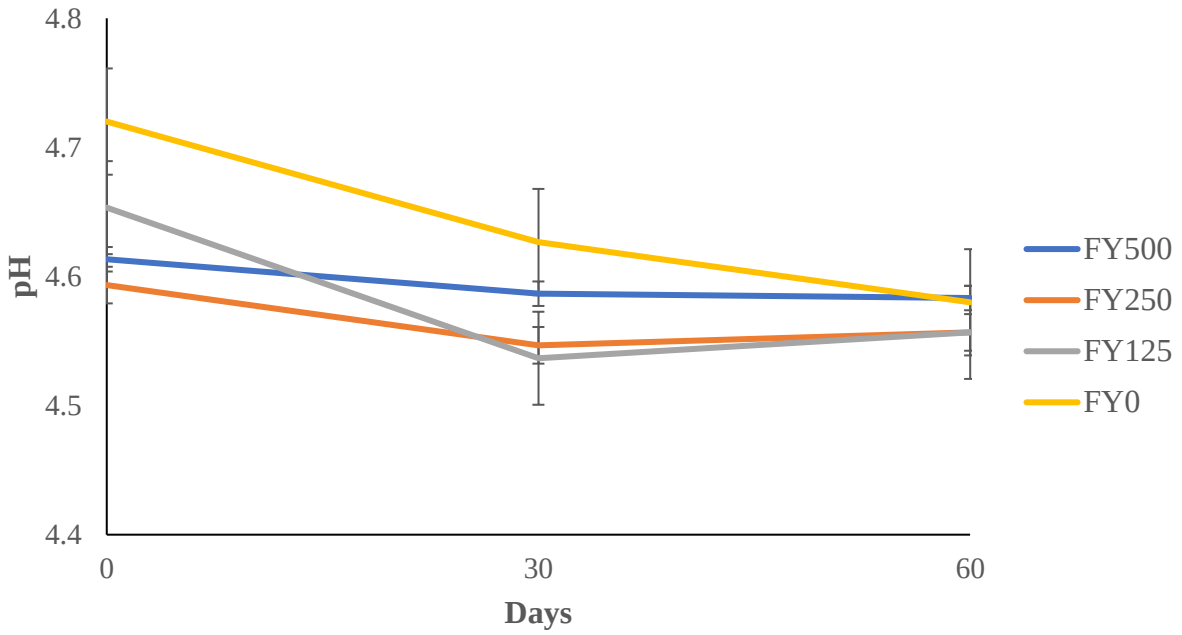

Figure 3.4. pH measurements in frozen yogurt with hesperidin added over 60 days of storage. FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

Two factors might explain the lack of significance ($P < 0.05$) for pH. First, the controlled endpoint of the frozen yogurt manufacture fermentation which was settled at a pH of 4.7± 0.1 and the second factor which is the stability of chemical and microbial reaction in freezing environment (Erkmen

and Faruk, 2016). These results are very similar to what Inoue *et al*. (1998) found when pH in frozen yogurt kept stable for six months of storage.

3.5. Color

L* value indicates the lightness of a sample on scale from 0 to 100 (Jung *et al.,* 2017). In table 3.5 are shown the Pr > F for the main effects and interaction. Statistical differences ($P < 0.05$) were found for treatments effect.

L* value was increased when more hesperidin was used (Table 3.6). This could be explained due to the different concentrations of hesperidin that were used. The darkest treatment was FY500 where the highest hesperidin concentration was used while the whitest frozen yogurt was FY0 which had no hesperidin. This result means that the ingredient changed how white the frozen yogurt was. These results can be related to the ones obtained by Binkowska (2020) where this author established that the more hesperidin that was used in a hesperidin-silica complex, the more L* value was decreased. In other words, several pigments apart from white might influence in the food color matrix, reducing how white the yogurt was.

Table 3.5. Probability (Pr > F) of treatment, day, and its interactions for L*, a*, and b* values in frozen yogurt with hesperidin added.

Effect	Pr > F		
	L* value	a* value	b* value
Treatment	<.0001	<.0001	<.0001
Day	0.2780	<.0001	0.0122
Treatment*Day	0.8428	0.0386	0.4950

Table 3.6. Means and standard deviations as influenced by treatment for L* values in frozen yogurt with hesperidin added.

Treatment	L* value
FY500	88.84 ± 1.27^{D}
FY250	92.67 ± 0.46^{C}
FY125	94.52 ± 0.77^{B}
FY0	97.13 ± 1.07^{A}

^{ABCD} Means ± standard deviations with different letters are significantly different ($P < 0.05$). FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

a* value of the frozen yogurt with hesperidin added appears in Figure 3.5. This property indicates the green-redness color. Statistical differences ($P < 0.05$) were found for treatment, day, day*treatment which can be observed in the Table 3.5. It can be observed that the higher the concentrations of hesperidin used, the higher a* value obtained (Table 3.7). Day effect results can be observed in Table 3.8. Moreover, the interaction time*treatment are visualized in Table 3.9. These slight differences could be derived due to the pigments that hesperidin had. It is important to mention that the differences found over time and between treatments ranged from -047 to 0.14 which represents less than one unit in the a* scale. Consequently, even though there are statistical differences they can be considered non-practical differences.

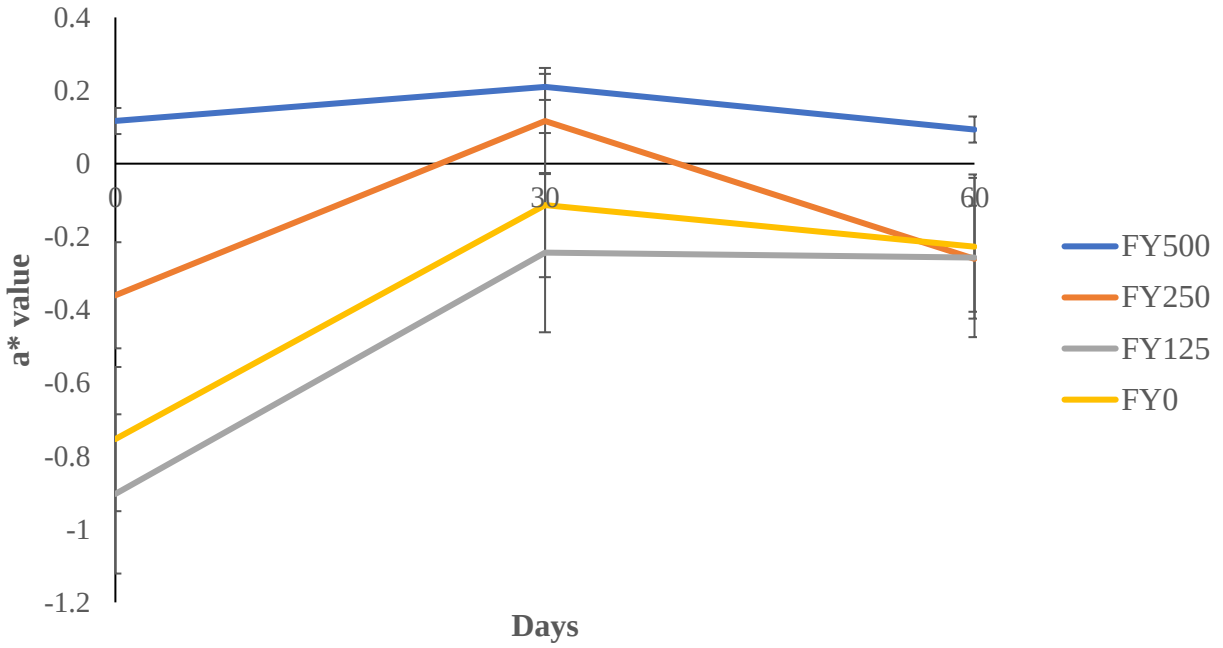

Figure 3.5. a* values in frozen yogurt with hesperidin added over 60 days of storage. FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

Table 3.7. Means and standard deviations as influenced by treatment for a* value in frozen yogurt with hesperidin added.

Treatment	a* value
FY500	0.14 ± 0.17^{A}
FY250	-0.17 ± 0.30^{B}
FY125	-0.47 ± 0.38^{C}
FY0	-0.36 ± 0.36^{BC}

^{ABCD} Means ± standard deviations with different letters are significantly different ($P < 0.05$). FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

Table 3.8. Means and standard deviations as influenced by day for a* value in frozen yogurt with hesperidin added.

Day	a* value
0	-0.48 ± 0.14^{B}
30	-0.01 ± 0.17^{A}
60	-0.16 ± 0.20^{A}

^{AB} Means ± standard deviations with different letters are significantly different ($P < 0.05$).

Table 3.9. Means and standard deviations as influenced by treatment*day for a* value in frozen yogurt with hesperidin added.

Treatment	a* value		
	Day 0	Day 30	Day 60
FY500	$0.11\pm0.26^{A,a}$	$0.21\pm0.09^{A,a}$	$0.09\pm0.15^{A,a}$
FY250	$-0.36\pm0.30^{C,b}$	$0.11\pm0.26^{AB,a}$	$-0.26\pm0.12^{B,b}$
FY125	$-0.90\pm0.36^{B,b}$	$-0.24\pm0.10^{C,a}$	$-0.25\pm0.05^{B,a}$
FY0	$-0.75\pm0.38^{B,b}$	$-0.11\pm0.17^{BC,a}$	$-0.22\pm0.02^{AB,a}$

^{ABCD} Means ± standard deviations with different letters within the column are significantly different ($P < 0.05$). ^{abcd} Means ± standard deviations with different letters within the row are significantly different ($P < 0.05$). FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

For b* value (blue-yellow), the effects that were statistical different ($P < 0.05$) were treatment effect and day effect. As expected, the b* value was higher in the treatments where the concentration of hesperidin was higher (Table 3.10). These results are like the ones found by Binkowska (2020), where the more concentration of hesperidin on silica complex showed a higher b* value. This could be due to the yellowish-brown color of the hesperidin (Sharma *et al*., 2013). After 30 days of storage b* value decreased slightly (Table 3.11), possibly due to oxidation process

during storage of components such as riboflavin (vitamin B2) and β-carothene present in milk fat (Popov, 2008). These results are very similar to the ones found by Kaur *et al.*, (2011) where they discovered a slight decrease of 2 units in b* value on ice cream for four months of storage.

Table 3.10. Means and standard deviations as influenced by treatment for b* value in frozen yogurt with hesperidin added.

Treatment	b* value
FY500	16.84 ± 0.95^{A}
FY250	13.64 ± 1.08^{B}
FY125	12.23 ± 0.94^{C}
FY0	8.27 ± 1.49^{D}

^{ABCD} Means ± standard deviations with different letters are significantly different ($P < 0.05$). FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

Table 3.11. Means and standard deviations as influenced by day for b* value in frozen yogurt with hesperidin added.

Day	b* value
0	13.53 ± 0.14^{A}
30	12.49 ± 0.17^{AB}
60	12.22 ± 0.20^{B}

^{AB} Means ± standard deviations with different letters are significantly different ($P < 0.05$).

3.6. Hardness

For hardness measurement, the only the main effect that had statistical difference ($P < 0.05$) was treatment as Table 3.12 shows. Results in Table 3.13 shows that the highest concentration of hesperidin caused the highest results in force (Newton) in Frozen yogurt. This could be related to the higher total solids that the products have, increasing the hardness in the final product (Kurultay

et al., 2010). Ghelich *et al., (*2022) demonstrated that the inclusion of wheat germ protein hydrolysates at 0.5, 1 and 1.5% concentrations significantly increase the hardness in frozen yogurt. Factors such as ice crystal sizes, fat content, overrun, and solid content affects melting rate (Muse and Hartel, 2004).

Table 3.12. Probability (Pr > F) of treatment, day, and its interaction in the hardness measurements of frozen yogurt with hesperidin added.

Effect	Pr > F
Treatment	0.0017
Day	0.1280
Treatment*Day	0.9960

Table 3.13. Means and standard deviations as influenced by treatment for hardness measurements in frozen yogurt with hesperidin added.

Treatment	Hardness (N)
FY500	340.52 ± 71.69^{A}
FY250	256.65 ± 53.76^{B}
FY125	211.37 ± 50.19^{B}
FY0	247.78 ± 80.74^{B}

^{AB} Means ± standard deviations with different letters are significantly different ($P < 0.05$). FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

3.7. Melting rate

Melting rate is a very important characteristic in frozen dairy desserts. This is defined as the resistance to melt which is influenced by content on fat, stabilizers, air cells, initial temperature of the product, ambient temperature, and air cells (Syed *et al.*, 2018). Statistical differences ($P < 0.05$)

were found in the effect of treatment and minute (Table 3.14). The treatment that had the lowest melting rate in this test was FY500 with 50.5 mL (Table 3.15) being statistically different ($P < 0.05$) from the control with 61.0 mL. As was expected, more minutes meant that more product was melted (Table 3.16). Hartel and Musse (2004) established that the main components that influenced melting rate are fat destabilization, ice crystal size, overrun and rheological properties. Moreover, Li *et al.* (2021) demonstrated that polyphenol-protein complexes have the potential to improve the heat stability (melting rate) of the sample. The results obtained in this study are like the ones found by Gabbi *et al*. (2017) where they tested the melting rate properties of an ice cream supplemented with ginger powder rich in polyphenols from 0.50 to 2.0%, and found differences from the control ice cream that did not have ginger. Also, Bilbao *et al.* (2019) added strawberry powder to a dairy frozen dessert enhancing the heat stability.

Table 3.14. Probability (Pr > F) of treatment, minute, day, and their interactions for melting rate measurements in frozen yogurt with hesperidin added.

Effect	Pr > F
Treatment	0.0114
Minute	<.0001
Day	0.3611
Treatment*day	0.8168
Treatment*minute	0.9314
Day*minute	0.4464
Treatment *day*minute	0.9994

Table 3.15. Means and standard deviations as influenced by treatment for melting rate measurements in frozen yogurt with hesperidin added.

Treatment	Melting rate (mL)
FY500	50.56 ± 27.80^{B}
FY250	59.22 ± 28.13^{A}
FY125	57.39 ± 28.37^{A}
FY0	61.06 ± 26.42^{A}

^{AB} Means ± standard deviations with different letters are significantly different ($P < 0.05$). FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

Table 3.16. Means and standard deviations as influenced by minute for melting rate measurements in frozen yogurt with hesperidin added.

Minute	Melting rate (mL)
60	32.00 ± 11.82^{B}
90	82.11 ± 9.52^{A}

^{AB} Means ± standard deviations with different letters are significantly different ($P < 0.05$).

3.8. Bile Tolerance of *Streptococcus thermophilus*

The results of bile tolerance for *S. thermophilus* are shown in Figure 3.6. This test consists in the measurement of the rate of survival of bacteria against bile salts (Ruiz *et al.,* 2013). For bile tolerance counts of *S. thermophilus* the effects that were statistically different ($P < 0.05$) were hour and the interaction day*hour as Table 3.17 shows.

Table 3.18 shows the counts of S. thermophilus as influenced by hour*day that the counts of *S. thermophilus* increased significantly ($P < 0.05$) by the hours passed but also increased when compared at day 7 to day 60 (hour*day interaction). Also, Table 3.19 evidenced that the counts increased significantly ($P < 0.05$) after 4 hours of essay. These results suggest that cold stress

improves the bile tolerance after 53 days of storage. This could be due to the production of cold shock proteins (CSPs) that are produced by lactic acid bacteria during cold stress (Papadimitriou 2016).

These results are similar to the ones that were obtained by Theegala *et al*. (2021) where she added flaxseeds rich in bioactive compounds that did not show a decrease in bacterial counts compared to the control without flaxseeds and remain stable during the 8 hours of measurement at 0.3% of bile salts. Moreover, Iyer *et al*. (2010) tested strains for *S. Thermophilus* for 3 hours at 0.5, 1 and 2% of bile salts in pure broth finding a consistent resistance of *S. thermophilus* against bile salts. *S. thermophilus* showed to be *a* very stable bacteria throughout time evidencing a good resistance against bile salts. Also, after 8 hours under bile salts conditions (Table 3.18), it can be observed that the log counts increased in 0.5 log CFU/g.

Table 3.17. Probability (Pr > F) of treatment, hour, day, and their interactions for counts of bile tolerance of *S. thermophilus* in frozen yogurt with hesperidin added.

Effect	Pr > F
Treatment	0.3587
Hour	<.0001
Day	0.1814
Treatment*day	0.5544
Treatment*hour	0.8009
Day*hour	<.0001
Treatment *day*hour	0.7843

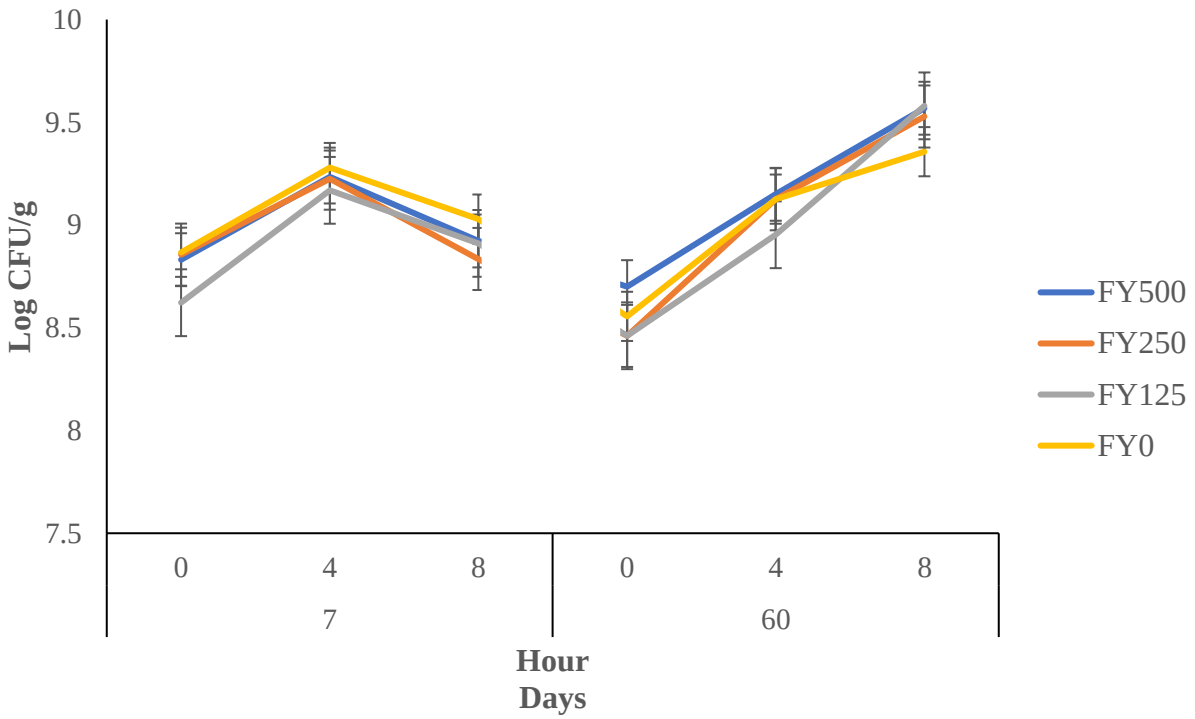

Figure 3.6. Counts of bile tolerance of *S. thermophilus* at day 7 and 60 in frozen yogurt with hesperidin added. FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

Table 3.18. Means and standard deviations as influenced by day*hour for counts of bile tolerance of *S. thermophilus* in frozen yogurt with hesperidin added.

Hour	*S. thermophilus* (Log CFU/g)	
	Day 7	Day 60
0	$8.79\pm0.18^{B,a}$	$8.54\pm0.16^{C,b}$
4	$9.23\pm0.17^{A,a}$	$9.09\pm0.24^{B,a}$
8	$8.92\pm0.30^{B,b}$	$9.51\pm0.15^{A,a}$

^{ABC} Means ± standard deviations with different letters within the column are significantly different ($P < 0.05$). ^{ab} Means ± standard deviations with different letters within the row are significantly different ($P < 0.05$).

Table 3.19. Means and standard deviations as influenced by hour for count of bile tolerance of *S. thermophilus* in frozen yogurt with hesperidin added.

Hour	*S. thermophilus* (Log CFU/g)
0	8.67 ± 0.21^{B}
4	9.16 ± 0.21^{A}
8	9.22 ± 0.38^{A}

^{AB} Means ± standard deviations with different letters are significantly different ($P < 0.05$).

3.9. Bile Tolerance of *Lactobacillus bulgaricus*

L. bulgaricus bile tolerance counts as influenced by hesperidin are shown in Figure 3.7. The main effects of day, hour, and day*hour were statistically different ($P < 0.05$) (Table 3.20). Counts influenced by day*hour effect are shown in Table 3.21. It is important to highlight that from day 7 to day 60 an increase was observed in the counts of *L. bulgaricus* (Table 3.22). On the other hand, after 8 hours under 0.3% of bile salts, the counts of this bacteria were decreased approximately 0.8 Log CFU/g, representing a reduction of 11% (Table 3.23). Once again, the differences were less than 1 Log CFU/g.

These results are different from the ones obtained by Vargas *et al.* (2015) where they studied the influence of whey protein (1, 2 and 3%) in the bile tolerance of *L. bulgaricus.* Vargas found that bacteria count in a period of 5 hours decreased approximately 3 to 4 log CFU/g. These differences could be explained due to the difference of matrixes where the bacteria were tested since the experiment conducted by Vargas was done in pure broth while the present experiment was done in yogurt matrix (Boke *et al.,* 2010). Additionally, Vargas highlighted the role of whey protein in the protection of *L. bulgaricus* since its survival rate increased. Moreover, Muramalla and Aryana (2011) tested bile tolerance of *L. bulgaricus* in different levels of homogenization in skim milk

finding a reduction of less than 1 Log CFU/g. This means that *L. bulgaricus* and *S. thermophilus* can be considered good probiotic bacteria in terms of bile resistance under a frozen yogurt food matrix. This resistance against bile salts could be explained due to the production of exopolysaccharides (EPS) produced by lactic acid bacteria (LAB) in yogurt manufacture (Suzuki, 2014).

Table 3.20. Probability (Pr > F) of treatment, hour, day, and their interactions for counts of bile tolerance of *L. bulgaricus* in frozen yogurt with hesperidin added.

Effect	Pr > F
Treatment	0.0638
Hour	0.0002
Day	0.0051
Treatment*day	0.8971
Treatment*hour	0.9626
Day*hour	0.0129
Treatment *day*hour	0.9678

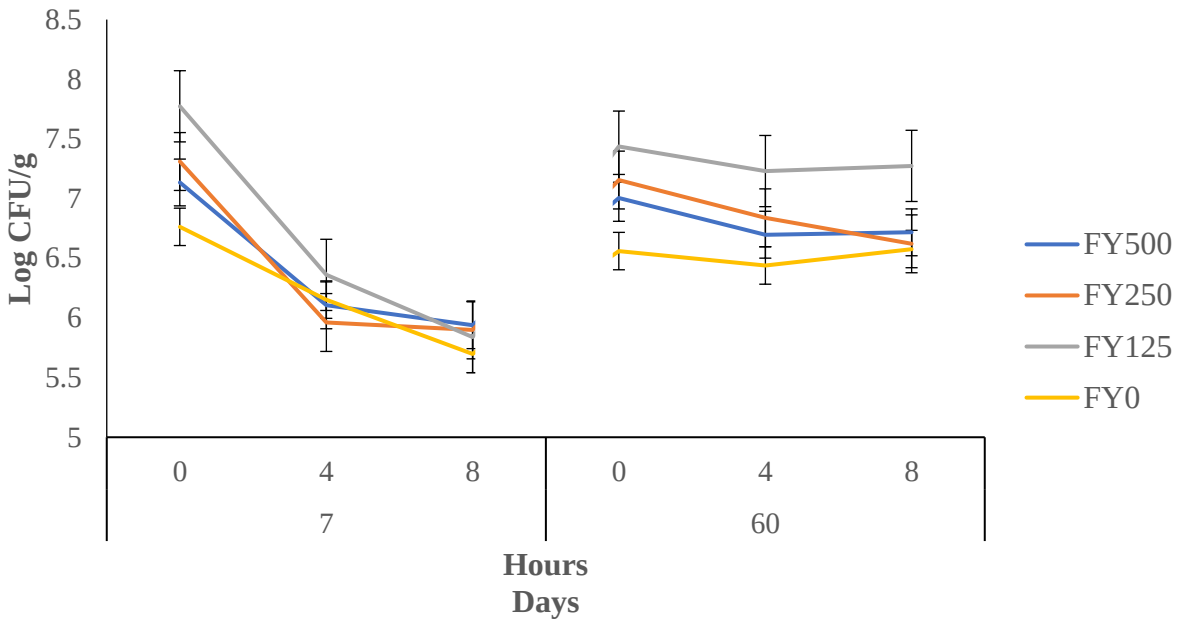

Figure 3.7. Counts of bile tolerance of *L. bulgaricus* at day 7 and 60 in frozen yogurt with hesperidin added. FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

Table 3.21. Means and standard deviations as influenced by day*hour for counts of bile tolerance of *L. bulgaricus* in frozen yogurt with hesperidin added.

Hour	*L. bulgaricus* (Log CFU/g)	
	Day 7	Day 60
0	$7.25\pm0.67^{A,a}$	$7.04\pm0.65^{A,a}$
4	$6.15\pm0.56^{B,b}$	$6.80\pm0.59^{A,a}$
8	$5.84\pm0.71^{B,b}$	$6.80\pm0.58^{A,a}$

[ABC] Means ± standard deviations with different letters within the column are significantly different ($P < 0.05$). [ab] Means ± standard deviations with different letters within the row are significantly different ($P < 0.05$).

Table 3.22. Means and standard deviations as influenced by day for counts of bile tolerance of *L. bulgaricus* in frozen yogurt with hesperidin added.

Day	*L. bulgaricus* (Log CFU/g)
7	6.41 ± 0.88^{B}
60	6.88 ± 0.60^{A}

^{AB} Means ± standard deviations with different letters are significantly different ($P < 0.05$).

Table 3.23. Means and standard deviations as influenced by hour for counts of bile tolerance of *L. bulgaricus* in frozen yogurt with hesperidin added.

Hour	*L. bulgaricus* (Log CFU/g)
0	7.14 ± 0.65^{A}
4	6.47 ± 0.66^{B}
8	6.32 ± 0.80^{B}

^{AB} Means ± standard deviations with different letters are significantly different ($P < 0.05$).

3.10. Acid tolerance of *Streptococcus thermophilus*

Acid tolerance can be defined as "the induced resistance to a normally lethal low pH challenge following growth or exposure at moderately low pH" (Dodd, 2014). Results of acid tolerance of *S. thermophilus* are shown in Figure 3.8. For *S. thermophilus* acid tolerance main factors influenced by hesperidin were day, hour, and the interaction day*hour were statistically different ($P < 0.05$) (Table 3.24). The counts resulted in the interaction day*hour appears in Table 3.25.

After storage period, the counts of *S. thermophilus* were reduced significantly by almost 2 logs CFU/g as Table 3.26 indicates. Moreover, from hour 0 to 8, the reduction of the bacterial count was more than 4 log CFU/g as Table 3.27 shows. This could be provoked due to the prolonged stress of freezing temperatures that bacteria were stored (Elhanafi *et al.*, (2004) making it more vulnerable to post stress conditions. The results obtained in this study are like the ones found by

Mena & Aryana (2020) where they studied the influence of lactose in *S. thermophilus* acid tolerance. They demonstrated that after 120 minutes of incubation at pH 2.0 the bacteria count was reduced by 4 log CFU/g.

Table 3.24. Probability (Pr > F) of treatment, hour, day, and their interactions for counts of acid tolerance of *S. thermophilus* in frozen yogurt with hesperidin added.

Effect	Pr > F
Treatment	0.8483
Hour	<.0001
Day	<.0001
Treatment*day	0.9318
Treatment*hour	0.9992
Day*hour	0.0032
Treatment *day*hour	0.9950

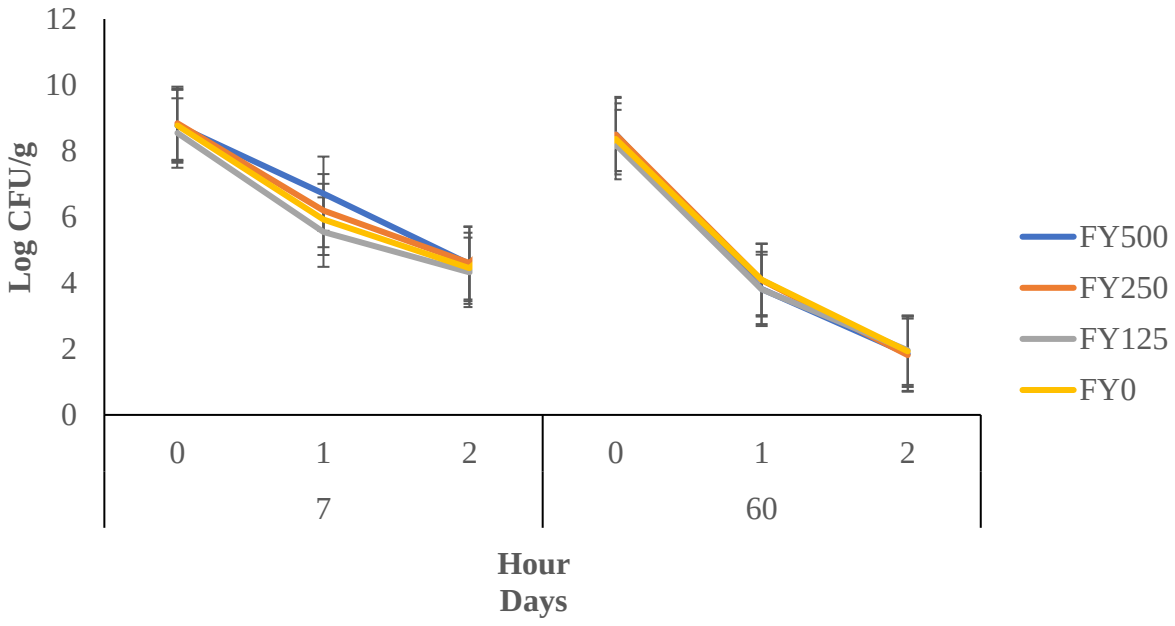

Figure 3.8. Counts of acid tolerance of *S. thermophilus* at day 7 and 60 in frozen yogurt with hesperidin added. FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

Table 3.25. Means and standard deviations as influenced by hour*day for counts of acid tolerance of *S. thermophilus* in frozen yogurt with hesperidin added.

Hour	*S. thermophilus* (Log CFU/g)	
	Day 7	Day 60
0	$8.73 \pm 0.19^{A,a}$	$8.39 \pm 0.24^{A,a}$
1	$6.09 \pm 1.52^{B,a}$	$3.95 \pm 0.50^{B,b}$
2	$4.49 \pm 2.46^{C,a}$	$1.89 \pm 0.50^{C,b}$

[ABC] Means ± standard deviations with different letters within the column are significantly different ($P < 0.05$). [ab] Means ± standard deviations with different letters within the row are significantly different ($P < 0.05$).

Table 3.26. Means and standard deviations as influenced by day for counts of acid tolerance of *S. thermophilus* in frozen yogurt with hesperidin added**.**

Day	*S. thermophilus* (Log CFU/g)
7	6.44 ± 2.41^{A}
60	4.75 ± 2.78^{B}

[AB] Means ± standard deviations with different letters are significantly different ($P < 0.05$).

Table 3.27. Means and standard deviations as influenced by hour for counts of acid tolerance of *Streptococcus thermophilus* in frozen yogurt with hesperidin added**.**

Hour	*S. thermophilus* (Log CFU/g)
0	8.56 ± 0.27^{A}
4	5.02 ± 1.55^{B}
8	3.19 ± 2.19^{C}

[AB] Means ± standard deviations with different letters are significantly different ($P < 0.05$).

3.11. Acid tolerance of *Lactobacillus bulgaricus*

L. bulgaricus acid tolerance as influenced by hesperidin is shown in Figure 3.9. Statistical differences ($P < 0.05$) were found in the effects of treatment, minute, and day*minute (Table 3.28). The treatment FY125 had the highest counts in this measurement as Table 3.29 shows.

As Table 3.30 shows, the more minutes passed, the less counts of *L. bulgaricus* were observed. The results of the interaction minute*day are shown in Table 3.31 representing the same trend. A low resistance of *L. bulgaricus* against pH adverse conditions was observed. Similar results were found by Boke *et al.* (2010) where two out of four strains of *L. bulgaricus* tested in pH 2. Two strains did not survive (0%) and the other two survived by less than 50%. This makes evident the poor resistance of *Lactobacillus bulgaricus* against pH. Even the buffer capacity of proteins contained in the frozen yogurt were not enough to prevent the kill of bacteria under these conditions (Nadal *et al*., 2010).

Table 3.28. Probability (Pr > F) of treatment, minute, day, and their interactions for counts of acid tolerance of *L. bulgaricus* in frozen yogurt with hesperidin added.

Effect	Pr > F
Treatment	0.0200
Minute	<.0001
Day	0.5368
Treatment*day	0.7373
Treatment*minute	0.9596
Day*minute	0.0035
Treatment *day*minute	0.8752

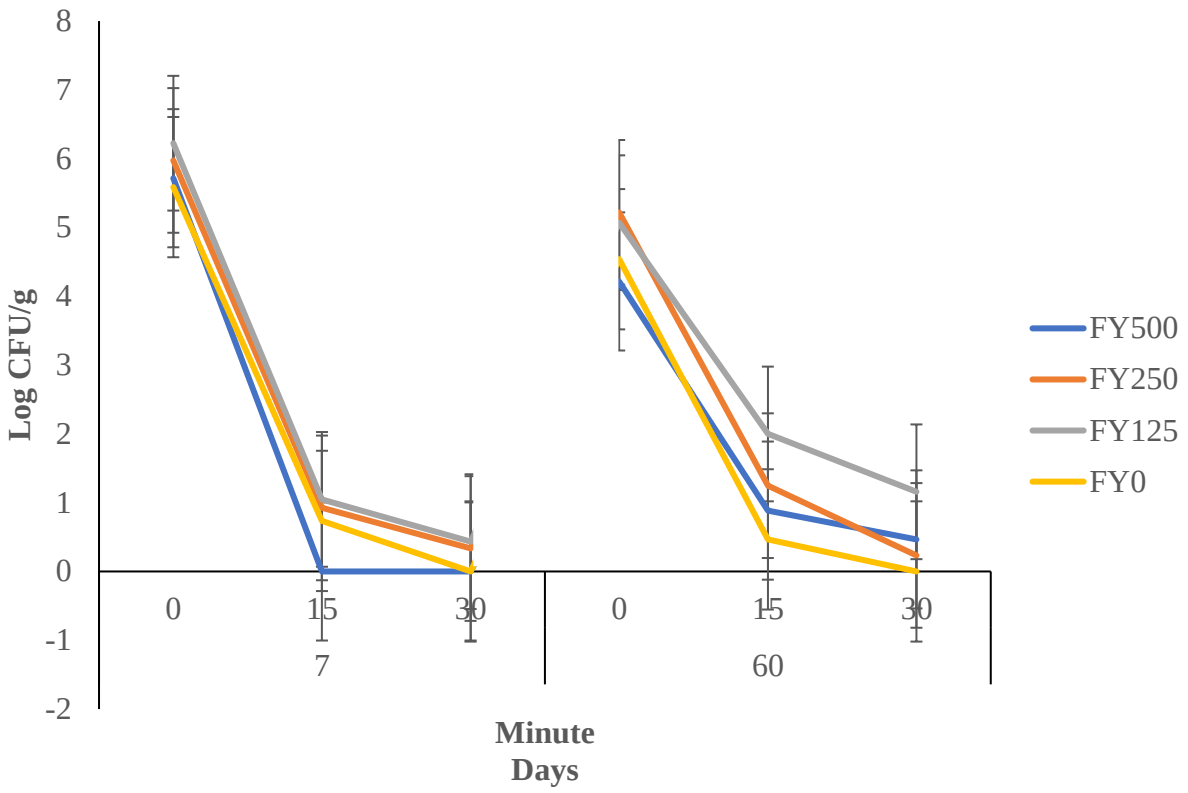

Figure 3.9. Counts of acid tolerance of *L. bulgaricus* at day 7 and 60 in frozen yogurt with hesperidin added. FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

Table 3.29. Means and standard deviations as influenced by treatment for counts of acid tolerance of *L. bulgaricus* in frozen yogurt with hesperidin added.

Treatment	*L. bulgaricus* (Log CFU/g)
FY500	1.89 ± 2.47^{B}
FY250	2.32 ± 2.58^{AB}
FY125	2.66 ± 2.49^{A}
FY0	1.88 ± 2.36^{B}

^{ABCD} Means ± standard deviations with different letters are significantly different ($P < 0.05$). FY500 = Frozen yogurt with 500 mg of hesperidin per serving of 90 g. FY250 = Frozen yogurt with 250 mg of hesperidin per serving of 90 g. FY125 = Frozen yogurt with 125 mg of hesperidin per serving of 90 g. FY0 = Frozen yogurt without the addition of hesperidin.

Table 3.30. Means and standard deviations as influenced by minute for counts of acid tolerance of *L. bulgaricus* in frozen yogurt with hesperidin added**.**

Minute	*L. bulgaricus* (Log CFU/g)
0	5.32 ± 1.12^{A}
15	0.91 ± 1.13^{B}
30	0.33 ± 0.65^{C}

^{ABC} Means ± standard deviations with different letters are significantly different ($P < 0.05$).

Table 3.31. Means and standard deviations as influenced by minute*day for counts of acid tolerance of *L. bulgaricus* in frozen yogurt with hesperidin added.

Minute	*L. bulgaricus* Log CFU/g	
	Day 7	Day 60
0	$5.88 \pm 1.04^{A,a}$	$4.76 \pm 0.93^{A,b}$
15	$0.68 \pm 1.24^{B,a}$	$1.15 \pm 0.99^{B,a}$
30	$0.19 \pm 0.45^{C,a}$	$0.46 \pm 0.80^{C,a}$

^{ABC} Means ± standard deviations with different letters within the column are significantly different ($P < 0.05$). ^{ab} Means ± standard deviations with different letters within the row are significantly different ($P < 0.05$).

3.12. Sensory analysis

The most relevant attributes (appearance, color, aroma, texture, iciness, flavor, sourness, and overall liking) results are shown in Table 3.32. No statistical differences ($P < 0.05$) were found on the attributes of appearance, color, aroma. On the other hand, texture, iciness, flavor, sourness, and overall liking were different statistically ($P < 0.05$.). The highest concentrations of hesperidin (FY500) influenced on how the consumers reacted on the sourness and overall liking, obtaining lower scores. This could happen because the flavonoid concentration masked the sweet-sour taste

by its bitterness (Soares *et al*., 2013). This can be related due to the bitter taste that hesperidin and other polyphenols have (Huang *et al.*, 2021).

It is important to highlight that treatment FY125 and control were not statistically different ($P < 0.05$) in liking scores of any sensory attributes. Making it statistically the same ($P < 0.05$) in all the parameters studied in this analysis.

Table 3.32. Consumer liking score means and standard deviations of frozen yogurts with hesperidin added.

Attributes	FY500	FY250	FY125	FY0
Appearance	6.37 ± 1.59^{A}	6.74 ± 1.53^{A}	6.50 ± 1.72^{A}	6.72 ± 1.34^{A}
Color	6.43 ± 1.51^{A}	6.69 ± 1.53^{A}	6.67 ± 1.47^{A}	6.74 ± 1.32^{A}
Aroma	5.79 ± 1.45^{A}	5.87 ± 1.70^{A}	5.57 ± 1.57^{A}	5.66 ± 1.73^{A}
Texture	6.58 ± 1.66^{AB}	6.25 ± 1.80^{B}	6.84 ± 1.55^{AB}	7.10 ± 1.58^{A}
Iciness/Sandiness	6.20 ± 1.87^{AB}	6.11 ± 1.78^{B}	6.41 ± 1.59^{AB}	6.77 ± 1.69^{A}
Flavor	5.40 ± 2.23^{B}	5.32 ± 2.23^{B}	5.83 ± 2.23^{AB}	6.43 ± 2.14^{A}
Sourness	4.99 ± 2.18^{B}	5.10 ± 2.10^{B}	5.48 ± 2.19^{AB}	6.00 ± 2.06^{A}
Overall liking	5.66 ± 2.09^{B}	5.42 ± 2.12^{B}	5.96 ± 2.03^{AB}	6.63 ± 1.88^{A}

^{ABC} Means ± standard deviations with the same letter within the row are not significantly different ($P < 0.05$).

Statistical differences were found ($P < 0.05$.) (Table 3.33) for purchase intent before and after the health claim in all the treatments which contained hesperidin. As expected, health claims have a positive impact on purchase intent of the consumers (Kaur *et al*., 2017).

Table 3.33. Distribution and probability (Pr > F) of the purchase intent before and after a health statement of the frozen yogurt with hesperidin added.

Treatment	Purchase intent before health claim (%)		Purchase intent after health claim (%)		Pr > F
	Yes	No	Yes	No	
FY500	38.83	61.17	58.25	41.75	<.0001
FY250	38.83	61.17	53.40	46.60	0.0007
FY125	50.49	49.51	65.05	34.95	0.0007
FY0	66.99	33.01	65.05	34.95	0.7905

CHAPTER 4. SUMMARY AND CONCLUSION

This study showed that the effect addition in three different levels of hesperidin (500, 250 and 125 mg/90g of product) did not influence the fermentation process, overrun and viscosity. The higher concentration of hesperidin statistically increased the characteristics of hardness and decreased melting rate. Usage of hesperidin did not decrease significatively counts of *S. thermophilus* and *L. bulgaricus* for 60 days of storage. L* and b* values were affected due to the color of this flavonoid. Moreover, the addition of hesperidin did not impact negatively on the survival rate of probiotic bacteria under pH and bile salts adverse conditions. The concentration 125 mg/90g of hesperidin had no statistical differences compared to the control on all the liking attributes in the sensory analysis. Finally, the addition of hesperidin positively impacted on the purchase intent after a health claim. Hesperidin seems to be an alternative for a market who demands healthier products. It is recommended to try other types of polyphenols and concentrations *in vitro* and *in vivo* models.

APPENDIX A. CONSENT FORM FOR CONSUMER STUDY

Consent frozen Yogurt

Q1 Research Consent Form
I, _____, agree to participate in the research entitled **"Consumer perception of Frozen Yogurt"** conducted by Dr. Aryana Kayanush, Professor of the School of Nutrition and Food Sciences at Louisiana State University, Agricultural Center, phone number (225) 578 4380.

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. For this particular research, about 5-10 minutes of participation will be required for each consumer.

Exclusion criteria:
- Children under the age of 18
- Pregnant Women

The following points have been explained to me:

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

2. The reason for the research is to gather information on consumer perception of frozen yogurt. The benefit that I may expect from it is the satisfaction that I have contributed to the solution and evaluation of problems relating to such examinations.

3. The procedures are as follows: 4 coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on the online questionnaire. 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 that of an allergy or an adverse reaction to dairy products, maltodextrin, hesperidin, and sugar.** However, because it is known to me beforehand that the food to be tested contains these common food ingredients, 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, Agricultural Center, 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 Keenan, Chair of LSU AgCenter IRB, (225) 578-1708.

I agree with the terms above and acknowledge. Please type in your first and last name below if you agree with the terms above and acknowledge:

_____

APPENDIX B. SENSORY STUDY EXEMPTION FORM

TO:	Kayanush J Aryana LSUAG | Dept | Nutrition and Food Sciences | CC00942
FROM:	Michael Keenan Chair, Institutional Review Board
DATE:	19-Aug-2022
RE:	IRBAG-22-0076
TITLE:	Influence of a polyphenol on the sensory characteristics of frozen yogurt.
SUBMISSION TYPE:	Initial Application
Review Type:	Exempt
Risk Factor:	Minimal
Review Date:	19-Aug-2022
Status:	Approved
Approval Date:	19-Aug-2022
Approval Expiration Date:	18-Aug-2025
Re-review frequency:	(three years unless otherwise stated)
Number of subjects approved:	300
LSU Proposal Number:	

By: Michael Keenan, Chair

Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU's Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the

individual participants, including notification of new information that might affect consent.

6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
7. Notification of the IRB of a serious compliance failure.
8. **SPECIAL NOTE: When emailing more than one recipient, make sure you use bcc. Approvals will automatically be closed by the IRB on the expiration date unless the PI requests a continuation.**

** All investigators and support staff have access to copies of the Belmont Report, LSU's Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents.*

Mike Keenan O 225-578-1708
209 Knapp Hall
Baton Rouge, LA 70803

O 225-578-1708
F 225-578-4443

APPENDIX C. QUESTIONARE FOR CONSUMER STUDY

Gender

Male

Female

Non-binary / third gender

Prefer not to say

Age:

18-25

26-35

36-45

46-59

60+

Race/Ethnicity

White / Caucasian

Black or African American

American Indian or Alaska Native

Asian

Native Hawaiian or Other Pacific Islander

Latino

From multiple races

Other

Please drink water and eat unsalted crackers to cleanse your palate.

Without tasting,

Please rate you liking of the **APPEAREANCE** of sample **420**

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Please rate you liking of the **COLOR** of sample **420**

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Please rate you liking of the **AROMA** of sample **420**

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

PLEASE TASTE SAMPLE 420 AND ANSWER THE FOLLOWING QUESTIONS:

Please rate you liking of the **TEXTURE** of sample **420**

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Please rate you liking of the **SANDINESS** of sample **420**

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Please rate you liking of the **FLAVOR** of sample **420**

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Please rate you liking of the **SOURNESS** of sample **420**

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Please rate **OVERALL LIKING** of sample **420**

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

How likely would you purchase this product?

YES NO

Sample 420 contains hesperidin which is a polyphenol that might provide health benefits as an antioxidant.

Knowing this additional information, how likely would you purchase this product?

YES NO

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