DEVELOPMENT OF A CO-FERMENTED YOGURT-LIKE PRODUCT USING LACTIC ACID-PRODUCING YEAST AND FLAVOR-SPECIFIC YEAST STRAINS

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Master of Food Science

by

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ABSTRACT

Advances in fermentation are changing how food products are created and present a chance at developing novel dairy products. Newly engineered yeasts that produce lactic acid have yielded to the development of new yogurt-like products. Based on recent work, this study aimed to create a more complete flavor profile to this yogurt-like product by adding an additional yeast strain known to produce aromatic compounds in beer or wine. After preliminary tests, two flavor producing strains were selected to be scaled up. Fermentation profiles were monitored for pH, and cell populations were verified with plating before and after the fermentation. Additionally, using the Cornell Dairy Plant's yogurt base, the yogurt-like product was scaled up and evaluated for consumer acceptance in a sensory evaluation. Based on the results of the sensory study and fermentation profiles, there exists a market for such products, though some further development can still be implemented.

BIOGRAPHICAL SKETCH

Marisa Morán Cepeda graduated from the University of California, San Diego with a Bachelor's Degree in Nanoengineering focused on Material Science and a Minor in Environmental Systems in 2022.

She began her professional journey as an R&D intern at a plant-based protein manufacturer, later being promoted to a Research Associate. There she cultivated duckweed and developed experiments pertaining to decontamination and strain evalutation for a year. From there, Marisa transitioned to the biotechnology industry, where she worked as a Fermentation Research Associate for six months. Her experience at Debut Biotech made Marisa take an interest in fermentation.

In the Fall of 2023, she started her professional Master's Degree in Food Science at Cornell University. She was a member of the Alcaine Research Group and aimed to expand her technical knowledge and understanding of food science.

Quiero dedicar este estudio a mi familia, quienes son mi mayor apoyo. Gracias por siempre estar a mi lado. Los amo más que todos los números.

ACKNOWLEDGMENTS

First, I would like to express my gratitude to my advisor, Sam Alcaine. Sam, thank you for your support and guidance. I always appreciated the enthusiasm and ideas you brought to this project. Your support, advice, and mentorship have been integral to my success in the Master of Food Science program.

I would also like to give a special thanks to my lab mates, the members of the Alcaine Research Group. A special thanks to Tim DeMarsh and Paola Gamboa Moreno, I will forever be so appreciative for all of your support, kind words, and for always keeping me grounded when things inevitably went wrong. Your mentorship did not go unnoticed and I will take the skills, both of you taught me with me in my future endeavors. A big thank you is also due to everyone at the Sensory Evaluation Center, who helped make our study a success.

My deepest gratitude goes out to the friends I have made at Cornell. I am so grateful to have found such an incredible community who supported me every step along the way. Thank you for all the memories and for being by my side on this journey. That being said, thank you to all my friends back home, who though physically far were always in my thoughts. Thank you for all the video calls, sweet messages, and unwavering support.

Lastly, I would like to express my sincerest appreciation to my family. Your support and faith in me has been such a source of motivation. I am forever grateful for your encouragement to change career paths and for always motivating me when I need it the most. I would especially like to thank my brother for always believing in me and pushing me to be the best version of myself. I don’t think I would’ve taken the leap of faith to even consider this master’s degree without his encouragement.

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INTRODUCTION

Historically, lactic acid bacteria (LAB) have been used in the production of dairy products like yogurt, cheese, and kefir. The presence of LAB in these products can be attributed to spontaneous fermentation or inoculated started cultures. These species help drive fermentation and improve food safety, sensory attributes, and nutritional profiles of food products. Homofermentative species of LAB convert sugars in milk mostly into lactic acid, while heterofermentative species convert lactose into lactic acid, acetic acid, ethanol and CO_2. Hence the fermentation process acidifies the milk which prevents the growth of spoilage and pathogenic microorganisms due to competition among species (Widyastuti et al., 2014). However, it is key to note that LAB cultures are still susceptible to viruses such as bacteriophages.

Bacteriophages or phages are viruses that can infect and replicate within bacterial cells. Phages attack bacterial DNA and ultimately kill the culture (Szczepankowska et al., 2013). Overall, bacteriophages negatively affect the safety and quality of fermented milk products. Phage outbreaks yield huge economic losses, especially since affected products must be discarded and large efforts must go into sanitation. Mitigation strategies include stringent hygiene, compartmentalization of dairy plant production units, use of multi-strain starters, and rotation of starter cultures (Kleppen et al., 2013). One promising solution to reduce the bacteriophage impact on the dairy industry is the use of phage resistant cultures, like lactic acid-producing yeast (LAY).

Saccharomyces cerevisiae is a widely used yeast strain in the production of alcoholic beverages such as wine, beer, ciders, among others. It can ferment sugar into alcohol and produce a wide range of flavors and aromas (Walker et al., 2016). Wild-type *S. cerevisiae* cannot utilize lactose as a carbon source. However, Sourvisiae®, a bioengineered strain of *S. cerevisiae*, is capable of producing lactic acid as well as ethanol during fermentation. This strain contains a lactate dehydrogenase gene from a food microorganism which allows the yeast to produce lactic acid on its own and alongside other brewing yeast strains. According to the manufacturer, Sourvisiae® does not produce flavor compounds associated with *Lachancea*, *Brettanomyces*, or LAY (Lallemand Brewing, 2024).

The commercially available LAY strains can't utilize lactose in the same way LAB can, therefore the addition of lactose is needed to the culture in order to begin the fermentation process. This could serve as a control point for the fermentation since some lactases inactivate at lower pHs. These pH sensitive lactases could lose activity once the LAY-based products drop below pH 4. Based on previous work done by Bihongee Sarkar and Paola María Gamboa Moreno, two members of ARG, they determined that the optimal lactase enzyme was Maxilact A4, the best pitch rate for just the Sourvisiae® yogurt was 7 logs, and the ideal temperature is 30°C.

Furthermore, LAY strains, like Sourvisiae®, could be the basis for developing novel yeast-fermented yogurt-like products. New flavors associated with yeasts could be incorporated into these products. Yeast strains can produce a wide range of aromatic compounds from floral to pepper notes, which often contribute to the complex flavors and aromas in wines and beers. Furthermore, by combining an acidifying LAY strain with a flavor producing yeast strain new dairy products can be produced with complex flavor profiles. This is similar to the work products fermented with current LAB cultures, yet these yeast strains used for flavor, would also not be susceptible to bacteriophage either.

For this study, the flavor-producing yeast strains were selected based on common flavors seen in yogurt products, along with commercial availability. All five strains used in this study were bioengineered from *S. cerevisiae.* Table 1 summarizes the different flavor notes associated with each strain.

Name	Flavor Notes	Distributor
Bavarian Wheat (OYL-034)	Banana, light pear, apple/plum, clove and vanilla	Omega Yeast
French Saison (OYL-026)	Pepper and citrus	Omega Yeast
Cosmic Punch (OYL-402)	Grapefruit, passion fruit and guava	Omega Yeast
Sunburst™	Pineapple	Berkeley Yeast
Superbloom™	Orange blossom, geranium, lime peel, and lemon zest	Berkeley Yeast

TABLE 1. Summary of flavors produced by each flavor-producing strain and their distributors

The aim of this study was to develop a co-fermented yogurt-like product using Sourvisiae® and a flavor-producing yeast. The objective was to determine the ideal concentrations needed to use these yeasts as starters so that it can mimic the fermentation of traditional bacterial starter culture. Furthermore, once the products were developed, the process was scaled-up to a 10L scale and a consumer panel was run to determine the acceptability of these new fermented products.

MATERIALS AND METHODS

Materials

Active dry form of Sourvisiae® (bioengineered beer brewing yeast strain of Saccharomyces cerevisiae) was obtained from Lallemand Brewing and stored in the 4°C refrigerator in the Alcaine Research Group (ARG). Sunburst™ and Superbloom™ (bioengineered beer brewing yeast strains of Saccharomyces cerevisiae) liquid cultures were obtained from Berkeley Yeast and stored in the -80°C refrigerator in glycerol stocks in the ARG lab. Bavarian Wheat II, Cosmic Punch, and French Saison (bioengineered beer brewing yeast strain of Saccharomyces cerevisiae) were obtained from streaked plated from the Gibney Lab, but originally those were purchased from Omega Yeasts. These strains were also prepared into glycerol stocks and stored in the -80°C refrigerator in glycerol stocks in the ARG lab. A commercial brand of Ultra-High Treated (UHT) skim milk was purchased from Amazon. The lactase enzyme, Maxilact A4, was purchased from DSM Food & Beverage and was stored in the 4°C refrigerator of the ARG lab. The Cornell Dairy Plant yogurt base was obtained from the Cornell Dairy Plant and stored in the 4°C refrigerator of the ARG lab.

Growing up the Cultures

First, the corresponding isolates (not including the dried Sourvisiae®) used for that run's experiment were streaked onto potato dextrose agar plus chloramphenicol (PDA+CAM) plates. The plates were incubated plates at 30°C until colonies were present, which occurred approximately 24 hours later. 12°Plato dry malt extract (DME) broth was prepared and sterilized using a 0.2µm filter. In 5mL tubes of DME, one colony of each streaked isolate was placed in one "over-night" (ON). In addition, one negative control tube was always prepared, to ensure no contamination had occurred and only the yeast colonies were present. The tubes were then all incubated at 30°C with shaking at 200 rpm. About 24 hours later, the tubes were inspected for turbidity. The negative control was always verified to have no turbidity. Each ON tube was then pitched into a flask with 400mL of DME and incubated at 30°C with shaking at 200 rpm for another 24 hours. For each culture, the cell concentrations were quantified using a hemocytometer. When concentrations reach usable levels, they are transferred to the 4°C refrigerator.

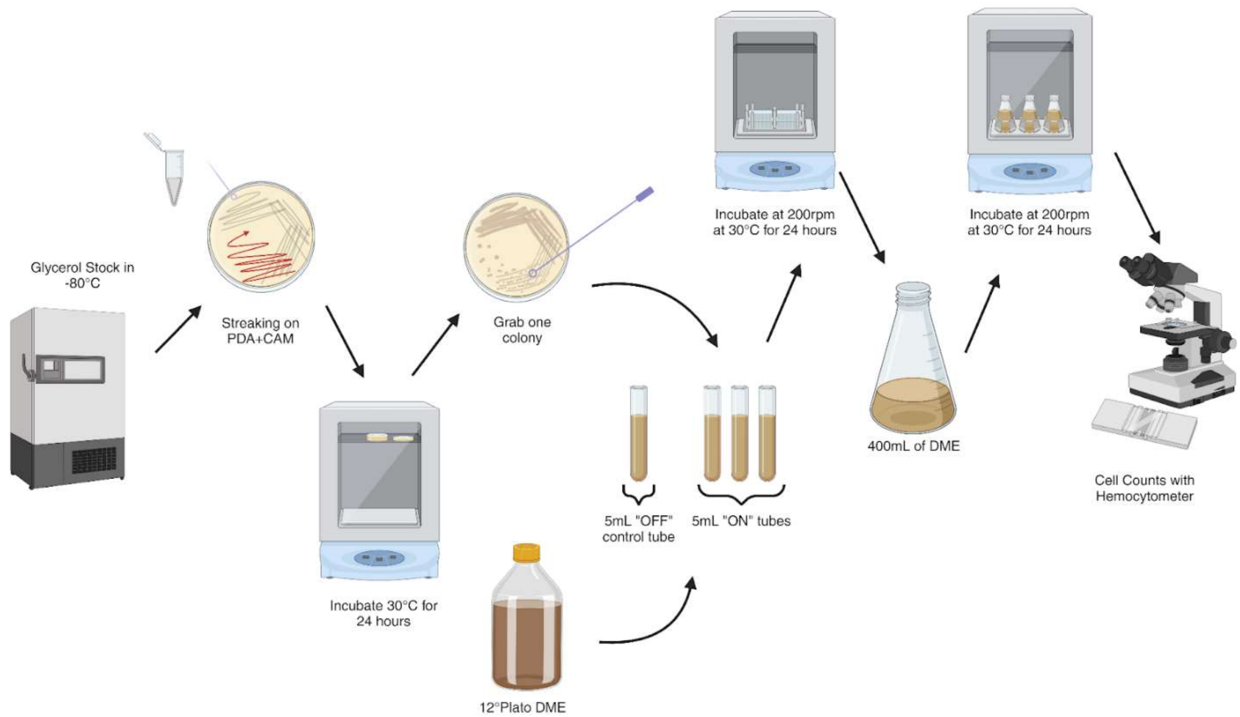

FIGURE 1. Method to grow flavor-producing yeasts from glycerol stock

Fermentation Setup

Before the fermentation was set up, all the necessary supplies were procured. The total UHT milk necessary was transferred to one vessel and homogenized by being inverted twenty-five times. The UHT milk was aliquoted into specified amounts and incubated in a 30°C water bath. For the initial trials, each treatment was filled with 200mL of UHT milk, but this amount was later scaled-up to 2 liters and 10 liters. The iCinac pH probes were calibrated and a 10% bleach solution was prepared to be later used to sterilize the probes.

Next to a flame, each starter was measured and transferred into conical tubes. They were then spun down in a centrifuge at 4000 rpm for 2 minutes. After centrifuging all the starters, the supernatants were poured out and discarded. The pellets were resuspended in 50mL of sterile 1x phosphate-buffered saline (PBS) solution. During this time, the appropriate amount of dry Sourvisiae® was weighed, and rehydrated in a conical tube with 50mL of sterile 1x PBS solution. The conical tubes were once again centrifuged at 4000 rpm for 2 minutes. During this time, the milk bottles were removed from their water baths and the previously calibrated iCinac pH probes were placed in a tube of 10% bleach for 10 minutes. The supernatants were all poured out and discarded. Each starter was then pitched into the appropriate substrate bottle and a calculated amount of lactase was added. Each bottle was then inverted twenty-five times to be homogenized. A 1mL sample was then taken from each treatment, transferred to an autoclaved microcentrifuge tube, and placed in the 4°C refrigerator.

Next, an additional setup was attached to each treatment bottle to make sure continuous pH measurements, temperature control, and a sterile environment was maintained during the entire fermentation period. Each bottle was equipped with a sterile polyvinyl chloride septum with a larger hole in its center and an additional small orifice. A pipette tip was attached aseptically to a sterile high-grade polyvinyl chloride (PVC) flexible tubing and fitted to the small septum orifice. The end of the PVC tube was placed inside of an airlock water bottle. A calibrated and sterilized pH probe was inserted vertically in the larger center hole of the septum. This probe was then attached and to an iCinac unit, which measured the pH for each treatment throughout the run. The bottles were placed in a clean water bath at 30°C for the duration of the experiment. The 1ml samples of inoculated milk samples were then serially diluted in 1x PBS solution based on appropriate concentration. These samples were then plated in replicates on PDA+CAM plates. Each replicated concentration plate was incubated at 30°C for a duration appropriate for about 48 hours.

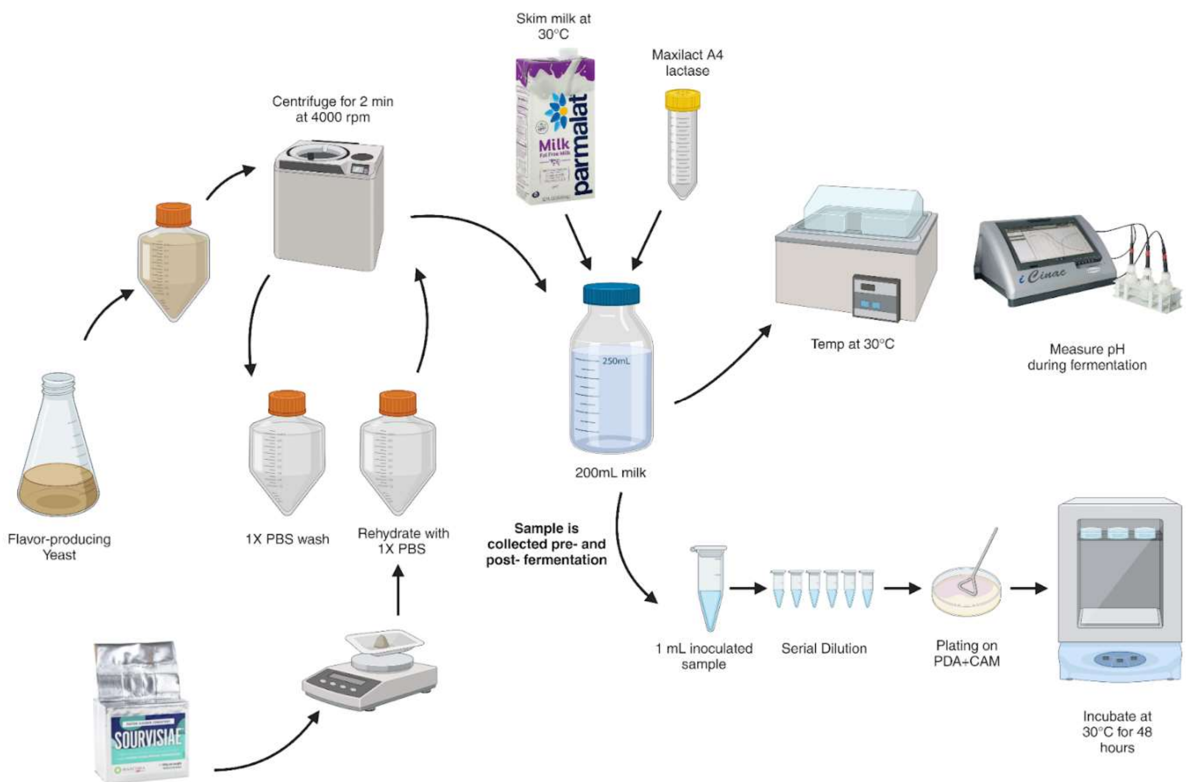

FIGURE 2. Design of co-fermentatation experiments

Post-Fermentation

Next to a flame, the remaining fermentate was transferred to a stomacher bag. The bag was inserted into the stomacher for 1 minute at 230 rpm. This made a homogeneous mixture of the whey and curd. A 1mL sample was taken to be serially diluted and similarly, using the protocol mentioned above. Duplicate 5mL samples were taken to measure lactose, glucose, and galactose. Duplicate 50mL samples were taken to measure titratable acidity. These samples were stored at -80°C, but not analyzed for this study.

Data Collection

The pH was measured using iCinac throughout the incubation period every 15 minutes. Using an iCinac pH probe, the pH was taken every 15 minutes. Samples for cell counts were taken at the beginning of the incubation and after the fermentation was completed. These samples were plated in duplicates according to the protocol above. Once the replicate plates contained the appropriate numbers of CFUs within the countable range, they were counted using a Chemopharm®Color QCount model 530.

Preliminary Test

Once the five flavor-producing yeast strains were obtained, a small preliminary test was run to determine the amount of starter needed to inoculate 200mL of milk. This experiment also served as a way to discard any strains whose aroma was unpleasant or redundant in the study. First, all five flavor producing yeast isolates were streaked onto PDA+CAM plates. The plates were incubated plates at 30°C until colonies were present, which occurred approximately 24 hours later. In 5mL tubes of DME, one colony of each streaked isolate was placed in one "over-night" (ON). In addition, one negative control tube was always prepared. The tubes were then all incubated at 30°C with shaking at 200 rpm. About 24 hours later, the tubes were inspected for turbidity. The negative control was always verified to have no turbidity. Each ON tube was then pitched into a flask with 150mL of DME and incubated at 30°C with shaking at 200 rpm for another 24 hours. For each culture, the cell concentrations were quantified using a hemocytometer.

Inoculation Rate of Yeasts

The purpose of these experiments was to determine the inoculation rate of both Sourvisiae® and the flavor-producing yeasts. Over nine different weeks, different pitch rates were tested along with some additional small tests as seen in Table 2. This process included much trial and error so not every experiment and treatment had a biological replicate.

Name	Treatments	Scale	Fermentation Length
Week 1	Sourvisiae® from prepared glycerol stock at $1x10^8$ CFU/mL and flavor strains (Sunburst™, Superbloom™, Cosmic Punch) at $1x10^8$ CFU/mL	200mL	24 hours
Week 2	Sourvisiae® from prepared glycerol stock at $1x10^8$ CFU/mL and flavor strains (Sunburst™, Superbloom™, Cosmic Punch) at $1x10^8$ CFU/mL	200mL	24 hours
Week 3	Dry Sourvisiae® $1x10^8$ CFU/mL and the flavor strains (Sunburst™, Superbloom™, Cosmic Punch) at $1x10^7$ CFU/mL	200mL	24 hours
Week 4	Dry Sourvisiae® $1x10^8$ CFU/mL and the flavor strains (Sunburst™, Superbloom™, Cosmic Punch) at $1x10^7$ CFU/mL	200mL	Once reaching pH 4.6
Week 5	Dry Sourvisiae® at $1x10^7$ CFU/mL and the flavor strains (Sunburst™, Superbloom™, Cosmic Punch) at $1x10^8$ CFU/mL. Adding an additional treatment of Superbloom™ was fermented with the Cornell Dairy Plant Yogurt Base instead of UHT milk.	200mL	After 27 hours (Only Control reached pH 4.6)
Week 6	Dry Sourvisiae® at $1x10^8$ CFU/mL and the flavor strains (Sunburst™, Superbloom™, Cosmic Punch) at 10^8 CFU/mL. Adding an additional treatment of Sunburst™ with Post-Fermentation Heat Treatment	200mL	Once reaching pH 4.6
Week 7	Dry Sourvisiae® at $1x10^8$ CFU/mL and the flavor strains (Superbloom™) at 10^8 CFU/mL.	2L	Once reaching pH 4.6

Week 8	Dry Sourvisiae® at $1x10^7$ CFU/mL and the flavor strain (Superbloom™) at $1x10^7$ CFU/mL. Cornell Dairy Plant Yogurt Base was used instead of UHT milk	10L	Once reaching pH 4.6
Week 9	Dry Sourvisiae® at $1x10^7$ CFU/mL and the flavor strains (Sunburst™, Superbloom™) at $1x10^7$ CFU/mL. Cornell Dairy Plant Yogurt Base was used instead of UHT milk	10L	Once reaching pH 4.6

TABLE 2. Summary of all pitching rate experiments and treatments

Sensory Study

The sensory study protocol was reviewed and approved by the Cornell University Institutional Review Board prior to the sensory evaluation. A recruitment email was sent through the Cornell Sensory Evaluation Center. Word of mouth and flyers were also used to recruit participants. The study was labeled as a "Fermented Milk" study to avoid any bias participants had. All panelists were issued informed consent forms before participation. The participants were asked to accept the consent form to confirm no allergies or intolerances to dairy or little amounts of alcohol. Each participant was reward a $5 gift card for their contribution.

Scale Up Protocol

First, Sunburst™ and Superbloom™ were streaked onto PDA+CAM plates. The plates were incubated plates at 30°C until colonies were present, which occurred approximately 24 hours later. Two 5mL tubes of DME were prepared for each strain, where one colony of each streaked isolate was placed in one "over-night" (ON). In addition, one negative control tube was prepared. The tubes were then all incubated at 30°C with shaking at 200 rpm. About 24 hours later, the tubes were inspected for turbidity. The negative control was always verified to have no turbidity. Each ON tube was then pitched into a flask with 400mL of DME, two of these 400mL flasks were combined, and incubated at 30°C with shaking at 200 rpm for another 24 hours. In summary, two ON tubes were added to 800mL of DME. For each culture, the cell concentrations were quantified using a hemocytometer. When concentrations reach usable levels, they are transferred to the 4°C refrigerator.

Before the fermentation was set up, all the necessary supplies were procured. About 30 liters of Cornell's Yogurt Base was obtained through the Cornell Dairy Plant a week before the study. 10L of the Cornell Yogurt Base was transferred to three separate large vessels with lids (that were previously sterilized with StarSan sanitizer). The vessels were then placed in a 30°C water bath. The iCinac pH probes were calibrated and a 10% bleach solution was prepared to be later used to sterilize the probes.

Next to a flame, each starter was measured and transferred into conical tubes. They were then spun down in a centrifuge at 4000 rpm for 2 minutes. After centrifuging all the starters, the supernatants were poured out and discarded. The pellets were resuspended in 50mL of sterile 1x PBS solution. During this time, 20 grams of dry Sourvisiae® was weighed, and rehydrated in a conical tube with 50mL of sterile 1x PBS solution. The conical tubes were once again centrifuged at 4000 rpm for 2 minutes. During this time, the previously calibrated iCinac pH probes were placed in a tube of 10% bleach for 10 minutes. The supernatants were all poured out and discarded. Each starter was then pitched into the appropriate substrate bottle and a calculated amount of lactase was added. Using a sterile spoon, the fermentate was mixed and homogenized. A 1mL sample was then taken from each treatment, transferred to an autoclaved microcentrifuge tube, and placed in the 4°C refrigerator.

Once the treatments had reached a pH of 4.6, the vessel was removed from the water bath. About 32oz of the fermentate was added at a time into a kitchen blender (Oster Classic Series 8-Speed Blender) sanitized with StarSan. The fermentate was blended until no clumps were visible and consistency was smooth. The blended yogurt products were added to sanitized 5 gallon buckets and transferred to the 4°C refrigerator. Two additional commercial products were purchased and placed into the 4°C refrigerator. The samples tested were as followed:

1. Dry Sourvisiae® at $1x10^{6.5}$ CFU/mL (Prepared by Paola María Gamboa Moreno using the same protocol mentioned above)
2. Dry Sourvisiae® at $1x10^{7}$ CFU/mL and Sunburst™ at $1x10^{7}$ CFU/mL
3. Dry Sourvisiae® at $1x10^{7}$ CFU/mL and Superbloom™ at $1x10^{7}$ CFU/mL
4. Lifeway Lowfat Milk Plain Kefir, 32 fl oz
5. Great Value Plain Nonfat Yogurt, 32 oz

A day before the sensory evaluation, 50mL of each of the samples were placed into 2 oz labeled plastic cups with lids and left inside the 4°C refrigerator.

Individual Online Questionnaire

Using the RedJade program, a questionnaire was generated to measure consumer acceptance. In the questionnaire, participants were asked to rate the appearance and aroma of each product, prior to consumption. Next, the participants were asked to taste the samples and to rate their overall preference and the intensity of the sensory attributes of the samples on a JAR Scale. After tasting all samples, participants were asked to rank the samples. Each sample was labeled with a 3-digit code and were presented to the participants in a randomized order generated by the RedJade program. The participants were not informed about the ingredients of the samples to receive their unbiased feedback.

Data Analysis

JMP® Pro 170.0 was used to calculate the means and standard deviations for the biological replicates in experiment. The program was also used to produced the cell count and fermentation profile graphs. The responses to the sensory study questionnaire were recorded and analyzed using RedJade. Mean score, standard deviations, and spider plots were also conducted using RedJade.

RESULTS AND DISCUSSION

Preliminary Tests

Based on the result of this preliminary experiment, about 350mL of DME is needed inoculate in 200mL of milk and achieve a concentration of 10^8 CFU/mL. Hence for the rest of the experiments, 400mL of DME was used for one 5mL overnight to tube. These tests also showed Sunburst™ and Superbloom™ had the strongest and most pleasant aromas. The Bavarian Wheat strain smelled very sour, hence it was instantly cut. The Cosmic Punch strain and French Saison strains smelled fairly similar, so to limit redundancy the French Saison strain was cut. All experiments excluding the sensory test scale up included Sunburst™, Superbloom™, and Cosmic Punch. The scale up excluded Cosmic Punch since Sunburst™ and Superbloom™ had very similar fermentation profiles and exhibited stronger aromas and flavors.

Inoculation Rate of Yeasts

Week 1 Trial

Based on previous experiments done by Paola María Gamboa Moreno, 10^8 CFU/mL was the first pitch rate tested for both yeast strains in the co-ferment. During this initial trial, Sourvisiae® was grown from a glycerol stock using the same protocol as the flavor-producing strains. To reach pH 4.6, it took the strains the following amount of time: the Sourvisiae® control took 2.25 hours, the Sunburst™ and Superbloom™ co-ferments took 5.75 hours, and Cosmic Punch co-ferment took 6.5 hours as displayed in Figure 5. These initial trials used UHT milk which produced a distinct whey and curd layer as seen in Figure 3. When plating the cells, there was no way to distinguish individual populations found within the co-ferment. However, based on this first trial, all strains experienced a decrease in population throughout the fermentation dropped, as seen in Figure 4.

FIGURE 3. From Left to Right: Sourvisiae® at 1x10^8 CFU/mL, Sunburst™ at 1x10^7 CFU/mL and Sourvisiae® at 1x10^8 CFU/mL, Superbloom™ at 1x10^7 CFU/mL and Sourvisiae® at 1x10^8 CFU/mL, Cosmic Punch at 1x10^7 CFU/mL and Sourvisiae® at 1x10^8 CFU/mL, Superbloom™ at 1x10^6 CFU/mL and Sourvisiae® at 1x10^8 CFU/mL

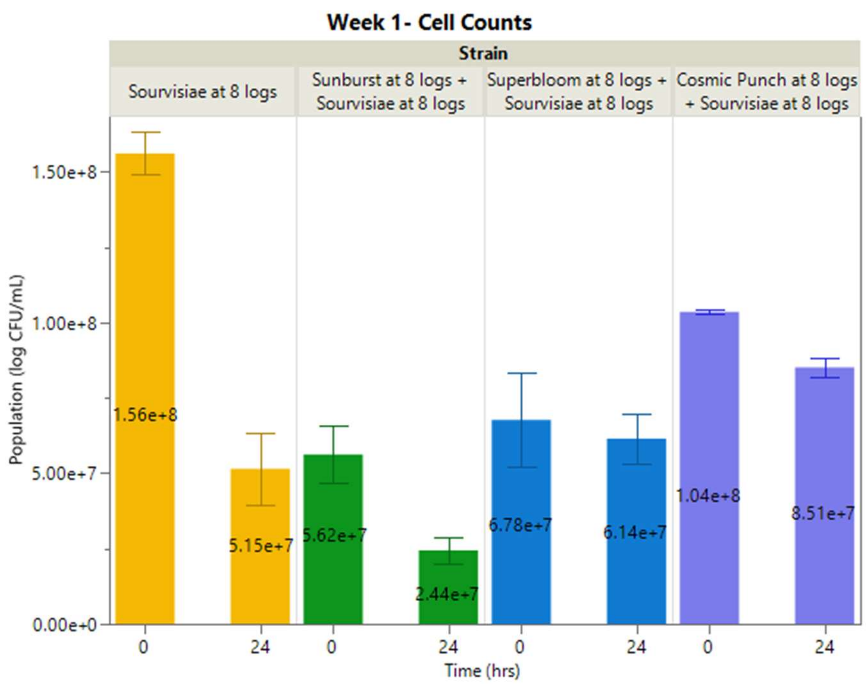

FIGURE 4. Population of all strains at different pitch rates before and after fermentation for Week 1. Values represent means ± standard deviations.

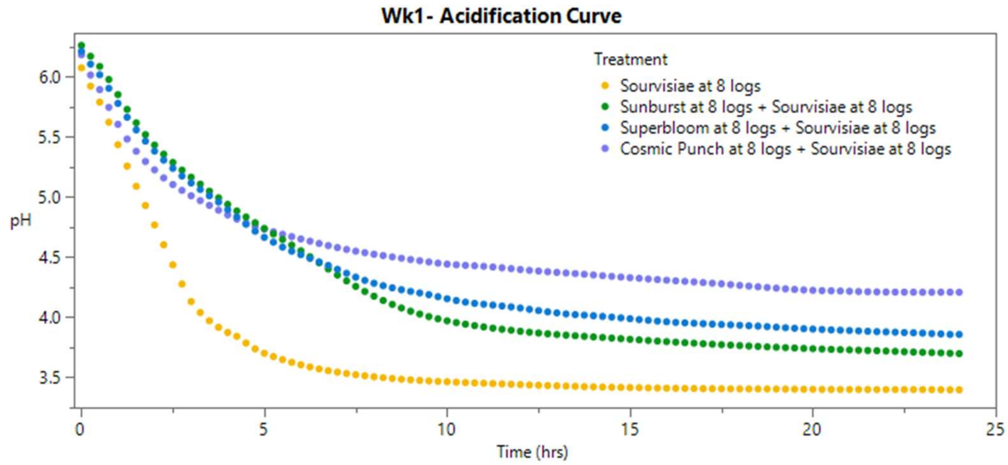

FIGURE 5. Acidification curves of strains at different pitch rates for Week 1

Week 2 Trial

This second trial was initially meant to be a biological replicate for the first week. However, the results were very different from the first and there seemed to have been something wrong which is evident as per Figure 7. The control Sourvisiae® yogurt took almost five times as long to reach pH 4.6 compared to the previous week's results. Additionally, Cosmic Punch co-fermented yogurt never reached pH 4.6. Unlike the previous week, all strains experienced a decrease in population throughout the fermentation, as seen in Figure 6.

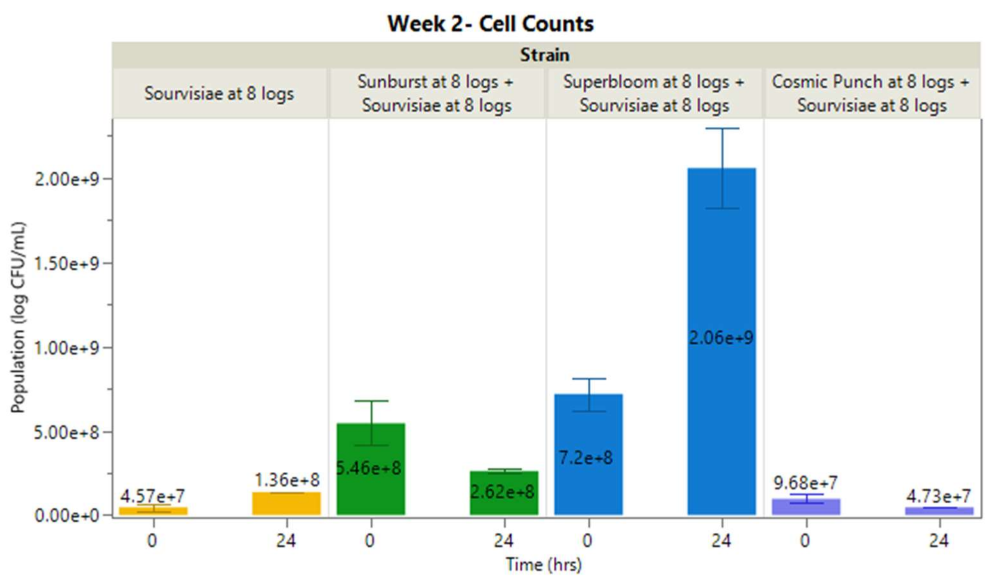

FIGURE 6. Population of all strains at different pitch rates before and after fermentation for Week 2. Values represent means ± standard deviation

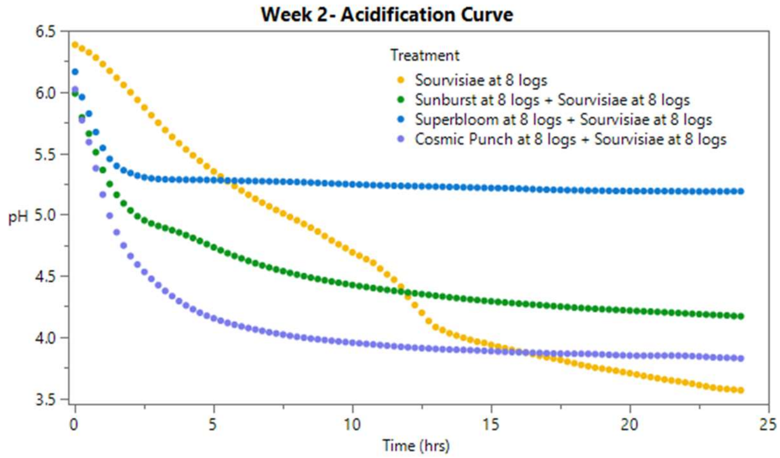

FIGURE 7. Acidification curves of strains at different pitch rates for Week 2

Week 3 Trial

The aim of the week 3 treatments was to understand if pitching the flavor strain at a lower concentration would work. This would make the process more cost-effective, especially once the process would be scaled up. Additionally, from this point onward dry Sourvisiae® was used since it was less labor intensive and more economical to use when scaling up. Based on aroma only, there was little detectable added aroma in the co-ferments compared to the control Sourvisiae® yogurt. All strains experienced a decrease in population throughout the fermentation, as seen in Figure 8. Furthermore, all treatments acidified very similar to each other.

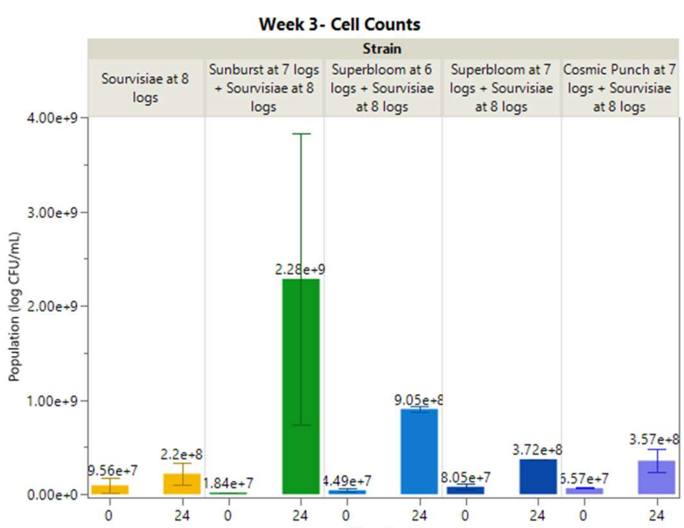

FIGURE 8. Population of all strains at different pitch rates before and after fermentation for Week 3. Values represent means ± standard deviations

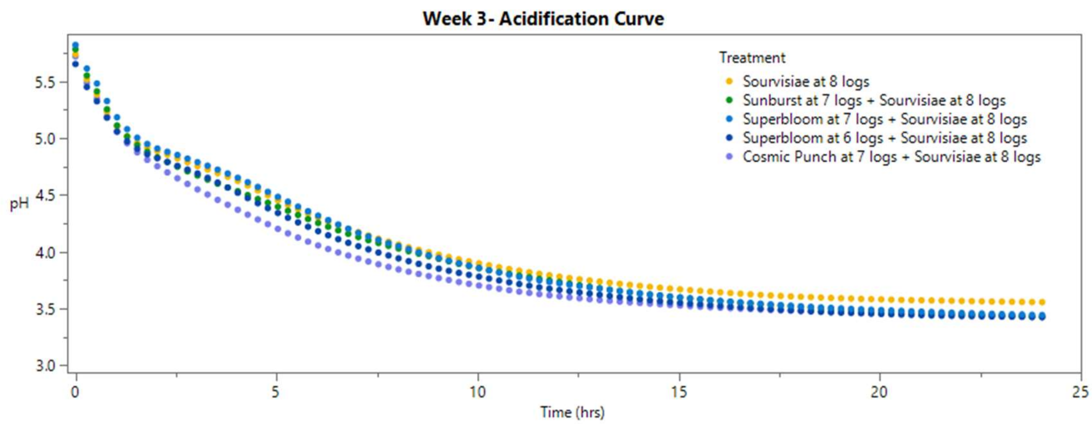

FIGURE 9. Acidification curves of strains at different pitch rates for Week 3

Week 4 Trial

Originally, this week had twice as many treatments and its aim was to compare preparing the co-fermented yogurt with pitch rates of dry Sourvisiae® $1x10^8$ CFU/mL and the flavor strains at $1x10^7$ CFU/mL and dry Sourvisiae® $1x10^8$ CFU/mL and the flavor strains at $1x10^8$ CFU/mL. However, user error when using the centrifuge yielded in the loss of all the flavor strains that were meant to be pitched at $1x10^8$ CFU/mL. The fermentation was ended once the yogurts reached pH 4.6 to give them a taste test that would be more comparable to the samples later prepared for the sensory evaluation. Additionally, plating validation is not available for this week since all plates had too many colonies and were not countable. A blank PDA+CAM plate was incubated to see if the plates themselves were contaminated, but after 48 hours nothing grew on the blank plate.

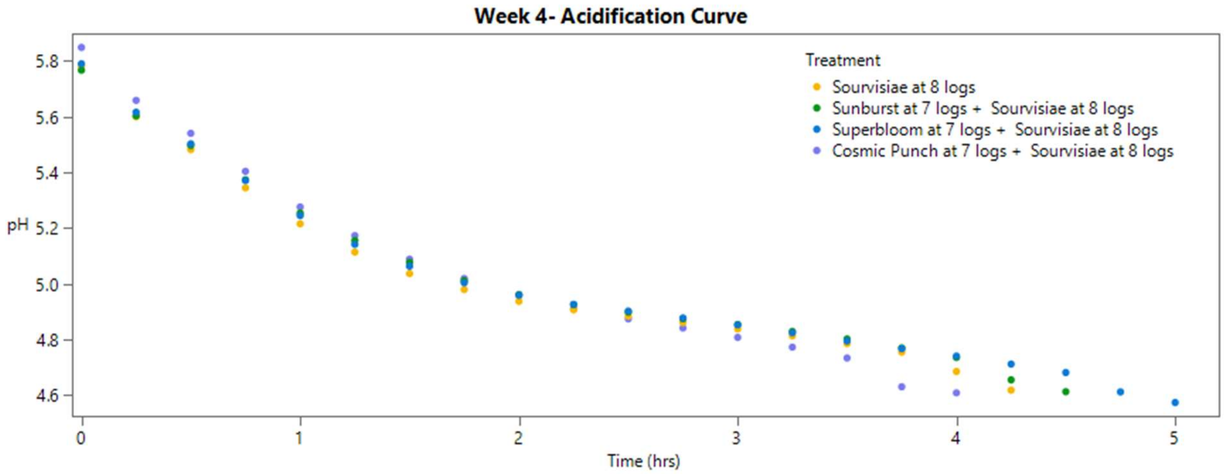

FIGURE 10. Acidification curves of strains at different pitch rates for Week 4

Week 5 Trial

Since the aroma and flavor was so weak in the week 4 treatments, the flavor producing strains were pitched at a higher pitch rate than Sourvisiae®. This intended to produce more flavorful product that could mask the yeast smell and taste of the Sourvisiae®. However as seen for the fermentation curves in Figure 11, the co-ferments did not reach pH 4.6 under 27 hours. Additionally, plating validation is not available for this week since all plates had too many colonies and were not countable. However, this is probably due to insufficient homogenization when adding in the dry Sourvisiae®. The following weeks prior to inoculating the milk with the dry Sourvisiae®, the yeast would be rehydrated with 1x PBS as per the protocol above.

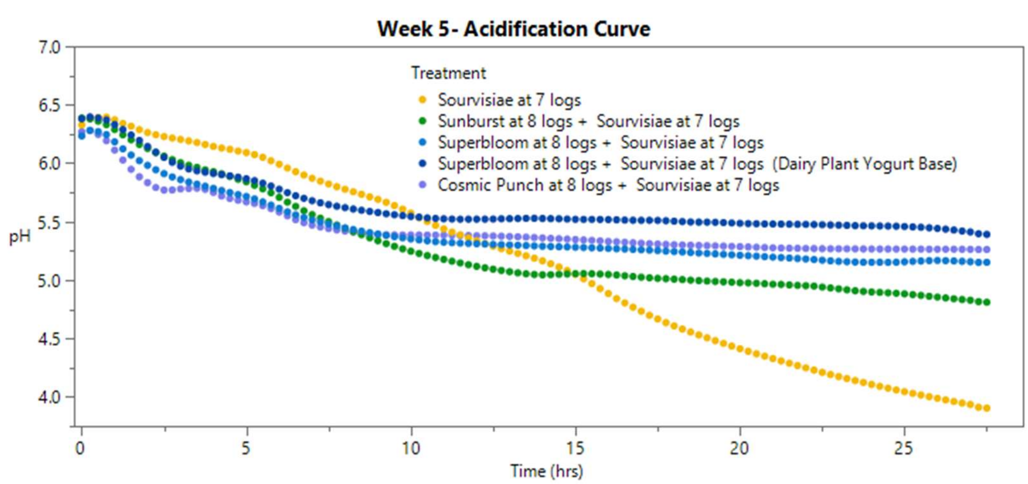

FIGURE 11. Acidification curves of strains at different pitch rates for Week 5

Week 6 Trial

Since the Week 1 and Week 2 co-ferments were acidified past the target pH of 4.6 and fermented for 24 hours, this week's treatment meant to stop the fermentation at pH 4.6 and retest those conditions. This helped confirm that the Week 2 data was an anomaly. Furthermore, this week included a heat treatment to attempt to kill the yeast and fully prevent the product from acidifying even in refrigeration. Hundreds of logs of *S. cerevisiae* in Pilsen beer could be inactivated if held at a temperature of 60°C for 1 minute (Reverón et al., 2003). Hence, an extra treatment was put into a water bath set at 60°C once it was finished fermenting. However, it is key to note that the increase in temperature did further acidify the co-ferment. This trial was performed at 200mL scale, hence it would not be feasible to heat treat at a larger scale in the same method since it would take too long to heat a larger quantity of yogurt and the product would continue acidifying past pH 4.6.

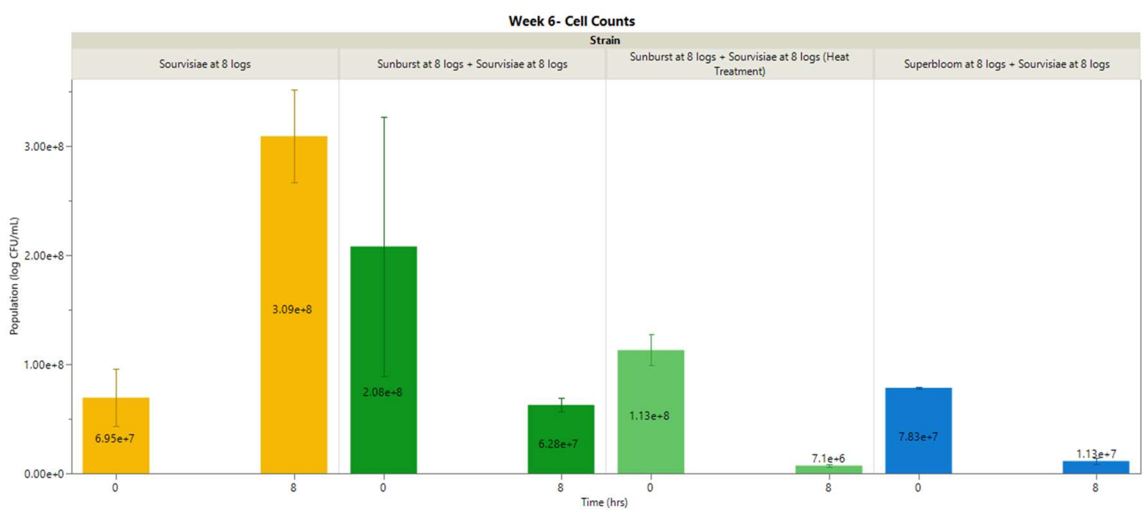

FIGURE 12. Population of all strains at different pitch rates before and after fermentation for Week 6. Values represent means ± standard deviations.

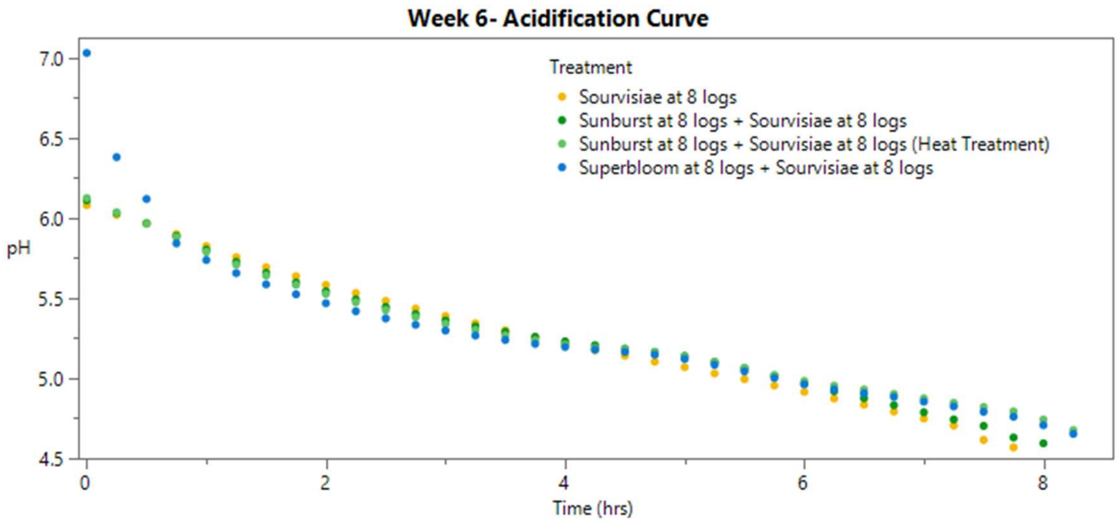

FIGURE 13. Acidification curves of strains at different pitch rates for Week 6

Week 7 Trial

This week focused on scaling up one strain to a 2 liter scale. The preparation for this experiment showed that it was not feasible to scale up a co-ferment in which both yeasts (Sourvisiae® and the flavor-producing strain) were pitched at 8 logs, since too much DME would be needed to scale up and the workload would make it very difficult to produce 2 co-fermented yogurts. Therefore, though both yeasts (Sourvisiae® and the flavor-producing strain) hadn't been pitched at 7 logs, the following week attempted to make a co-ferment with this rate since it seemed like the last viable pitch rate combination to try and scale up.

FIGURE 14. Sourvisiae® at 1x10^{8} CFU/mL and Superbloom^{TM} 1x10^{8} CFU/mL at 2L scale

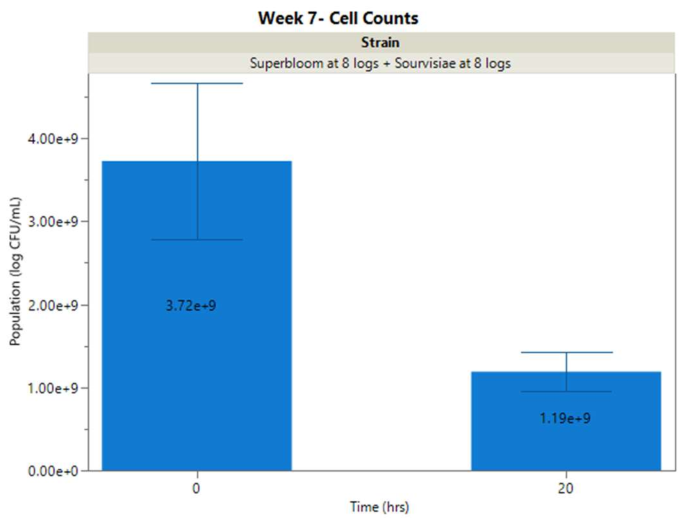

FIGURE 15. Population of Sourvisiae® at 1x10^{8} CFU/mL and Superbloom^{TM} 1x10^{8} CFU/mL before and after fermentation for Week 7. Values represent means ± standard deviations.

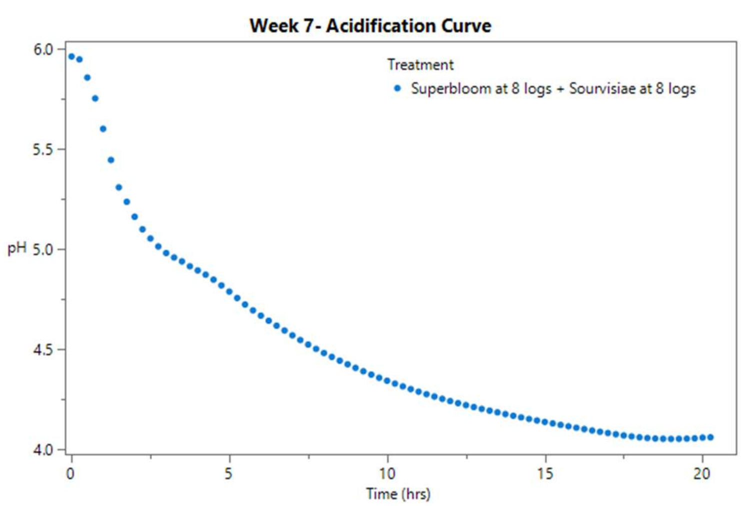

FIGURE 16. Acidification curves of Sourvisiae® at 1x10^{8} CFU/mL and Superbloom^{TM} 1x10^{8} CFU/mL for Week 7

Week 8 and 9 Trials for Sensory Study

The Cornell Dairy Plant Yogurt Base is heat treated and has some thickeners added to it. Therefore, in order to minimize the workload, the Week 8 and 9 runs switched over to the base over UHT milk. When the UHT milk was used, the co-ferments had an apparent phase separation between the curd and the whey as seen in Figure 14. When blended, the curd would simply reduce in particulate size but would not be well incorporated with the whey. On the other hand, when the co-ferments were used with the Cornell Dairy Plant Yogurt Base a smoother, more incorporated texture was achieved even post-blending. Additionally, this week’s treatment was a practice scale up for the way the co-fermented yogurt would be prepared in the sensory evaluation. In addition to Superbloom™, Sunburst™ was added as an additional treatment.

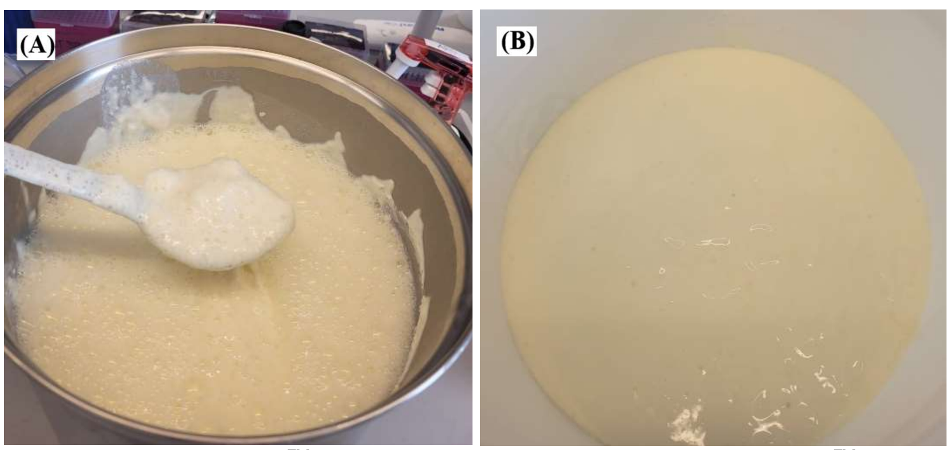

FIGURE 17. (a) Pre-Blending Superbloom™ Co-Ferment 10L Scale (b) Post-Blending Superbloom™ Co-Ferment 10L Scale

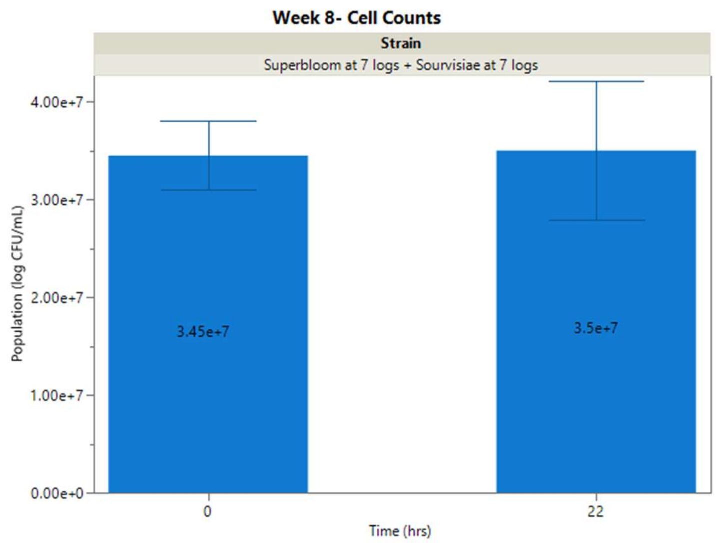

FIGURE 18. Population of Sourvisiae® at 1x10^7 CFU/mL and Superbloom^{TM} 1x10^7 CFU/mL before and after fermentation for Week 8. Values represent means ± standard deviations.

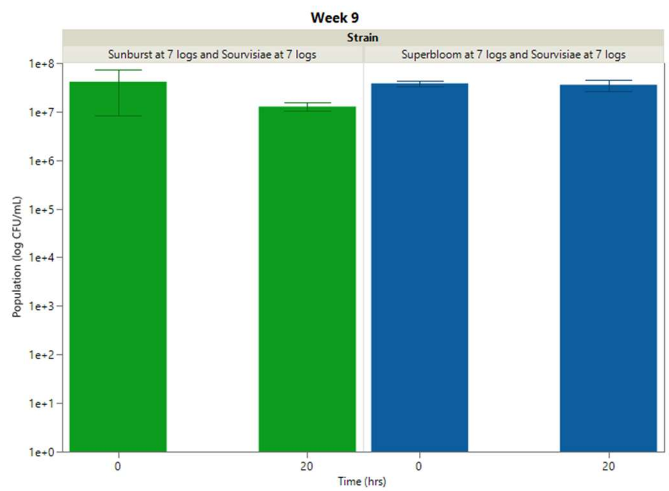

FIGURE 19.Population of co-ferments for Week 9. Values represent means ± standard deviations.

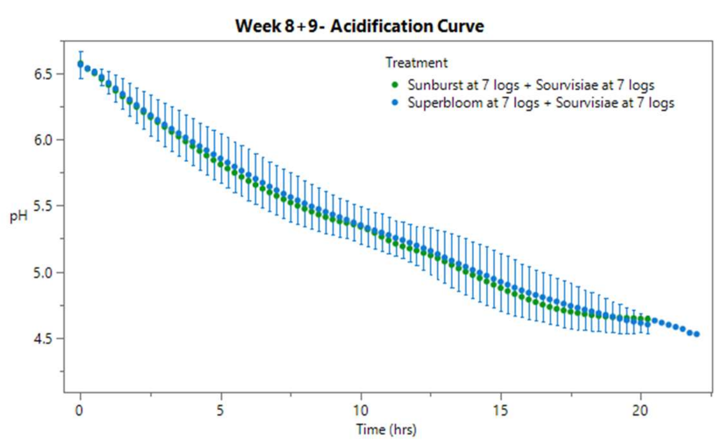

FIGURE 20. Acidification curves of co-ferments for Week 8 and 9

Sensory Study

A central location test (CLT) of n=88 was conducted with the sample prototypes to determine the consumer acceptance of the product and avenues for product development. Out of the 88 participants, 64% were female and 36% were male and between the ages of 19 to 71. Two commercial products were purchased to compare the viability of these fermented products. The kefir was selected based on its similar sour profile to the co-ferments and the plain non-fat yogurt was selected due to its similarity in texture to the co-ferments.

FIGURE 21. (a) Appearance liking on a 9-point hedonic scale (b) Texture liking on a 9-point hedonic scale (c) Aroma liking on a 9-point hedonic scale (d) Taste liking on a 9-point hedonic scale

Before tasting the samples, participants were asked to rank the appearance and aroma on a 9-point hedonic scale, as seen in Figure 21 (a) and (c). After tasting the samples, participants were asked to rank the texture and taste on a 9-point hedonic scale, as seen in Figure 21 (b) and (d). The appearance, aroma, and texture of the co-ferments were perceived as being acceptable and performed closely to that of just the Sourvisiae®. Among the four sensory modalities evaluated fruity apple aroma is consistent with the fact that this particular yeast strain is known to produce a pineapple aroma. The sample by Superbloom™ was characterized as having aromas of floral scents, sourness, yeast, citrus and acidity. The perceived floral and citrus aroma is consistent with the scents expected from this strain. However, both samples were overwhelmingly labeled as sour by consumers.

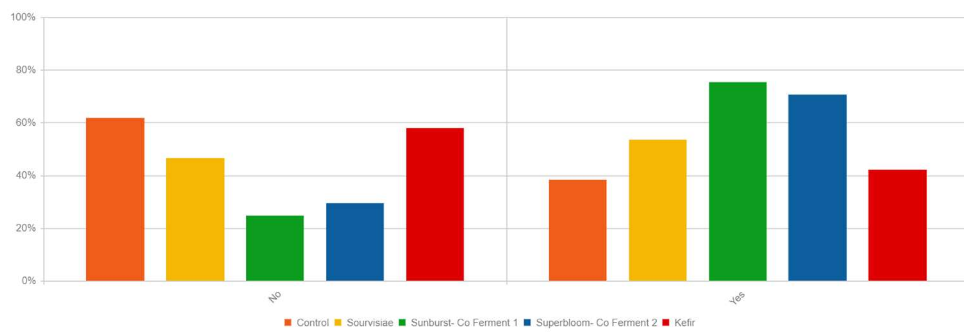

FIGURE 22. Aftertaste perception

CONCLUSION AND NEXT STEPS

Findings from this study provide a foundational understanding of the potential of a co-fermented yogurt-like product that uses Sourvisiae® as a starter culture for fermenting milk. It also helped conceptualize that yeast strains have the potential to be used as alternate cultures to traditional bacterial starter cultures. Moreover, it provided an insight into the behaviors and resulting sensory characteristics of the prototype developed by the Sourvisiae® culture and another flavor-producing yeast strain. The co-ferment with Superbloom™ had a tart, mildly sweet aftertaste when freshly prepared, whereas the co-ferment with Sunburst™ had a more bitter flavor palette. Both co-ferments were highly carbonated, and more carbonation was present than in the sample which just had Sourvisiae®. There was a limitation on how the samples could be heat treated, since only a water bath was able to heat treat them. At larger scales, it would take too long to heat hence the yogurts would continue acidifying and the texture became very tough. If produced in a commercial facility, the samples could be heat treated instantly, which would kill the yeast and carbonation. This could improve both the flavor and texture of the product. This was an flavored prototype designed to understand consumers' comments on this new type of fermented milk-based beverage. However, consumers were uninformed about the way the samples were fermented, hence future sensory studied could include adding additional information to see if that changes the way the product is perceived.

With the knowledge gained from this study, a shelf-life study could help to understand the changes in the fermentation parameters and sensory profiles of the yogurts with storage. In the future, different flavor producing strains could be utilized, but this study proves that a co-fermentation of this sort is possible. Furthermore, glucose supplementation of the co-ferments is yet to be tested similarly to past experiments conducted at ARG. The lactose, galactose, ethanol, and titratable acidity levels of the co-ferments still needs to be studied.

Overall, these trials showed that producing a co-fermented product in which Sourvisiae® is pitched at $1x10^7$ CFU/mL and the flavor producing strain is pitched at $1x10^7$ CFU/mL is possible. It is key to note that these samples had no added sugar or flavor, hence both could be added to produce a more complete product that could be more appealing towards consumers.

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