

**UTILIZATION OF LACTIC ACID-PRODUCING YEAST TO CREATE NOVEL,
YOGURT-LIKE DAIRY PRODUCTS**

A Thesis

Presented to the Faculty of the Graduate School
of Cornell University

in Partial Fulfillment of the Requirements for the Degree of
Master of Food Science

by

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BIOGRAPHICAL SKETCH

Bihongee is a native of India. She completed her Bachelor's and Master's degree in Food and Nutrition from two renowned universities in India. She wanted to become a Dietitian until she came to the USA. She started volunteering in a small startup which was her first step in the food industry, and she realized she would like to pursue her career in Food Science. Moving forward she worked mostly as a Food Safety/Quality Assurance/Regulatory Affairs. However, she felt a keen interest in R&D and Product Development. Whenever she got a chance she assisted in any of the ongoing R&D-related projects. She realized to give her passion for Food Science a deeper breadth of understanding and she decided to further explore this passion at Cornell.

In Fall 2021, Bihongee joined the Alcaine Research Group as a Master's student. Her research project was focused on 2 components 1) to understand the kinetics of a transgenic yeast when used as a single strain for milk fermentation instead of the traditional bacterial cultures; 2) to understand the potential of the fermentate sample to be developed into a product like refreshing beverage and analyze the consumer acceptability.

Through the interdisciplinary research projects and courses that she participated in at Cornell, Bihongee discovered her passion for Food microbiology, and she hopes to pursue a Ph.D. in Food Microbiology or Fermentation in the next few years.

I'd like to dedicate my work to my mom, dad, and brother who loved me, supported me, and believed in me, and to my partner who has always motivated me to choose this path and stay focused on the path that I am supposed to embark on moving forward in my journey

ACKNOWLEDGMENTS

I would like to thank my advisor, Prof. Sam Alcaine for being extremely supportive and providing his valuable guidance throughout the start of the program from selecting coursework that would help me pursue my career in R&D. I am forever grateful for his patient guidance and constant encouragement during the past one year when my life was full of unknowns and I was trying to figuring things out. I would also like to thank the Alcaine Research Group for being the best lab mates I could ever ask for. I will miss being a part of the lab. Also, I cannot thank enough Tim for being so patient throughout and teaching me every small thing in the lab that I was unaware of. He helped me familiarize myself with every lab technique to such detail that now I feel confident working in a lab alone. Also, a big thanks to Dana for being there whenever I needed her help whether it was giving me a ride or helping me with media preparation in the lab, or during the sensory test. I would also like to thank Deanna, who has been my mentor and a great mental support from the first day I started my internship under her supervision. Moreover, I am thankful to Alina for making me understand about sensory analysis in a better way and also providing her support during the sensory study.

Cornell has been an eye-opening experience for me. This was the riskiest and most challenging decision I have ever made for myself. However, I feel proud that I could take this challenge which turned out to be the most rewarding experience. I feel blessed and humble being a Cornellian. Each and every person I have met on this journey inspired me to work harder and dream bigger. For the first time, I was able to push myself beyond my limit and accomplish goals that I would just have dreamt of. My journey at Cornell would not be an easy one without the support of my family, Pankaj, my Cornell friend and professors, course TAs, ARG and Deanna. My heartfelt thanks to everyone for being there for me through the ups and downs and making this journey an easy one for me

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UTILIZATION OF LACTIC ACID-PRODUCING YEAST TO CREATE NOVEL, YOGURT-LIKE DAIRY PRODUCTS

ABSTRACT

Bacteriophages are environmental viruses omnipresent in the air, on plants, animals, and liquids. They need a host culture to grow. The bacterial starter cultures that are commonly used in the fermented dairy industry become their potential host. Bacteriophage infects the starter cultures and changes their functionality disabling them to produce lactic acid from lactose as they should. Thus, the texture of the fermented product gets affected. Later, phages kill the host culture, reproduce more phages and lead to a fermentation failure.

Yeasts (*Saccharomyces cerevisiae*) do not naturally produce lactic acid but can be used as an organism that can be engineered to exchange ethanol with lactic acid as a fermentation by-product. Sourvisiae® is a genetically modified strain of *S.cerevisiae* engineered to develop sour beer using one strain of yeast at a faster rate with fewer steps involved. Sourvisiae® has a single gene modification, a lactate dehydrogenase gene, that enables the yeast to produce high levels of lactic acid.

For this project, we studied the fermentation kinetics of this engineered *S.cerevisiae* strain in milk. We aim to standardize the fermentation parameters using this yeast strain so that it can mimic the fermentation properties of traditional bacterial starter culture. This would allow us to find a potential solution to address the bacteriophage issue in the fermented dairy industry and also would help create a novel line of fermented dairy products using yeast as a single strain starter culture.

One of the major parameters to consider for proper fermentation is the time taken to reach the food-safe pH of 4.6. To achieve that we considered the fermentation parameters to be standardized as incubation temperature,

using different lactase enzymes, no lactase enzyme, and the addition of excess glucose to understand the conversion rate of glucose to lactic acid by the yeast, and the inoculation rate of the yeast. The parameters were standardized using UHT Skim milk and the fermentation was set up for 24 hours. The fermentation markers like pH, ethanol concentration, lactic acid production, and cell counts (before and after fermentation) were recorded for each trial conducted. All the experiments were conducted in triplicates. The final standardized parameters were found to be 30°C as the incubation temperature of the inoculated milk sample along with Maxilact A4 as the lactase enzyme and the inoculation rate of the yeast to be 10^8 CFU/ml. These parameters would help to reach a pH of 4.6 within a set timeframe. Following these parameters, the comparison between the viscoelastic properties of the fermentate using a traditional starter culture and Sourvisiae® as the starter culture was observed using the ElastoSens™ Bio. The results showed that both the fermentates had a similar viscoelastic property. Therefore, Sourvisiae® has the potential to be considered as a starter culture and replace the traditional culture based on the set parameters. A sensory study was consulted with the prototype to understand the consumer's thoughts about this new product. An unflavored fermented sample was tasted against a commercial store-bought kefir drink which had a similar ingredient panel with no additives. The results showed a huge difference in the acceptability. The store-bought was more acceptable in visual appearance and taste compared to the Sourvisiae® fermented prototype.

Further shelf-life study to be conducted to understand the rate at which the pH and other parameters change with storage. Also, further product development to be done based on the sensory data to understand consumer preferences and to optimize the sensory profiles accordingly.

INTRODUCTION

In fermented dairy manufacturing industries lactic acid bacteria (LAB) is mostly used as the starter cultures. These cultures grow and produce lactic acid by converting milk sugar (lactose) into lactic acid via lactose fermentation, leading to a rapid decrease in pH [1]. The growth and metabolic activities of these starter cultures play an important role in assuring a high-quality final product [1]. It is also crucial to controlling the growth of spoilage and pathogenic microorganisms [1]. However, there are bacterial viruses or Bacteriophages (also known as “phages”) which are present in the same ecosystem as bacteria. These phages when introduced into the starter culture environment, interfere with the production of lactic acid and negatively impact the flavor, yield, and texture of dairy products like yogurt or cheese. Phages attack the DNA of the bacteria, change the function of the culture, and ultimately kill it. The invaded culture then multiplies, and more phages are released into the environment. As the starter culture gets killed by the phage, there are no acid or flavor production in the cheese or no coagulation in yogurt, buttermilk, or sour cream production. Once phage is present in the starter culture, it is usually too late to salvage the fermentation. There is no way to reintroduce culture into cheese or to effectively add it to fermented products without causing structural damage to the milk protein.

The fermented dairy industry has taken a variety of practical approaches to controlling phages. Some of the approaches include adapted factory design, adequate ventilation, process changes, improved starter medium, use of sanitizers, thermal and chemical treatment, strain rotation regimes, and spontaneous mutant phage-resistant strains [1][2]. Despite these efforts, phage infection of starter cultures remains the most common cause of slow or incomplete fermentation in the dairy industry. The cultured products have to be discarded or downgraded, which causes economic loss for the dairy processor. Therefore phage attack is considered a major issue in the fermented dairy industry.

In this study, our objective is to address this issue of bacteriophage contamination by finding out an alternative microbial strain to LAB which can mimic the process of traditional fermentation by converting lactose to lactic

acid as the byproduct and would be less vulnerable to phage attack [1]. Also, the new strain could unleash the possibility of developing a novel yeast-fermented yogurt-like product.

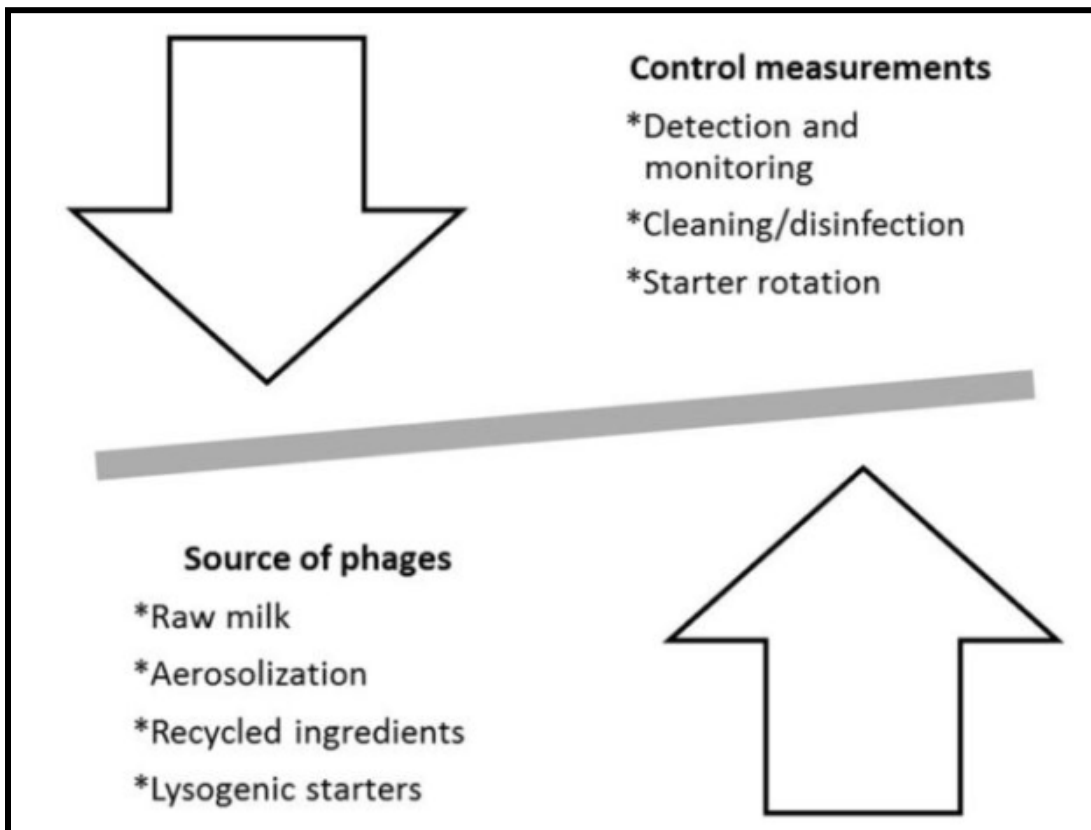


Figure 1: Source: [Bacteriophages in the Dairy Environment: From Enemies to Allies](#), National Center for Biotechnology Information

Saccharomyces cerevisiae is a common yeast species with diverse applications in the food and beverage industry. It is well known for its role in the production of fermented beverages, including beer, wine, cider, and distilled beverages. Currently, there are a number of commercially available *S. cerevisiae* strains, each of which is used preferentially for specific categories of fermented beverages [3]. Although *S. cerevisiae* is incapable of fermenting lactose, studies have demonstrated that it can utilize both sugar monosaccharides-glucose and galactose resulting from the hydrolysis of lactose for alcohol fermentations [4].

Lactic acid is not a natural fermentation product of *S. cerevisiae*. However, a cytoplasmic D-LDH (lactate dehydrogenase) was identified in previous studies where the expression was stimulated by damage to mitochondria [5]. However, metabolically and biochemically it is possible to replace the fermentation from pyruvate to ethanol to lactate. The regeneration of NAD⁺ which is the driving force is generated in both cases and the reactions are balanced. Moreover, for lactic acid bacteria, the theoretical yield of lactic acid (g/g) on glucose is 100%, while in ethanol production it is only 50%. This is because part of the carbon is lost as CO₂. Previous research has confirmed the partial redirection of pyruvate to lactate when the construction of a recombinant *S. cerevisiae* strain expressing the *Lactobacillus casei* L-LDH gene was described [5][6]. The resulting mixed lactic acid-alcoholic fermentation can be desired in some processes, like in wine making [5].

Sourvisiae®, a genetically engineered strain of *S. cerevisiae* was developed with a single gene modification, a lactate dehydrogenase gene, which would enable the yeast to produce high levels of lactic acid. Sourvisiae® is created for developing an easy method of brewing mono-cultured sour-style beer. The gene modification in Sourvisiae® makes it capable of producing lactic acid during fermentation which is the main compound that imparts the flavor of the sour beers. Sourvisiae® helps to ferment and sour the beer in one step, thereby reducing cross-contamination risks, lowering costs, and total processing time, and achieving a consistent product with every production. Brewing with Sourvisiae® does not require any modification; the yeast is inoculated at a similar rate to conventional yeast and takes a normal fermentation time. Sourvisiae® also helps produce a cleaner and more reproducible souring process, with much shorter fermentation times with no production of off-flavor, unlike the wild microbe varieties.

(Source: <https://www.lallemandbrewing.com/en/united-states/product-details/sourvisiae/>)

For our study, we used this bioengineered strain of *S. cerevisiae* as it is capable of producing lactic acid during fermentation. The aim of this study was to understand its kinetics with milk. The objective was to standardize the fermentation parameters using this yeast strain so that it can mimic the fermentation of traditional bacterial starter culture. This would allow us to find a potential solution to address the bacteriophage issue in the fermented dairy industry and also would help create a novel line of fermented dairy products using a single strain starter culture.

MATERIALS AND METHODS

Fermentation Characterization

Active dry form of Sourvisiae® (bioengineered beer brewing yeast strain of *Saccharomyces cerevisiae*) was obtained from LALLEMAND BREWING and stored in 50ml vials at the 4C refrigerator in the Alcaine Research Group (ARG) (Cornell University, Food Science Department). A commercial brand of Ultra-High Treated (UHT) skim milk was purchased and the Pasteurized skim milk was purchased from the Cornell Dairy Bar. The lactase enzyme Maxilact A4 and Maxilact LGX 5000F was purchased from DSM Food & Beverage. The lactases were stored in the 4C refrigerator of the ARG lab. They were filtered before being used in the fermentation setup. A low-protein binding syringe filter (VWR 0.2um Cellulose Acetate) was used for the filtration process. Dextrose was present at the Alcaine Research Group (Cornell University, Food Science Department).

Fermentation Setup

Before the fermentation setup, all the necessary supplies were procured: Pasteurized skim milk and UHT skim milk measured and incubated in a 30°C water bath, pH probe setup materials, 250 ml bottles, measuring cylinders, and epi tubes autoclaved, pH probe calibrated and sterilized in 10% bleach solution. 250 ml borosilicate glass round bottles were used as fermentation vessels for each trial conducted. Each fermentation bottle was filled with 200ml milk. To the bottles measured amount of *S.cerevisiae* “Sourvisiae®” and liquid lactase was added. 1ml of the inoculated milk sample was stored in an autoclaved labeled epi tube for serial dilution and plating to the appropriate concentration.

To set up the trial bottles with the iCinac for continuous pH measurement during the entire fermentation period, each bottle was equipped with a sterile polyvinyl chloride septum with a hole in its center and

an orifice. A pipette tip was attached aseptically to a sterile high-grade polyvinyl chloride flexible tubing as fitted to the septum orifice and another tubing end was put inside the airlock water bottle. This system served as an airlock, to create and maintain an anaerobic environment. To the center hole of the septum, an InLab Smart Pro-ISM pH probe (Mettler Toledo; Columbus, OH, USA) was inserted vertically. The probe was connected to an iCinac (AMS Alliance; Rome, Italy), providing measurements of the pH for each trial throughout the experiment. The bottles were placed in a clean water bath at an appropriate temperature for the duration of the experiment. The temperature was fixed within the range of optimal fermentation temperatures for *S.cerevisiae* (~ 25°C to 35) [5]. Further, the 1ml of inoculated milk sample was serially diluted in phosphate-buffered saline solution based on appropriate concentration and then plated in replicates on potato dextrose agar media supplemented with chloramphenicol. Each replicated concentration plate was incubated at 30°C for a duration appropriate for the growth of the strain's colonies.

Post-fermentation, a 5ml sample was carefully pipetted to get the whey for ethanol analysis. For homogenous mixing of the whey and the curd, the rest of the fermentate sample was mixed using a sterile loop with the whey, poured into a filter bag, and stomached in a stomacher at 230 RPM for 1 min. 1 ml of the stomached sample was stored for post-fermentation cell count following the similar procedure mentioned above. 5ml of the fermentate was used to measure titratable acidity.

Data Collection and Analysis

Each fermentation trial were carried out in triplicate. During each trial period, for each fermentate, samples for ethanol, cell count, and titratable acidity were taken. The pH was measured using Icinac throughout the incubation period every 10 minutes. The pH probe was inserted into the inoculated milk sample to gather the real-time pH reading. Samples for ethanol analysis were taken post-fermentation,

and samples for cell count were taken at the beginning of the incubation and after the fermentation is complete. Titratable acidity was also measured in the fermentate sample. Titratable acidity was analyzed using Titrator (HI931 Automatic potentiometric Titrator by Hanna Instruments). Cell count samples were serially diluted in phosphate-buffered saline solution and then plated in replicates on potato dextrose agar supplemented with chloramphenicol. The plates were incubated at 30°C for a duration appropriate for the growth of the colonies. The replicate plates containing the appropriate numbers of CFUs within the countable range were counted using a Chemopharm® Color QCount model 530 (Advanced Instruments, Inc., Norwood, MA, USA).

5 mL post-fermentation sample were collected at the end of the fermentation and centrifuged. The supernatant was collected and sent for quantification along with the skim milk used for that trial. Concentrations of percent ethanol, organic acids (lactic and acetic), and sugars (lactose, glucose, and galactose) in g/l of these samples were analyzed using high-performance liquid chromatography (HPLC) at the Cornell Craft Beverage Analytical Laboratory (Cornell AgriTech, Geneva, NY, USA). JMP® Pro 16.0.0 (SAS Institute, Cary, NC, USA) was used to calculate the means and standard deviations for the three biological replicates in experiment.

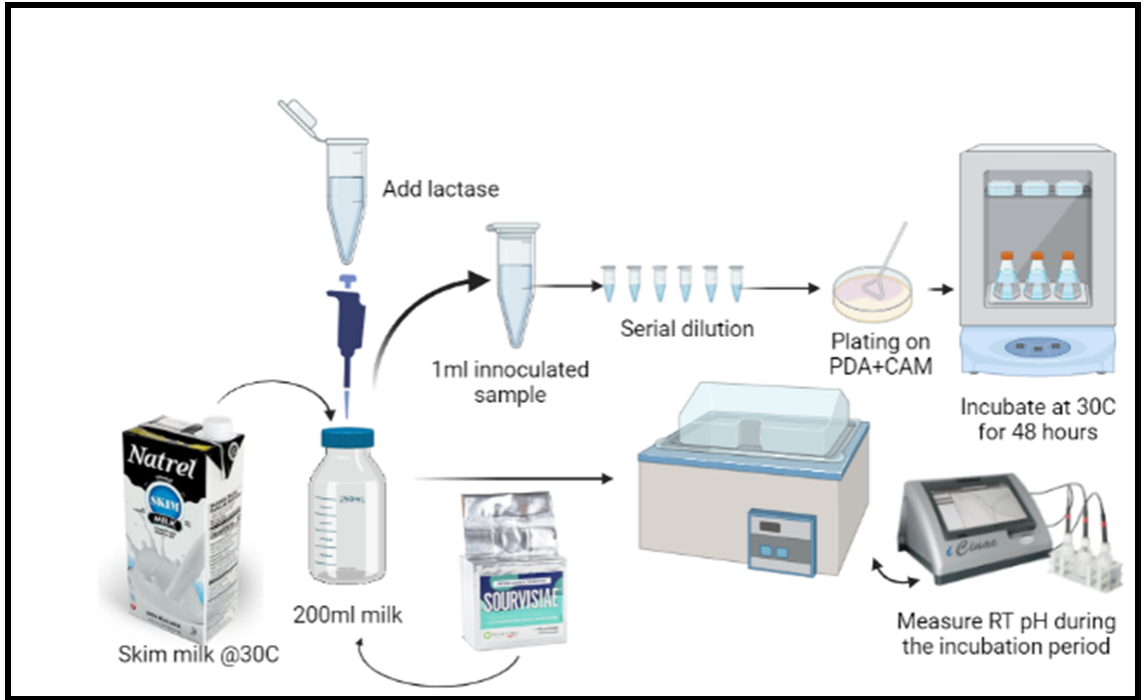


Figure 2: Design of the experiment before setting up the fermentation (0H)

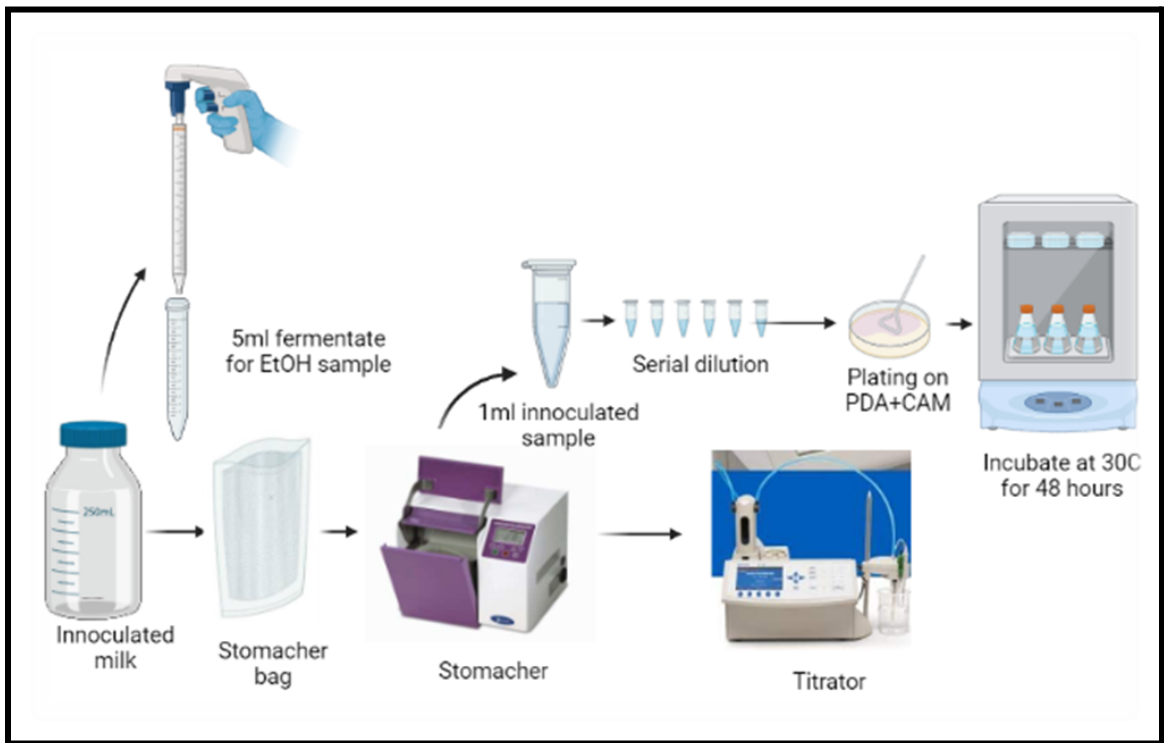


Figure 3: Design of the experiment after the fermentation is complete (24H)

a) Temperature of Incubation:

A range of temperature from 25°C-40°C was considered to determine the incubation temperature. Walsh and Martin 1997[8], and Merrit 1965 [7] studied the effect of temperature on the growth rate of different yeast species including *S.cerevisiae*. Moreover, at the ARG lab, 30°C is considered the optimum temperature to analyze the exponential growth of any yeast species. So, combining all these factors, the following temperatures were decided: 25°C on the lower end where the growth is slow; 30°C and 35°C as the optimum temperatures for the most rapid initial growth [8] and 40°C which is considered as a nearly fatal temperature [9]. Four trials were conducted: 200ml UHT skim milk was inoculated with 10^8 CFU/ml yeast and was incubated at 4 water baths of different temperatures: 25°C, 30°C, 35°C, 40°C. For this experiment, Maxilact LGX 5000F was used as the lactase enzyme. It was added to the milk at the manufacturer's recommended dose of 6-12 IU/g lactose before adding the yeast.

b) Enzyme Used:

The purpose of this study was to understand how closely the transgenic *S. cerevisiae* strain can mimic the fermentation parameters of traditional LAB cultures. One of the key parameters was to control the acidification of the milk using this Sourvisiae® culture. In order to determine that, we conducted 4 trials starting with the same inoculation rate of the yeast and incubation temperature of 30°C with the following variations:

- a) No lactase: To determine the acidification of the milk by the yeast without any exogenous hydrolyzing enzyme;
- b) Maxilact LGX 5000F: To replicate the standard experiment;

- c) Maxilact A4: To determine the acidification of the milk using an acid lactase enzyme;
- d) Maxilact LGX 5000F and Maxilact A4: To determine the acidification of the milk using the neutral lactase enzyme and acid lactase enzyme together.
- e) 40 g/L glucose: To determine the acidification of the milk with the addition of glucose so that the yeast can convert the glucose to lactic acid.

c) Inoculation Rate of the yeast:

In order to determine the inoculation rate of the yeast, we need to consider the substrate which would also influence the rate of inoculation. Most yeasts are inoculated at the concentration of 10^6 CFU/mL [10][11] or 10^7 CFU/ml [10]. 3 trials were conducted using the inoculation rate of yeast as 10^6 CFU/ml, 10^7 CFU/ml, and 10^8 CFU/ml, respectively along with Maxilact A4 in UHT skim milk and incubated at 30°C throughout the experiment. The experiment was conducted in triplicate.

Comparative study on the viscoelastic properties between traditional yogurt cultures and transgenic *S.cerevisiae* strain “Sourvisiae®”

Lactic acid bacteria (LAB) plays an important role in the fermentation of lactose to lactic acid and providing the fermented milk and related products its health benefits [12]. *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* are the major bacterial strains that form the typical yogurt flavor during sugar fermentation (glycolysis), protein degradation (proteolysis), and fat degradation (lipolysis) [13]. We tried to investigate how similar are the viscoelastic property of the LAB fermentate and the Sourvisiae® fermentate.

The experiment was designed in a similar manner as previous experiments: the fermentation was set up with 200ml UHT skim milk and pasteurized skim milk. 2 trials were conducted for each skim milk: One inoculated with Maxilact A4 and 10^8 CFU/ml of dry yeast and the other with commercial yogurt culture inoculated per manufacturer recommendation 500units per 2500 liters (CHR-Hansen Yo-Fast 16). The yeast inoculated milk was incubated at 30°C and the bacterial inoculated milk was incubated at 43°C [14]respectively. Both the inoculated samples were added to ElastoSens™ Bio following the manufacturer protocol and set at the respective incubation temperatures to anaerobically ferment the milk. The viscoelastic data were collected throughout the fermentation.

Sensory Study

The study protocol was reviewed and approved by the Cornell University Institutional Review Board (Ithaca, NY, USA) before the commencement of the study. A recruitment email was sent through the Cornell Sensory Evaluation Center, and through the Cornell Food Science undergraduate and graduate listservs. The study was mentioned as a “Refreshing Drinkable Yogurt” as that was the closest familiar beverage for general consumers. Flyers were distributed in Stocking Hall, Cornell University (Ithaca, NY, USA). An online screening questionnaire, facilitated by Cornell Qualtrics (Qualtrics, Provo, UT, USA), was used to select eligible participants. Of the 78 participants, those who were selected to participate indicated that they were above 21 years of age and have no allergies or sensitivities to dairy or alcohol. The Cornell University Institutional Review Board evaluated the study for Human Participants, and all panelists issued informed consent before participation.

Sample Preparation

To prepare the prototype of the fermentate to be served to the individuals participating in the sensory study, four autoclaved 2000ml Ernehmeyer flasks were taken. Since the same autoclaved aluminum foil was supposed to be used to cover the inoculated milk, the foil was removed and secured aseptically. The entire amount of milk along with yeast and enzyme was mixed in the same ratios used in the fermentation trials, as described previously in a 5000ml glass bottle to ensure homogeneous mixing. The inoculated pasteurized skim milk (1000ml) was then transferred to each of the 2000ml Ernehmeyer flasks and was covered loosely with aluminum foil so that the CO₂ produced during the fermentation could get released easily. The bottles were kept in 30°C water baths till they reached the pH of 4.6. A StarSan solution, (Five Star Chemicals, Arvada, CO, USA) was prepared in a 5-gallon bucket and the loose parts of the kitchen blender (Oster Classic Series 8-Speed Blender) that was used to blend the fermentate and a few spoons were submerged for a minimum of 1 minute of contact time. The fermentate in the flasks was then blended in the kitchen blender (Oster Classic Series 8-Speed Blender) for 15 secs initially and then 10 seconds for a final blend using the “whip” mode. It was then stored in the sanitized bucket closed in the 4°C refrigerator in the ARG lab until the day of the sensory study. A 200 ml of milk was separately incubated with the same inoculated milk following the usual fermentation protocol and was used for testing the pH during the incubation process, cell count of the 0H inoculated sample, and for the post-fermentation data collection (ethanol sample, titratable acidity and cell count). A reference sample that has only skim milk, live and active cultures and Vitamin D added as ingredients were selected to represent the traditional bacterial culture inoculated drinking yogurt. The reference sample was also lactose-free to be as close to the yeast-fermented sample. The participants received 30 ml of both the samples (reference and test) at a refrigerated temperature during

the study. The consumers were asked to accept the consent form to confirm no allergies to dairy or little amounts of alcohol.

Individual Online Questionnaire

The selected parameters were included in a questionnaire generated using the RedJade program (RedJade Software Solutions, LLC, Redwood City, CA, USA), and were used as metrics against which the samples were rated. In the questionnaire, participants were asked to rate the intensity of the sensory attributes of the provided samples (control and test sample) on a categorical scale and JAR Scale. The samples were 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. They were each rewarded with \$5 cash for this session.

Analyses

Responses to the questionnaire were recorded and analyzed using RedJade. Mean Score, Standard deviations, and Tukey's honestly significant difference posthoc test (Tukey's HSD) were also conducted using RedJade.

RESULTS AND DISCUSSION

Fermentation Profile

The important markers to track fermentation progress are pHs, cell count, ethanol content, and the amount of organic acid such as lactic acid produced. Each experiment was set up in triplicate to standardize the fermentation parameters. 24 hours of fermentation was set up initially to determine the temperature of incubation, and pitch rate of the yeast, and to determine the amount and the type of lactase to be used.

Impact of Incubation temperature on the fermentation markers:

The incubation temperature played an important role in reaching the pH of 4.6 (Figure 4). There is a difference in the acidification rates throughout the incubation period. From the graph, we are able to determine that 35°C and 40°C initially dropped to a pH of around 5.3. However, both of the acidification curves flattened out and never reached the pH of 4.6. 30°C was seen to have the highest acidification rate among all the temperatures considered. 25°C was also found to drop to pH 4.6, but it took more time compared to the 30°C. The cell counts before and after fermentation at 40°C dropped significantly post-fermentation, implying that the temperature is higher than its optimum temperature and that at 40°C the cells die (Figure 5). So, 30°C seems to be an optimal temperature for incubation of the fermentate. Lactic acid production is also found to be higher in the treatments that dropped below pH 4.6 (Figure 6).

Impact of Enzyme and Sugar on the fermentation markers:

The rate of acidification of the milk was indicated by the dropping of the pH to 4.6 and below (Figure 7). This is influenced by the amount of lactic acid produced by the yeast after converting the glucose.

There was a difference in the acidification rates throughout the incubation period. From the graph, we are able to determine that the trial with Maxilact A4 showed a better drop in the pH (4.6 within 8 hours). The cell counts (Figure 8) of all the treatments using 40g Glucose, Maxilact A4, Maxilact LGX 5000F, Maxilact A4 plus Maxilact LGX 5000F, and no lactase were similar compared to the pre and post-fermentation. Also, the lactic acid production was highest in the sample with just Maxilact A4 (Figure 9). This indicates that Maxilact A4 facilitates the fermentation process by most effectively converting the milk's lactose to its monomer constituents, which the yeast can then convert to lactic acid, thereby decreasing the pH below 4.6.

Impact of Inoculation rate on the fermentation markers:

The inoculation rate of the yeast determined the drop of pH to 4.6 at a faster rate (Figure 10). The pH dropped below 4.6 within 7.5 hours when the inoculation rate of the yeast is 10^8 CFU/ml. The other two inoculation rate of yeast does not seem to drop the pH as efficiently as 10^8 CFU/ml. The cell counts at 0H and 24H were higher with the treatment of 10^8 CFU/ml considering the incubation temperature of the yeast plates to be 30°C for all treatments (Figure 11). Also, the lactic acid concentration production after the fermentation was also found to be higher for the treatment with 10^8 CFU/ml compared to the other treatments (Figure 12). Hence, 10^8 CFU/ml provided the best results compared to the other treatments.

Viscoelastic Properties of traditional starter cultures and Sourvisiae®

Figure 13 shows the viscoelastic properties of the fermentate produced using a traditional bacterial starter culture and Sourvisiae®. The figure shows the results for both the cultures when treated with both UHT skim milk and pasteurized skim milk. The viscoelastic properties of the fermentates from

both the starter cultures seem to be comparable when treated under similar conditions as per their standard parameters.

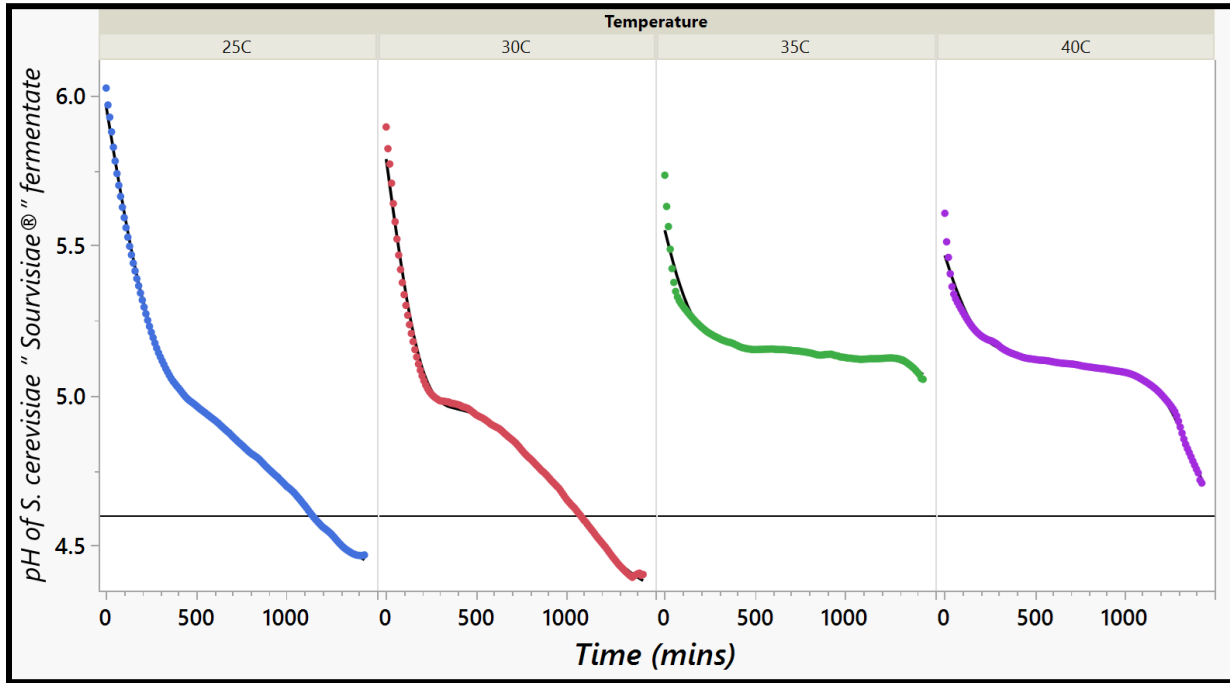


Figure 4. The pH profiles of the fermentate of *Saccharomyces cerevisiae* "Sourvisiae®" during the entire fermentation period.

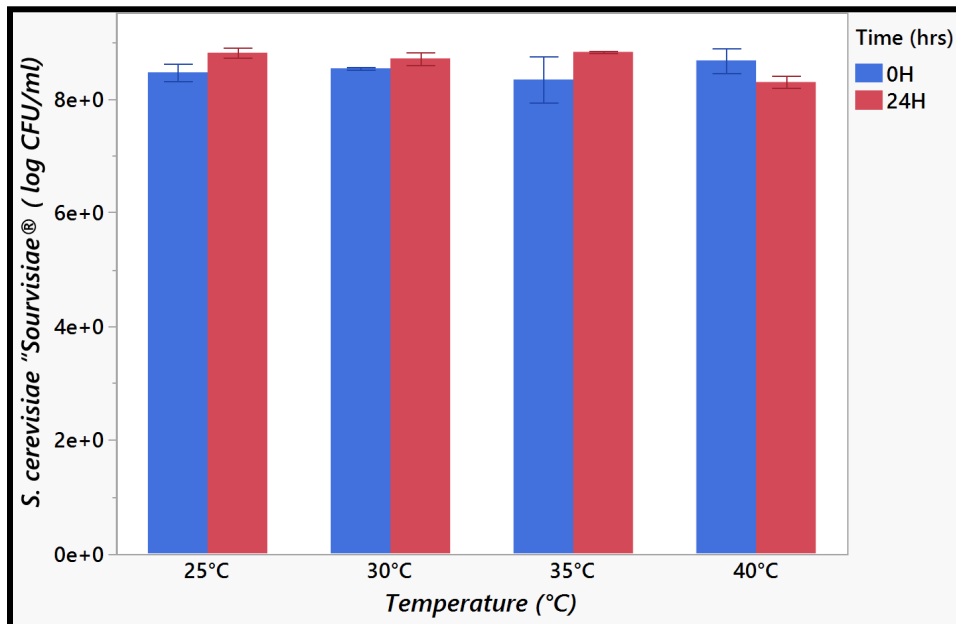


Figure 5. The concentration of *S. cerevisiae* "Sourvisiae®" before and after incubation at different incubation temperatures. Values represent means \pm standard deviations.

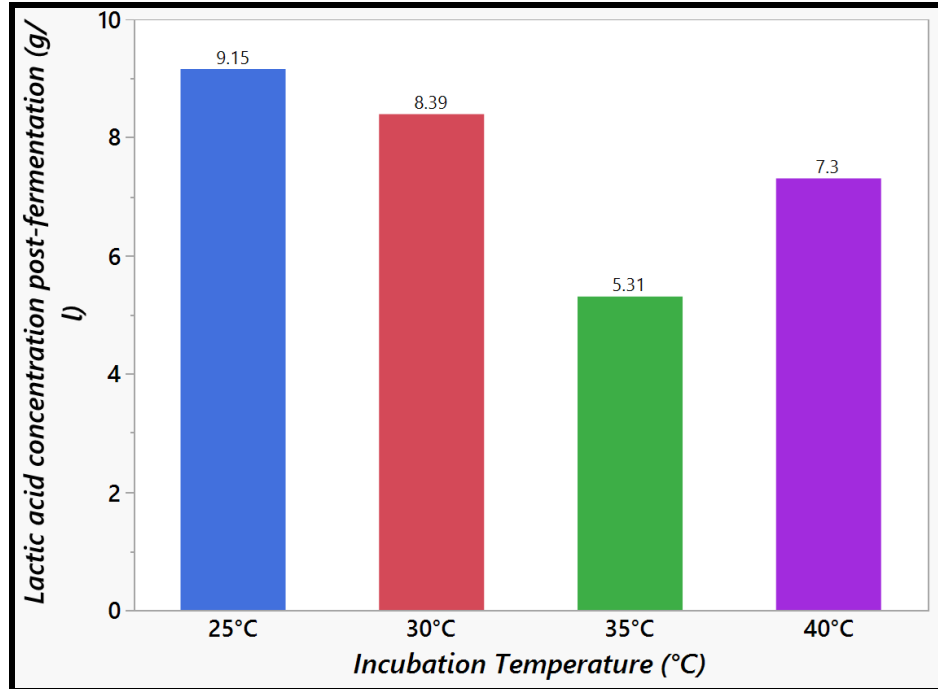


Figure 6. Amount of lactic acid concentration post-fermentation with the *Saccharomyces cerevisiae* "Sourvisiae®" when incubated at different temperatures.

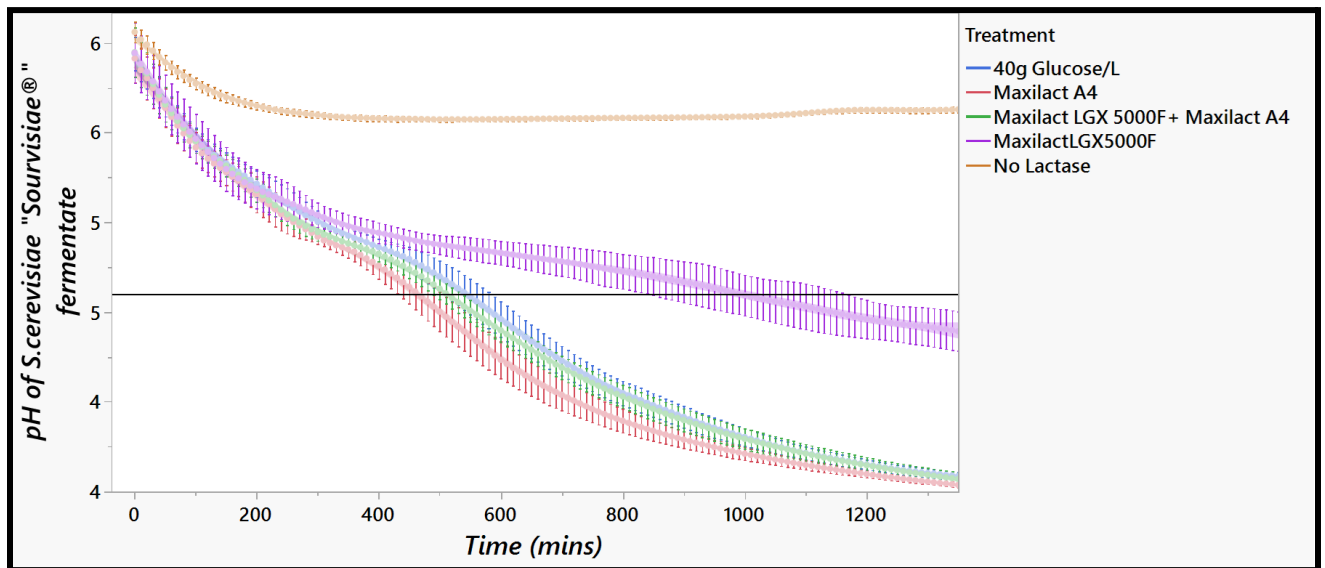


Figure 7. pH profiles of the fermentate of *Saccharomyces cerevisiae* "Sourvisiae®" during the entire fermentation period when treated with different lactase enzymes, no lactase, and glucose. Values represent means \pm standard deviations.

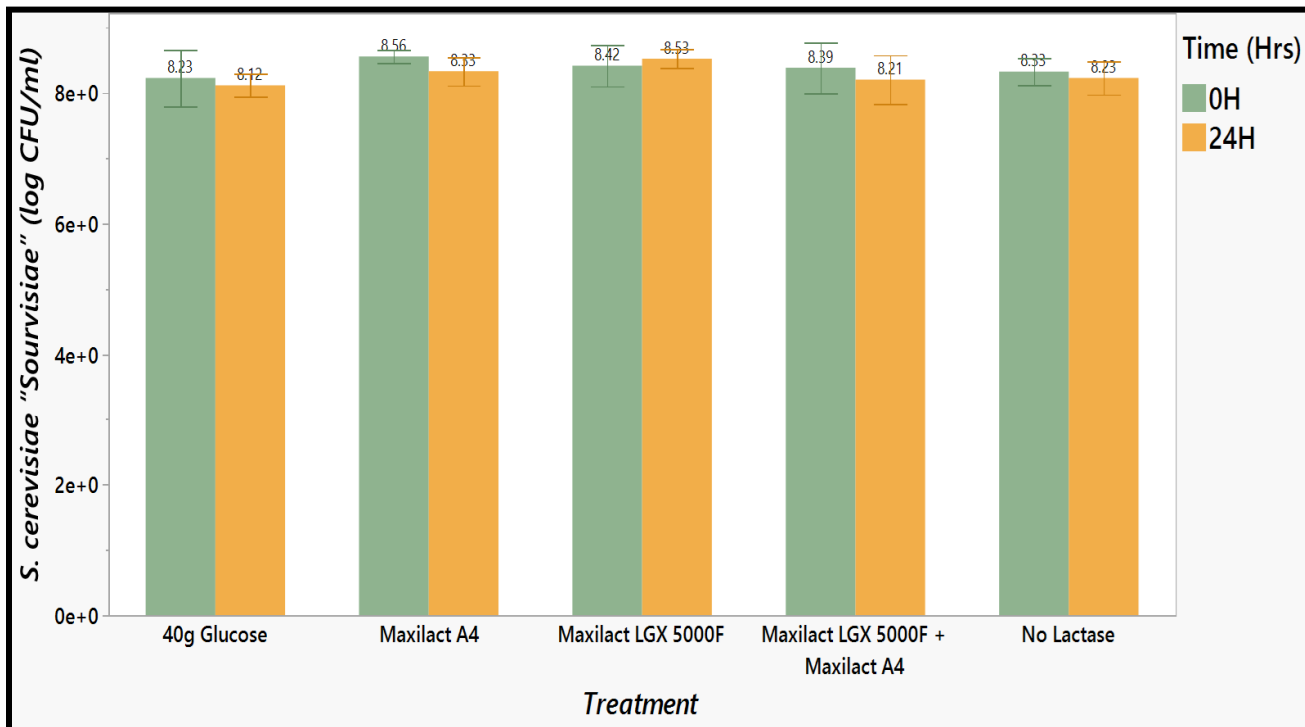


Figure 8. The concentration of *S. cerevisiae* “Sourvisiae®” before incubation (0H) and after fermentation (24H) with different enzymes was used. Data expressed as mean \pm standard deviation of biological triplicates.

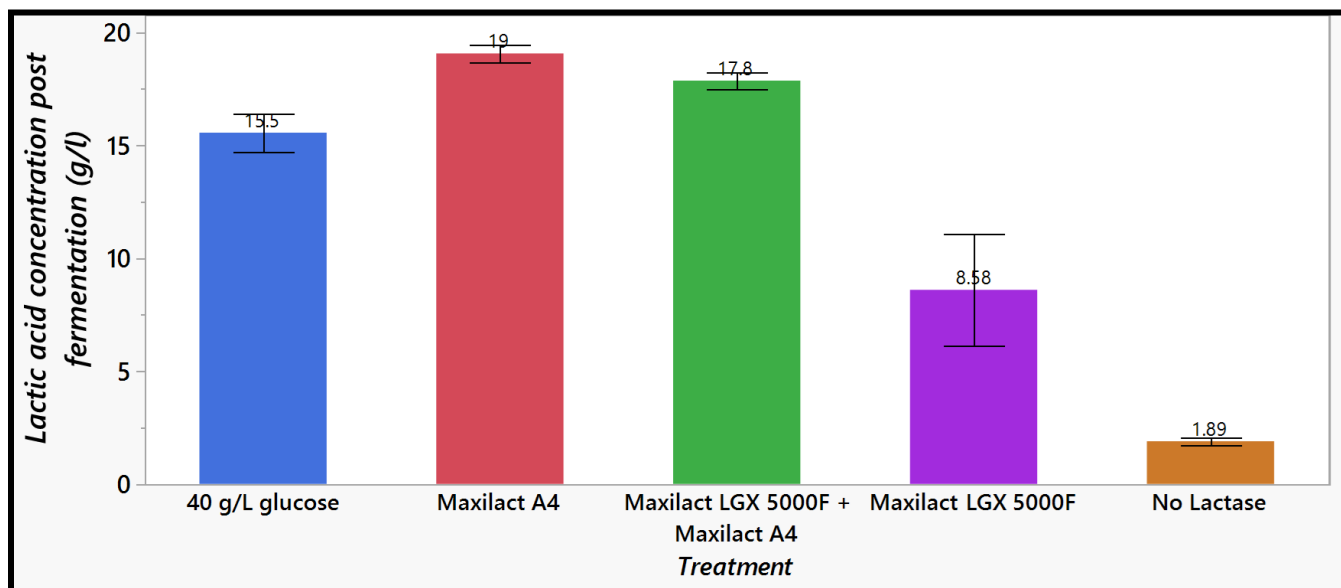


Figure 9. Amount of lactic acid produced by *S. cerevisiae* “Sourvisiae®” post-fermentation with different enzymes used. Data expressed as mean \pm standard deviation of biological triplicates.

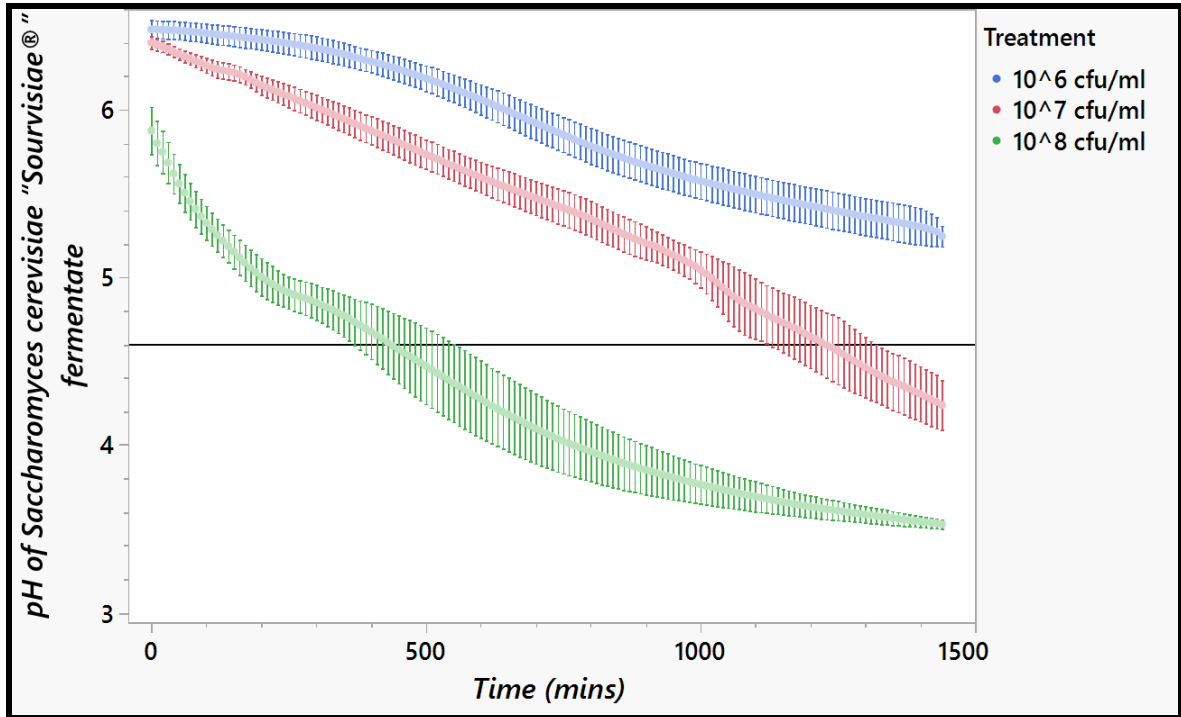


Figure 10. pH profiles of the fermentate of *Saccharomyces cerevisiae* “Sourvisiae®” during the entire fermentation period when treated when pitched at different rates. Values represent means \pm standard deviations.

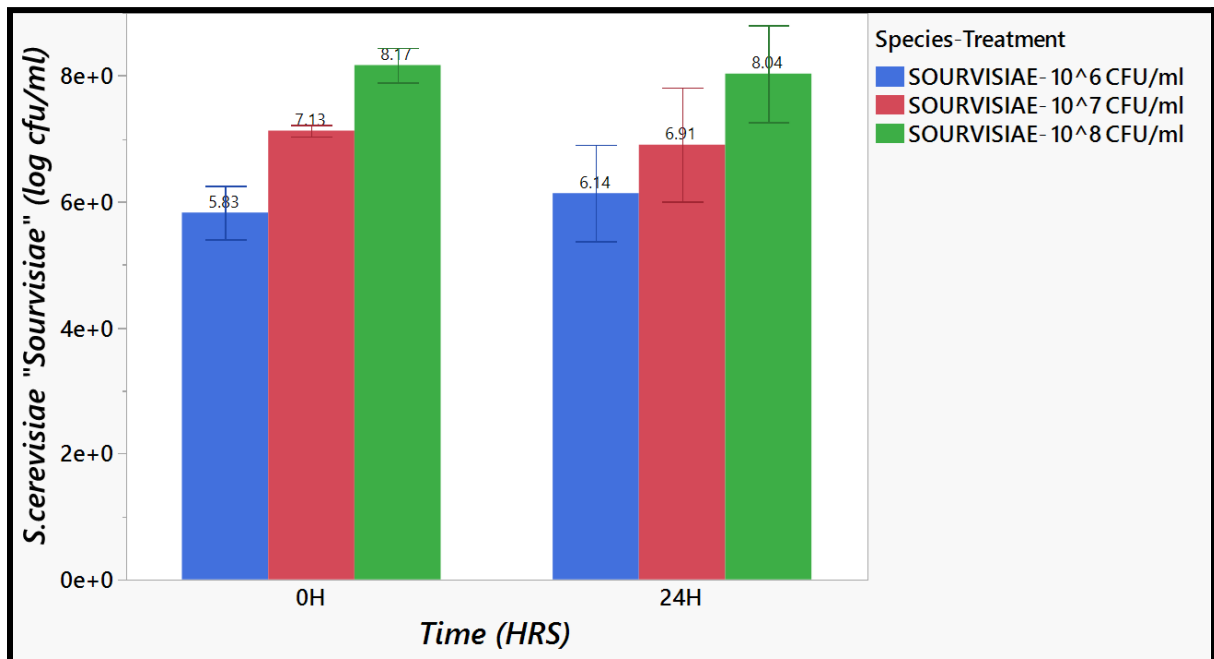


Figure 11. The concentration of *S. cerevisiae* “Sourvisiae®” before incubation (0H) and after fermentation (24H) with different yeast inoculation rates. Data expressed as mean \pm standard deviation of biological triplicates.

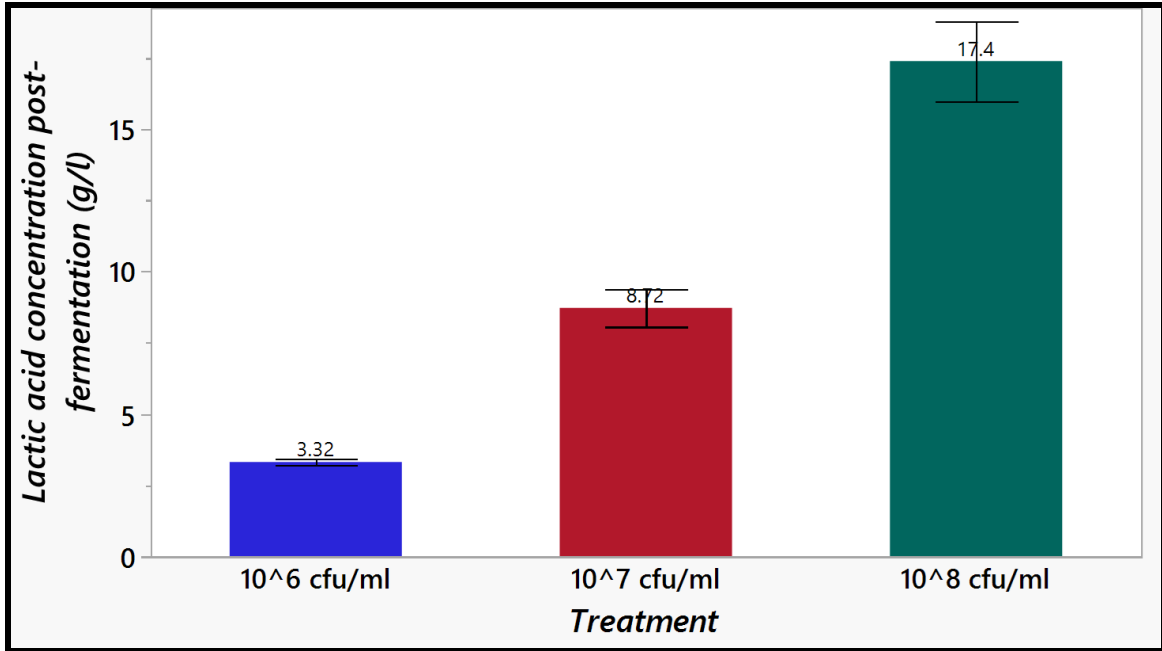


Figure 12. Amount of lactic acid produced by *S. cerevisiae* “Sourvisiae®” post-fermentation with different inoculation rates of yeast used. Data expressed as mean ± standard deviation of biological triplicates.

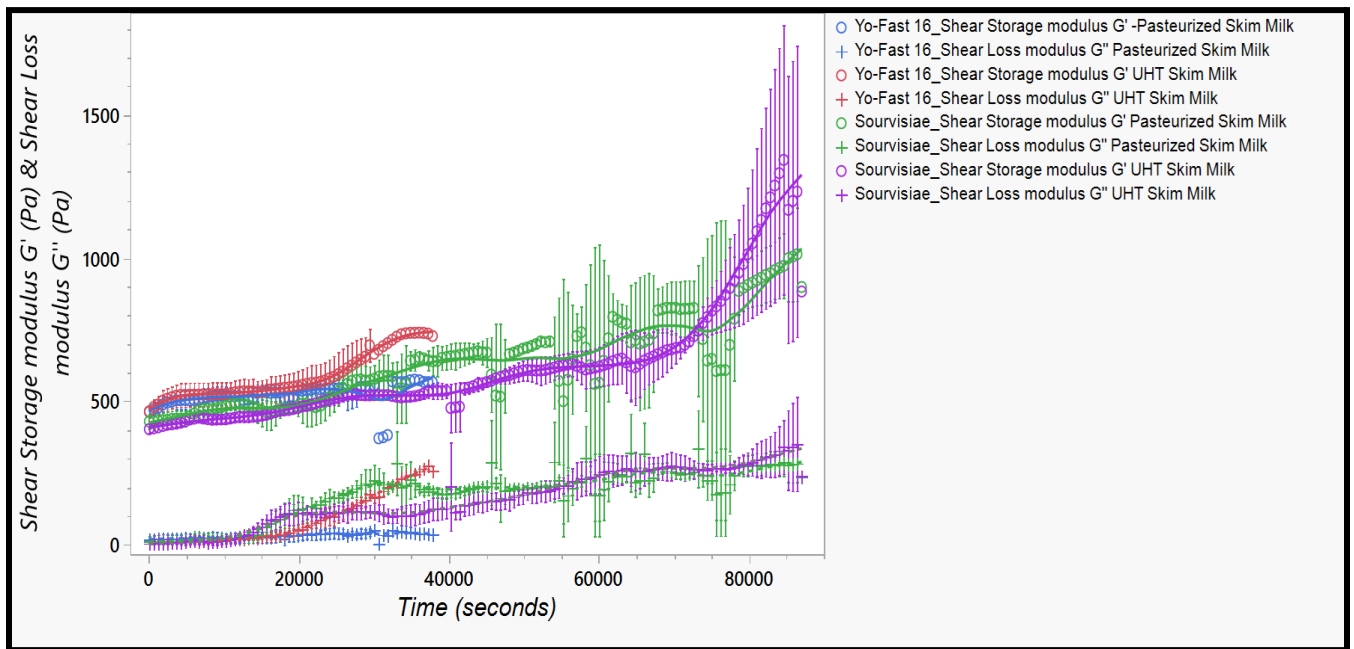


Figure 13: Comparative analyses between fermentation with *S. cerevisiae* “Sourvisiae®” and traditional bacterial starter culture “Yo-Fast 16”. The trial was conducted with both UHT skim milk and Pasteurized milk. Data expressed as mean ± standard deviation of biological triplicates.

Sensory Profile:

A Central location test (CLT) of n=78 was conducted with the sample prototype to determine the consumer insights on the ideation of the product. Two blind samples were provided- one was Lactose-Free Organic Plain Kefir - Lowfat and the other was yogurt beverage made with Sourvisiae®. Of the 78 participants, 59% were female, and 39.7% were male between 21-70 years of age. 50% of the participants never consumed a yogurt drink and 39.7% is familiar with the product. The sensory attributes were selected based on documented sensory attributes for yogurt [15][16].

The top report's analysis from the sensory data was: that 77% of the participants with a mean drop of 2.5 felt that the product was “Too Tart”. 60% of the participants with a mean drop of 0.8 felt the product to not have “not enough body/mouthfeel”.

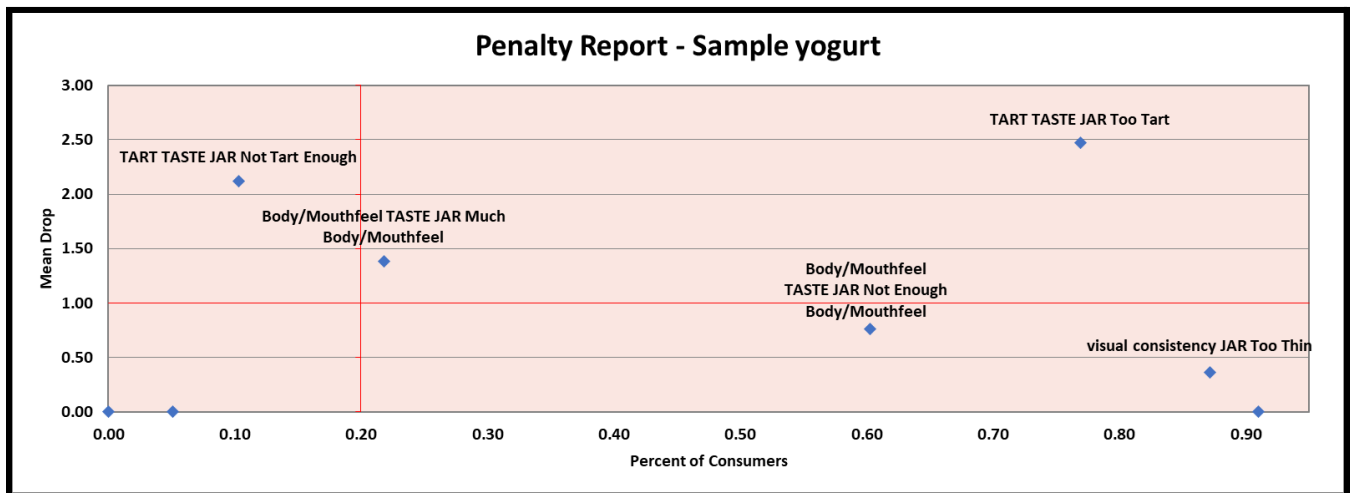


Figure 14: Penalty report analysis on the sensory profile of the Souvisiae fermented drinkable yogurt sample.

Appearance Liking				
Stat Method: Z-Test	Sample yogurt	Control	P Value	Confidence
Like it extremely	0%	1%	.316	68%
Like it very much	6%	10%	.385	62%
Like it moderately	23%	41%	.016	98%
Like It	29%	53%	.003	100%
Like it slightly	13%	19%	.275	72%
Neither like nor dislike it	21%	21%		
Dislike it slightly	14%	5%	.057	94%
Dislike It	23%	3%	.000	100%
Dislike it moderately	15%	3%	.005	99%
Dislike it very much	6%	0%	.023	98%
Dislike it extremely	1%	0%	.316	68%
Mean Score	5.09	6.27		
Visual Consistency JAR				
Slightly too thick	0%	9%	.007	99%
Too Thick	0%	9%	.007	99%
Just about the right texture	13%	74%	.000	100%
Too Thin	87%	17%	.000	100%
Slightly too thin	59%	14%	.000	100%
Much too thin	28%	3%	.000	100%
Mean Score	1.85	2.90		
OVERALL Liking of the drink				
Like it extremely	0%	0%		
Like it very much	0%	17%		
Like it moderately	3%	18%		

Like it slightly	3%	24%		
Neither like nor dislike it	5%	8%		
Dislike it slightly	8%	19%		
Dislike it moderately	18%	8%		
Dislike it very much	31%	5%		
Dislike it extremely	33%	1%		
Mean Score	2.38	5.55		
Post Hoc, HSD = 0.528	B	A		
Standard Deviation	1.48	1.82		
FLAVOR Liking				
Like it extremely	0%	1%	.316	68%
Like it very much	0%	17%	.000	100%
Like it moderately	0%	12%	.002	100%
Like It	0%	29%	.000	100%
Like it slightly	9%	22%	.026	97%
Neither like nor dislike it	4%	14%	.025	97%
Dislike it slightly	9%	21%	.042	96%
Dislike It	78%	14%	.000	100%
Dislike it moderately	14%	4%	.025	97%
Dislike it very much	32%	9%	.000	100%
Dislike it extremely	32%	1%	.000	100%
Mean Score	2.47	5.40		
SWEETNESS Liking				
Like it extremely	0%	1%	.316	68%
Like it very much	0%	15%	.000	100%
Like it moderately	0%	9%	.007	99%

Like It	0%	26%	.000	100%
Like it slightly	4%	19%	.003	100%
Neither like nor dislike it	23%	14%	.150	85%
Dislike it slightly	8%	23%	.008	99%
Dislike It	65%	18%	.000	100%
Dislike it moderately	15%	8%	.133	87%
Dislike it very much	24%	9%	.010	99%
Dislike it extremely	26%	1%	.000	100%
Mean Score	2.90	5.18		
SWEET TASTE				
Extreme SWEET taste	1%	0%		
Pronounced SWEET taste	4%	3%		
Moderate SWEET taste	1%	15%		
Slight SWEET taste	13%	42%		
No SWEET taste	81%	40%		
Mean Score	1.32	1.81		
Post Hoc, HSD = 0.251	B	A		
Bitter TASTE				
Extreme bitter taste	24%	0%	.000	100%
Pronounced bitter taste	22%	10%	.049	95%
Bitter Taste	46%	10%	.000	100%
Moderate bitter taste	9%	13%	.441	56%
Bitter Taste	45%	77%	.000	100%
Slight bitter taste	23%	31%	.279	72%
No bitter taste	22%	46%	.001	100%
Mean Score	3.04	1.87		

TART TASTE JAR				
Much too tart	54%	4%	.000	100%
Somewhat too tart	23%	26%	.709	29%
Too Tart	77%	29%	.000	100%
Just about the right level of tartness	13%	55%	.000	100%
Not Tart Enough	10%	15%	.338	66%
Somewhat not tart enough	3%	8%	.147	85%
Not tart enough	8%	8%		
Mean Score	4.13	3.10		
Texture Liking				
Like it extremely	0%	4%	.080	92%
Like it very much	3%	27%	.000	100%
Like it moderately	5%	29%	.000	100%
Like It	8%	60%	.000	100%
Like it slightly	12%	21%	.127	87%
Neither like nor dislike it	26%	8%	.003	100%
Dislike it slightly	14%	9%	.316	68%
Dislike It	41%	3%	.000	100%
Dislike it moderately	15%	1%	.001	100%
Dislike it very much	13%	1%	.005	100%
Dislike it extremely	13%	0%	.001	100%
Mean Score	3.95	6.60		
Body/Mouthfeel TASTE JAR				
Very much body/mouthfeel	5%	0%	.043	96%
Somewhat too much body/mouthfeel	17%	9%	.151	85%
Much Body/Mouthfeel	22%	9%	.026	97%

Just about the right amount of body/mouthfeel	18%	69%	.000	100%
Not Enough Body/Mouthfeel	60%	22%	.000	100%
Somewhat not enough body/mouthfeel	41%	21%	.006	99%
Not enough body/mouthfeel	19%	1%	.000	100%
Mean Score	2.47	2.86		
Refreshing Flavor liking				
Mean Score	1.74	2.14		
Aftertaste acceptability				
Yes	38%	81%	.000	100%
No	62%	19%	.000	100%
Mean Score	1.38	1.81		

Table 1: Sensory ratings for fermented unflavored drinkable yogurt developed using Sourvisiae (Sample) and commercial unflavored kefir (Control)



Figure 15. Word Cloud based on the sensory profile open-ended comments from participants based on the visual consistency of the Souvisiae fermented drinkable yogurt sample.



Figure 16. Word Cloud based on the sensory profile open-ended comments from participants based on the Overall Taste of the Souvisiae fermented drinkable yogurt sample.

During the fermentations outlined in the current research, Sourvisiae® caused the development of distinct sensory characteristics and transformed the sensory profile of the fermented beverage. Among the sensory modalities evaluated by the participants, the distinct areas of perception are appearance and overall flavor. As the product was advertised as a yogurt drink, participants came with an expectation to taste a thicker, milder tasting product. The yeasty, thin body and the tarty flavor were not acceptable to them. As we can see in the word cloud, most of the open-ended comments on visual consistency and overall taste were “thin”, “milk”, “watery” “yeast”, “sour”, “tart” and “bitter” respectively.

CONCLUSION

Findings from this study provide a foundational understanding of the fermentation parameters using Sourvisiae® as a starter culture for fermenting milk. It also helped conceptualize that Sourvisiae® has the potential to be used as an alternate culture 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. The prototype had a slightly tart, milk-like consistency with a refreshing fizziness, and a mildly sweet aftertaste when freshly prepared. However, with storage, it became more yeasty, tart, and tasted acidic imparting more of a bitter aftertaste which made the product unappealing to the consumers. This was an unflavored prototype designed to understand consumers' comments on this new type of fermented milk-based beverage. With the knowledge gained from this study, a shelf life study will help to understand the changes in the fermentation parameters with storage as well as its impact on nutritional and sensory profiles of the product. Further, a sensory acceptability test and a chemical analysis are required to better understand consumers' responses to these prototypes, and to optimize the sensory profiles accordingly.

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