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Effects of fermentation conditions on the physicochemical properties of plant-based yogurts

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Abstract

There is a growing demand for plant-based food alternatives in the industry with an increasing focus to simulate animal-based products. Plant-based yogurts have contributed significantly to this need, although there are several challenges that make it hard to imitate dairy yogurt's characteristics. Understanding the impact of different fermentation conditions is crucial for comprehending the production of such alternative food. The aim of this work was to understand the effect of different temperatures (39 °C, 42 °C and 45 °C) along with different times of fermentation (8, 16, and 24 hours) in two plant-based milks (almond and soy) on the physicochemical characteristics of yogurt. They were all compared with a dairy yogurt that served as the control. The physicochemical analysis included parameters such as pH, viscosity (mPas*s), color (L*, a*, and b*), dissolved oxygen (mg/L), brix (°), water holding capacity (%), and acidity (g/100mL). The findings revealed that these conditions have a significant effect on the physicochemical properties of almond and soy-based yogurts. For soy yogurt, the best options are those fermented at 39 °C for 24 hours and 42 °C for 24 hours, with the first one being more energy efficient. For almond yogurt, the ideal pH is achieved with fermentation at 39 °C for 16 hours, 39 °C for 24 hours, and 42 °C for 24 hours. The almond yogurt did not achieve ideal values of viscosity; therefore, thickeners agents should be used.

Keywords: almond, soy, temperature of fermentation, time of fermentation

Resumen

Existe una creciente demanda de alternativas alimentarias de origen vegetal en la industria, con un enfoque cada vez mayor de simular productos de origen animal. Los yogures a base de plantas han contribuido significativamente a esta necesidad, aunque existen muchos retos que dificultan la imitación de las características del yogur lácteo. Conocer el impacto de diferentes condiciones de fermentación es crucial para comprender la producción de este tipo de alimentos alternativos. El objetivo de este estudio fue demostrar el efecto de diferentes temperaturas (39 °C, 42 °C and 45 °C) junto con diferentes tiempos de fermentación (8, 16 and 24 horas) en dos leches a base plantas (almendra y soja). El análisis fisicoquímico incluyó parámetros como pH, viscosidad (mPas*s), color (L*, a* and b*), oxígeno disuelto (mg/L), Brix (°), capacidad de retención de agua (%) y acidez (g/100mL). Todos estos fueron comparados con un yogur lácteo que sirvió como control. Los resultados revelaron que estas condiciones tienen un efecto significativo en las propiedades fisicoquímicas de los yogures a base de soya y almendra. Para el yogur de soja, las mejores opciones son la fermentación a 39 °C durante 24 horas y 42 °C durante 24 horas, siendo la primera más eficiente energéticamente. Para el yogur de almendra, el pH ideal se logra con fermentación a 39 °C durante 16 horas, 39 °C durante 24 horas y 42 °C durante 24 horas. Sin embargo, el yogur de almendra no alcanzó valores ideales de viscosidad, por lo tanto, se deberían de usar agentes espesantes.

Palabras clave: almendra, soja, temperatura de fermentación, tiempo de fermentación

Introduction

The evolving consumer needs are driving continuous changes in food development. During the past decades, there has been a notable increase in the demand for plant-based alternatives. The projected value of the global plant-based food market is anticipated to reach USD 78.95 Billion by 2026 (Vantage Market Research, 2022). Also, plant-based foods sales grew 6.6% from 2021 to 2022 (Plant Based Foods Association, 2022).

Yogurt is the food produced by culturing milk and that contains lactic-acid-producing bacteria (Office of Federal Register, 2024). Its production involves a method where milk is acidified with specific bacteria, resulting in a creamy, often flavored product with an enhanced shelf life compared to its original form. It is rich in probiotics, fibers, vitamins and minerals (Fisberg & Machado, 2015).

The lactic acid bacteria (LAB) are gram-positive bacteria that mostly use carbohydrates as their main carbon source. They are usually round (cocci), or rod-shaped (Bacilli) and they can survive in low pH. There are more than 60 genera of LAB, however, the most used for food fermentation are *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Enterococcus,* and *Weisella* (Mokoena, 2017).

The standard procedure widely recognized for yogurt production typically involves milk pasteurization, followed by cooling to temperatures ranging from 43 to 45 °C. This cooled milk is then inoculated with a starter culture and undergoes fermentation at temperatures of approximately 42 to 45 °C until reaching the desirable pH (Dissanayake et al., 2014). However, detailed information regarding variations in temperatures and fermentation times remains limited.

In contrast to dairy yogurts, plant-based yogurt may exhibit weaker gel structures (McClements & Grossmann, 2022). Additionally, numerous other factors contribute to the challenges associated with producing plant-based yogurts, including variations in coagulation, the requirement for emulsifiers or thickening agents, and increased susceptibility to phase separation during storage (Montemurro et al., 2021).

Yogurts have many health benefits, the raw ingredients on their own have many benefits and nutrients, for example, soy-bean (*Glyxine max*) is abundant in folic acid, iso-flavonoids, and many other bioactive elements (Dukariya et al., 2020). However some individuals may be allergic to proteins found in milk [such as caseins (60%), β-lactoglobulin (9%), and α-lactalbumin (4%)] causing mild to severe reactions (Villafana et al., 2014). Typically, children exhibit a higher prevalence of allergy to cow's milk compared to soy milk. Cow milk allergy is observed in 2% to 3% of young children, whereas soy allergy affects around 0.4% of children (Savage et al., 2010). Besides, plant-based products are characterized by their low-calorie content, elevated unsaturated fatty acids, and the absence of cholesterol (Aydar et al., 2020). Also, many studies suggest that fermentation can reduce the allergenicity of plant-based foods (El Mecherfi et al., 2020).

Moreover, in countries where mammalian milk is both scarce and costly, plant-based dairy alternatives emerge as more economical products (Mäkinen et al., 2015). For individuals without access to dairy products, plant-based yogurts offer a valuable nutrient source. Consequently, in regions where animal-based protein is scarce due to production systems or socio-economic constraints, plant-based products offer a viable solution.

The plant-based yogurts are more environmentally friendly than the production of dairy since it stands as a big contributor to greenhouse gas emissions, contributing to climate change (Autio et al., 2023). Shifting to a diet with a greater emphasis on plant-based foods could lead to a substantial 48% decrease in greenhouse gas (GHG) emissions associated with food production (Clark et al., 2020).

In addition, animal welfare has become a pivotal factor leading individuals to shift away from consuming animal-derived products, prompting them to explore alternatives such as plant-based yogurts. Concerns regarding animal rights have become increasingly prominent among individuals. In fact, a study conducted in 2021 revealed that animal rights ranked as one of the primary social issues in America with a reported 33%, surpassing social justice at 28% and healthcare premiums at 22% (Feldmann et al., 2021).

The justification of this research is that the food industry is shifting as consumers increasingly seek plant-based alternatives for healthier and more sustainable choices. The substantial growth in the plant-based food market indicates this trend, driven by health consciousness, environmental awareness, and ethical concerns about animal welfare.

Understanding the elements in fermentation that affect the attributes and quality of plant-based yogurts is important in the context of production systems. Numerous factors can impact the final product, and this research focuses primarily on the fermentation temperature and duration time on two plant-based milks. The temperature is mainly defined by the microorganisms used (mesophilic and thermophilic) but it is important to know which of the temperature ranges described is the best for the development of quality attributes.

There is a limited understanding of the physicochemical structure of plant-based yogurt and this might impede greater adoption or substitution of their animal-based counterpart (Gupta et al., 2022). Studies have shown that soy yogurts have similar texture characteristics to traditional yogurts, from which, soy was the most favorite product by consumers (Grasso et al., 2020).

Therefore, this study aims to investigate how different fermentation conditions affect the physicochemical attributes of plant-based yogurts. The selected conditions assessed three fermentation temperatures (39 °C,42 °C, and 45 °C) and three fermentation durations (8 hours, 16 hours, and 24 hours), testing the combination of each on almond milk and soy milk. Also, the study analyzed the differences between the physicochemical properties of the plant-based yogurts compared to the dairy yogurt. The physicochemical characteristics of the different types of milk were also taken to determine the differences between milk and yogurt.

Materials and Methods

Study Location

The production of the plant-based yogurts and its corresponding physicochemical analysis was conducted at the University of Illinois at Urbana-Champaign in the United States of America.

Collection of Materials

The main raw materials utilized for yogurt preparation included Great Value brand soy milk and Silk brand almond milk, both purchased from Walmart in Urbana, Illinois. Also, Great Value brand dairy milk was bought from the same source to serve as the control group. These materials were chosen based on their availability, their reliability and consistency in preliminary trials. All the milk was refrigerated at 4 °C until used. The sugar used was Great Value Pure Cane Granulated Sugar.

The bacterial culture mixture used in the fermentation process was acquired from Cultures for health (Ohio, United States). The selected starter culture was composed of the following strains: *Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, and Bifidobacterium subsp. lactis*. The starter culture was refrigerated at 4 °C until it was used.

Ultimate Probiotic yogurt makers were acquired from the Ultimate company located at Cleveland, Florida, United States. This equipment was chosen for its efficiency and suitability for small-scale yogurt production.

Preparation of the Yogurt

The procedures for yogurt preparation were chosen following preliminary testing. Initially, yogurt was prepared using dairy milk as a base, with an 8-hour fermentation period at 43 ˚C, yielding yogurt with desirable qualities. Subsequently, yogurt was made using plant-based milk under the same conditions, with modifications made afterward to fermentation times. The combinations of the main experiment were determined based on the observations of the preliminary trials and a review of the literature.

Heating of the Milk

It should be noted that, as commercial products, all the milks used in the experiment were already pasteurized. The plant-based milks underwent a heat treatment process, raising their temperature to 85 °C for 15 minutes, following the recommendations of the commercial culture indications to make a yogurt with more viscosity (Cultures for Health, 2016). Throughout this procedure, constant agitation was applied.

For the dairy milk, which served as the control, a slight variation was introduced in the heating process. Instead of reaching 85 °C, the dairy milk was heated to 90 °C for 15 minutes. The temperature was chosen due to its ability to promote the aggregation of whey proteins with casein, thereby enhancing the gelation process (Xu et al., 2008). While the recommended heating time for milk is typically 10 minutes, a longer duration is required for thicker yogurt consistency (Smith, 2015). As a result, a duration of 15 minutes was selected.

Inoculation

The milk had to be cooled down to 45 °C to inoculate it with the starter culture. This cooling was necessary to prevent the bacteria from dying due to the elevated temperature. Ideally, the milk should be cooled down to a range of 43-45 °C (Cultures for Health, 2016).

In accordance with the methodology provided by Cultures for Health, one packet of the starter culture is used for approximately one liter of milk. To determine the appropriate amount of culture required for the experiment, one pack was weighed, which was 1.067 grams.

Each batch consisted of 500 mL of milk, from which 15 mL were set aside to create a paste with 15 grams of sugar and 0.53 grams of culture. The inoculated mixture or paste was prepared following the instructions provided in The Quick Start Guide for the Ultimate Probiotic Yogurt Maker (Brockie, n.d). Then each yogurt-maker cup was filled with 121 mL of milk and 6.5 mL of paste on each yogurt-maker cup.

Incubation/Fermentation

Each cup was placed in the yogurt maker, with specific time and temperature settings being applied. The treatments involved variations in time and temperature for fermentation, with three different temperature levels: 39 °C, 42 °C, and 45 °C and three different fermentation durations: 8 hours, 16 hours, and 24 hours. Also, the control was fermented with a fermentation temperature of 43 °C and 8 hours. Water at room temperature was added to each yogurt maker to ensure that the temperature of all yogurt cups remained consistent through convection. Treatments description is included in Table 1.

Table 1

Description of the treatments applied to plant-based yogurts.

Factor 1	Factor 2
Temperature of fermentation (°C)	Hours of fermentation
39	8
42	16
45	24

Note. °C: Degree Celsius

Physicochemical Analysis

The physicochemical analysis was performed on the plant-based yogurts and the dairy yogurt (control). The pH, viscosity, color, dissolved oxygen, and Brix were measured following 20 hours of refrigeration at 4 °C. The acidity and water holding capacity (WHC) were assessed after the samples had been frozen at -24 °C and then defrosted to 4 °C.

pH Measurements

The plant-based yogurts and the dairy yogurt (control) underwent pH analysis using a pH meter pH700 (APERA, Columbus, OH, USA), which operated within a range of 0 to 14. Prior to analysis, the instrument was calibrated and standardized using buffer solutions with pH values of 4.00 and 7.00.

Viscosity

The apparent viscosity (η) of the yogurts was measured at approximately 10 °C using a viscometer NDJ-5S (Bonvoisin, Shanghai, China). A 100 mL beaker was used to analyze thick yogurts, the beaker was filled with 80mL of samples. Thick plant-based yogurts underwent measurement with rotor number 3 at a speed of 6.0 revolutions per minute (rpm). The less viscous yogurt was analyzed with rotor 2 at the same speed. Soy milk and dairy milk were measured using rotor number 0 at a speed of 30 rpms, while almond milk was measured with the same rotor but at 12 rpms. The viscosity of each sample was measured following one minute of the equipment's operation.

Color

The same 30 mL of the samples were used to do the color analysis. The instrument used was the precise color reader (WR-10). A plastic wrap was used to cover the color meter sensor to prevent it from getting dirty from contact with the samples. The values L*, a*, and b* were recorded.

Oxygen

The dissolved oxygen analyzer (D09100) was utilized to measure the dissolved oxygen levels in milligrams per liter (mg/L). The sensor was introduced on each yogurt without moving it and the values were recorded after one minute had elapsed.

Brix

Brix levels were measured with a digital refractometer MA871 (Milwaukee). One drop of each sample was used for the measurement.

Water Holding Capacity (WHC)

The water holding capacity was determined using the centrifugation method (Feng et al., 2019). Ten grams were used of each sample and centrifuged at 2,500 rpm for 10 minutes. This coagulated the samples and separated the water from it. The samples were weighed after taking the separated water out of the testing tubes. The water holding capacity was determined with the following formula.

$$\text{WHC (\%)} = \frac{\text{Weight of the drained gels}}{\text{Weight of the sample (10 grams)}} \times 100 \qquad [1]$$

Acidity

For the acidity analysis, 0.5 grams of the sample were diluted with 9.5 grams of distilled water for plant-based samples and 1 gram of dairy yogurt with 49 grams of distilled water for the control. The acidity was determined by the acidity Meter (ATAGO) by adding three droplets in the instrument's sensor. A calibration curve was used to document the grams of acid per 100 mL of yogurt.

Experimental Design

The experiment included treatments with a factorial arrangement with a three-way ANOVA that included two types of milk, three fermentation times, and three fermentation temperatures, resulting in 18 treatments. Each treatment was replicated four times, leading to a total of 72 experimental units plus the control. A Duncan´s multiple range test was performed, incorporating repeated measures over time, and a Duncan´s separation was applied among the different fermentation temperatures. Samples underwent analysis for pH, viscosity, color, dissolved oxygen, brix, water holding capacity, and acidity. The statistical analysis was made using SAS Software, considering the different fermentation times (8,16, and 24 hours and temperatures (38,42, and 45 ˚C) along with the different kinds of milks used. The plant-based milks were compared to the control which was dairy yogurt with 43 ˚C and 8 hours of fermentation.

Results and Discussion

Physicochemical Properties of the Substrates

As shown in Table 2, each substrate (dairy, almond and soy milk) has specific physicochemical properties that influence the characteristics of the final yogurt. The pH of all three milks differs significantly. Dairy milk tends to be slightly more acidic, whereas almond milk is more alkaline, likely due to additives such as Calcium Carbonate, which raise pH levels. Almond milk exhibits higher viscosity compared to soy and dairy milk, possibly attributed to increased gellan gum content and addition of soy protein isolate. Dairy milk has the highest brix value, followed by soy milk, with almond milk having the lowest.

Table 2

Physicochemical analysis of milk before fermentation.

Milk	pH	Viscosity (mPa)	Color L*	Color a*	Color b*	Dissolved oxygen (mg/L)	Brix (˚)
Dairy milk	6.62±0.01^{c}	2.80 ±0.04^{b}	65.41±2.68^{a}	- 0.65±0.13^{b}	1.46±0.18^{b}	4.83 ±0.33^{a}	12.10±0.34^{a}
Almond milk	7.55±0.03^{a}	42.00±2.20^{a}	61.93±2.27^{a}	0.35±0.08^{a}	4.32±0.48^{a}	4.40±0.18^{a}	7.20±0.17^{c}
Soy milk	6.78±0.02^{b}	7.20±0.30^{b}	60.43±1.14^{a}	0.03±0.08^{a}	3.87±0.26^{a}	4.05±0.26^{a}	10.30±0.24^{b}
CV(%)	0.15	7.40	3.41	0.17	10.25	6.01	2.62

Note. $^{a-c}$: means with a different letter are statistically different ($P \leq 0.05$); mg/L: milligrams per Liter; mPa*s: millipascal second; °: degrees; CV(%): Coefficient of variation

Changes in Milk Composition Post-fermentation

Yogurt undergoes significant transformations compared to its raw material which can be milk or its plant-based alternative. As shown from Tables 3 to 8, following fermentation, the pH of all milks decreases, while their viscosity increases, and their Brix values decrease. This transformation is attributed to lactic acid bacteria, which generate acids during fermentation. The increase in viscosity is due to protein modifications and fat emulsification, whereas the utilization of sugars by bacteria leads to a reduction in Brix values.

Table 3

Physicochemical analysis of dairy milk vs. dairy yogurt.

Milk	pH	Viscosity (mPa)	Dissolved oxygen (mg/L)	Brix (˚)
Dairy milk	6.62 ±0.01 ^{a}	2.80±0.04^{b}	4.83±0.33^{a}	12.10±0.34^{a}
Dairy yogurt	4.31±0.06^{b}	1,4447±1,323.00^{a}	5.18±0.31^{a}	10.10±0.02^{b}
CV (%)	0.76	12.94	6.4	3.02

Note. Dairy yogurt: 42 ˚C and 8 hours of fermentation ^{ab}: means with a different letter are statistically different (P ≤ 0.05); mg/L: milligrams per Liter; mPa*s: millipascal second; °: degrees; CV (%): Coefficient of variation

Table 4

Color analysis of dairy milk vs. dairy yogurt

Milk	Color L*	Color a*	Color b*
Dairy milk	65.41±2.68 ^{a}	-0.65±0.13^{a}	1.46±0.18^{b}
Dairy yogurt	74.67±2.52^{a}	-1.01±0.17^{a}	3.55±0.17^{a}
CV (%)	3.72	0.25	12.41

Note. Dairy yogurt: 42 ˚C and 8 hours of fermentation ^{ab}: means with a different letter are statistically different (P ≤ 0.05); mg/L: milligrams per Liter; mPa*s: millipascal second; °: degrees; CV (%): Coefficient of variation

Table 5

Physicochemical analysis of almond milk vs. almond yogurt.

Milk	pH	Viscosity (mPa)	Dissolved oxygen (mg/L)	Brix (˚)
Almond milk	7.55±0.03^{a}	42.00±2.20^{b}	4.40±0.18^{a}	7.20±0.17^{a}
Almond Yogurt	4.55±0.03^{b}	3927.00±639.00^{a}	5.33±0.25^{a}	6.40±0.43^{a}
CV (%)	0.35	22.78	4.50	4.86

Note. The almond milk is being compared with almond yogurt made with 16 hours of fermentation and 39 ˚C; ^{ab}: means with a different letter are statistically different (P ≤ 0.05); mg/L: milligrams per Liter; mPa*s: millipascal second; °: degrees; CV (%): Coefficient of variation

Table 6

Color analysis of almond milk vs. almond yogurt

Milk	Color L*	Color a*	Color b*
Almond milk	61.93±2.27^{a}	0.35±0.08^{a}	4.32±0.48^{a}
Almond Yogurt	64.74±0.98^{a}	0.39±0.11^{a}	5.07±0.20^{a}
CV (%)	2.76	0.16	7.77

Note. The almond milk is being compared with almond yogurt made with 16 hours of fermentation and 39 ˚C; ^{ab}: means with a different letter are statistically different (P ≤ 0.05); mg/L: milligrams per Liter; mPa*s: millipascal second; °: degrees; CV (%): Coefficient of variation

Table 7

Physicochemical analysis of soy milk vs. soy yogurt

Milk	pH	Viscosity (mPa)	Dissolved oxygen (mg/L)	Brix (˚)
Soy milk	6.78±0.02^{a}	7.20±0.30^{b}	4.05±0.26^{a}	10.30±0.24^{a}
Soy yogurt	4.34±0.02^{b}	13,270.00±654.00^{a}	4.70±0.32^{a}	7.4±0.21^{b}
CV (%)	0.32	6.96	6.66	2.53

Note. The soy milk is being compared with soy yogurt made with 24 hours of fermentation and 39 ˚C; ^{ab}: means with a different letter are statistically different (P ≤ 0.05); mg/L: milligrams per Liter; mPa*s: millipascal second; °: degrees; CV (%): Coefficient of variation

Table 8

Color analysis of soy milk vs. soy yogurt

Milk	Color L*	Color a*	Color b*
Soy milk	60.43±1.14^{b}	0.03±0.08^{a}	3.87±0.26^{a}
Soy yogurt	73.85±2.27^{a}	-0.81±0.19^{a}	7.70±0.36^{b}
CV (%)	2.67	0.24	5.40

Note. The soy milk is being compared with soy yogurt made with 24 hours of fermentation and 39 ˚C; ^{ab}: means with a different letter are statistically different (P ≤ 0.05); mg/L: milligrams per Liter; mPa*s: millipascal second; °: degrees; CV (%): Coefficient of variation

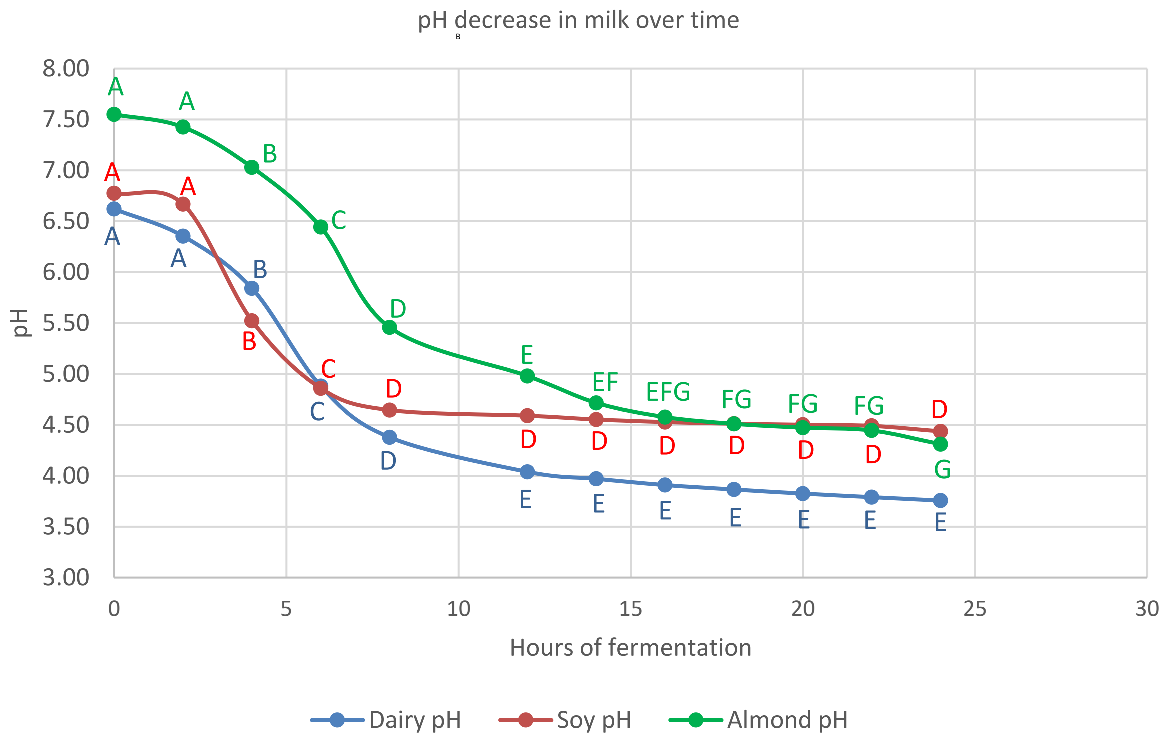
pH Behavior During Fermentation

As shown in Figure 1, dairy milk experiences its most significant pH drop between the second and eighth hours of fermentation. However, in the 12th hour, its pH becomes more stable, and it does not appear to have any significant changes. The soy milk shows the greatest decrease in pH between the second and eighth hours of fermentation, with significant pH changes during this period. However, from the 12th hour onward, the pH appears to stabilize with no significant changes. It seems that soy milk has the same pH behavior as dairy milk.

Figure 1 also illustrates the pH changes in almond milk over 24 hours of fermentation. The most significant pH decrease occurs between the second and 12th hours. Between the 14th and 24th hours, the pH decreases at a slower rate. In contrast, dairy and soy milk do not show significant changes in pH during the final hours of fermentation.

Figure 1

Decreasing pH of dairy, soy and almond milks.



Note. A-E: indicate statistically significant differences in pH in different fermentation hours in dairy yogurt; A-D: indicate statistically significant differences in pH in different fermentation hours in soy yogurt; A-G: indicate statistically significant differences in pH in different fermentation hours in almond yogurt.

Physicochemical Analysis

pH

The pH of yogurt decreases primarily through the production of lactic acid, and this is crucial for its distinctive taste (Harper et al., 2022). According to the standard of identity of yogurt, it must have a pH of 4.6 or lower (United States Food and Drug Administration, 2023). The interaction of the temperature of fermentation, duration of fermentation, and the type of milk used significantly impacted the pH values ($F=14.03$, $p<0.0001$). Also, a double interaction shows that the factors that have a more significant impact are the temperature and milk used ($F=63.17$, $p<0.0001$).

In general, a trend of lower pH values in lower temperatures of fermentations was shown. Understanding the temperature preferences of the primary yogurt-making bacteria, *S. thermophilus* and *L. delbrueckii*, is important. *L. delbrueckii* prefers temperatures closer to 30 °C. Lower temperatures favor greater population growth of this bacteria, leading to a more pronounced pH decrease. While *S. thermophilus* grows better in temperatures around 45 °C. Adjusting growth conditions to favor *Lactobacillus* over *Streptococcus* can yield a slightly more acidic product while prioritizing *Streptococcus* can enhance the product's aromatic qualities (Tojo Sierra et al., 2006).

The faster reduction of pH in soy-based yogurt can be attributed to its higher carbohydrate and sugar content compared to almond-based yogurt. The soy milk contains 9 g of carbohydrates and 7 g of sugars per serving (240mL), while almond milk contains 6 g of carbohydrates and 5 g of sugars per serving (240mL). The greater availability of substrate in soy provides bacteria with resources for fermentation, resulting in increased production of metabolites such as lactic acid. The dairy yogurt shows the lowest pH values while the almond yogurt presents the highest pH values. Dairy milk features a significant sugar and carbohydrate content, with 12 g of total sugars and 12 g of carbohydrates per serving (240mL). This amount is notably higher compared to both soy and almond milk.

Based on the analysis and separation of means as shown in Table 9, several plant-based yogurts were found to be like dairy yogurt in terms of pH. The soy yogurts that met this standard included those Fermented at 39 ˚C for 8 hours (4.80), at 39 ˚C for 16 hours (pH 4.52), at 42 ˚C for 16 hours (pH 4.56), at 39 ˚C for 24 hours (pH 4.34), at 42 ˚C for 24 hours (pH 4.5) and at 45 ˚C for 24 hours (4.63±0.02). Almond yogurts that also met the standard included those fermented at 39 ˚C for 16 hours (pH 4.55), at 39 ˚C for 24 hours (pH 4.63), and at 42 ˚C for 24 hours (pH 4.46). These indicate that both soy and almond yogurts can achieve pH levels comparable to dairy yogurt under specific fermentation conditions. However, according to the FDA´s standard of identity, which requires a pH below 4.6, the treatment at 45 ˚C for 24 hours resulting in a pH of 4.63 and the treatment of soy with

39 ˚C for 8 hours resulting in a pH of 4.8 do not comply with this standard and cannot be considered yogurts (United States Food and Drug Administration, 2023).

Table 9

pH result of the plant-based yogurts.

	Temperature of fermentation (˚C)	Substrates	Hours of fermentation		
			8	16	24
Treatments	39	soy	4.80±0.04^{cdefg}	4.52±0.10^{efg}	4.34±0.02^{g}
		almond	5.04±0.03^{cd}	4.55±0.03^{defg}	4.43±0.07^{fg}
	42	soy	4.86±0.04 ^{cdef}	4.56±0.10^{defg}	4.50±0.02^{efg}
		almond	5.74±0.44^{b}	4.97±0.28^{cde}	4.46±0.06^{efg}
	45	soy	5.15±0.26^{c}	4.89±0.07^{cdef}	4.63±0.02^{defg}
		almond	6.59±0.61^{a}	5.79±0.18^{b}	6.57±0.61^{a}
Control	dairy*				4.32±0.06 ^{g}
CV(%)					4.17

Note. *dairy: control made with 8 hours of fermentation and 43 °C; $^{a\text{-}g}$: means with a different letter are statistically different (P ≤ 0.001); °C: : Degrees Celsius; CV (%): Coefficient of variation

Viscosity

The ANOVA analysis indicates significant interactions between time and substrate used in the fermentation that impacts the final viscosity of the yogurt ($F=36.75$, $p<0.001$). Overall, soy-based yogurts exhibit higher viscosity compared to almond-based yogurts, likely due to differences in fat content. Both the heating pretreatment of the substrate and the fat content significantly impact the gelation rate and final rigidity of the yogurt, as fat molecules trap proteins, forming a more stable gel network (Zhao et al., 2021). As mentioned in other studies, the higher heating temperatures and fat content accelerate gelation onset and increase gel rigidity (Xu et al., 2008). Despite undergoing identical heat treatment, soy milk contains 4 g of fat per serving (240mL), whereas almond milk contains 2.5g of fat per serving (240mL), explaining the difference in viscosity because the increased amount of fat content results in the formation of larger fat globules contributing to a firmer gel.

Protein is a very important factor for yogurt's texture. The more protein in milk, the thicker the yogurt is. In plant-based milks the proteins undergo denaturation when heat treatment is applied. Two specific proteins found in soy, glycinin, and conglycinin, possess the ability to create ordered structures composed of strands 10-15nm thick (Hermansson, 1986). The soy and almond proteins precipitate at a pH level of around 4 to 5, known as the isoelectric point (Freitas et al., 2017). It is important to mention that the almond milk used contains soy protein isolate in its formulation to increase the amount of protein. Consequently, both types of milk contain 8 g of protein per serving (240mL). In dairy milk, the casein (protein) clusters in milk by forming a three-dimensional mesh when exposed to the lactic acid created by culturing. Heating milk before culturing denatures one of the main whey proteins, lactoglobulin (β-lacto-globulin), which allows it to join in the mesh and effectively increase the amount of protein in the milk that will be available to thicken the yogurt, these proteins interact with κ-casein.

Plant-based yogurts have a lower protein content, and the proteins coagulate differently, this makes it hard to achieve a similar texture compared to dairy yogurt. Therefore, it is recommended to use thickening agents to replicate its texture (Sim et al., 2021). As mentioned before, a greater quantity of protein corresponds to a denser consistency in yogurt. During the fermentation process, starter cultures reduce the milk pH to the isoelectric point of casein which makes the protein precipitate (pH 4.6) (Asaduzzaman et al., 2021). This explanation may clarify why dairy yogurts have higher values of viscosity than plant-based yogurts.

As shown in Table 10, there is a trend of increase of viscosity with longer fermentation durations, particularly in soy yogurts fermented at 39 ˚C and 45 ˚C from 8 to 16 hours. The production of exopolysaccharides (EPS) by the lactic acid bacteria contribute to a better viscosity in yogurts (Han et al., 2016). This may explain why at longer fermentations the yogurt shows a better viscosity because the bacteria have more time to produce EPS. According to the mean separation, the only yogurts with statistically similar viscosities as the dairy yogurt are the soy yogurts with the following combinations: 39 ˚C for 24 hours (13,270 mPa*s), 42 ˚C for 24 hours (13,340 mPas*s), and 45 ˚C for 24 hours (12,705 mPa*s). None of the almond yogurts had viscosities like dairy yogurts, indicating that achieving comparable thickness might require additional ingredients, such as thickening agents such as protein extracts or inulin (Montemurro et al., 2021). However, the almond-based yogurt achieved a viscosity like that of drinkable yogurt.

Table 10

Viscosity (mPas/s) results of the plant-based yogurts.

	Temperature of fermentation(˚C)	Substrates	Hours of fermentation		
			8	16	24
Treatments	39	soy	9,576±251de	11,095±330^{cd}	13,270±654^{abc}
		almond	3,070±289^{gh}	3,927±639^{g}	3,666±321^{g}
	42	soy	8,199±1,591^{ef}	10,459±964^{d}	13,340±1192^{ab}
		almond	2,649±126^{gh}	2,279±116^{gh}	2,534±189^{gh}
	45	soy	6,556±1,222^{f}	11,414±2,212^{bcd}	12,705±738^{abc}
		almond	78±22^{i}	2,104±157^{ghi}	1,061±763^{hi}
Control	dairy*				14,448±1,323^{a}
CV(%)					12.75

Note. *dairy: control made with 8 hours of fermentation and 43 °C; $^{a\text{-}i}$: means with a different letter are statistically different (P ≤ 0.001); mPa*s: millipascals seconds; °C: Degrees Celsius; CV (%): Coefficient of variation

Color

The L* values, which represent the level of lightness, are most significantly affected by the type of milk used (F=96.45, p<0.001). As shown in Table 11, soy yogurts present higher L* values than almond yogurts, indicating that soy yogurts are lighter in color. Conversely, the two treatments with the lowest L* value, suggesting darker colors are both almond yogurts. Only soy yogurts are statistically like the control.

The a* is relative to the green-red colors, where negative values are toward the green and positive toward the red colors. Among soy yogurts, the a*values generally range from -0.81 to -0.47, indicating a tendency towards greenness. In contrast, almond yogurts show a shift towards positive a* values, ranging from 0.06 to 0.40, indicating a tendency towards redness. The a* values also show significance depending on the type of milk used (F=293.69, p<0.001).

The b* values represent the color component indicating yellowness and blueness, where positive values indicate yellowness and negative values indicate blueness. The value b* was more significantly affected by the type of milk used (F=147.21, p<0.001). There is a tendency where soy yogurts have higher b* values ranging from 6.62 to 7.75 (yellowness) and the almond yogurts generally presented lower b* values, from 4.26 to 5.44 (blueness). Tables 11, 12 and 13 show the different values in lightness, yellowness and blueness of the yogurts in the different times of fermentation.

Table 11

Color analysis results of the plant-based yogurts in eight hours of fermentation.

Treatments		Color Lab		
Substrate	Temperature	L*	a*	b*
Dairy	43	74.67 ± 2.52^{a}	-1.01 ± 0.17^{c}	3.55 ± 0.17^{b}
Soy	39	73.24 ± 1.23^{ab}	-0.47 ± 0.12^{b}	6.62 ± 0.34^{a}
Soy	42	73.59 ± 3.10^{ab}	-0.58 ± 0.19^{bc}	6.97 ± 0.77^{a}
Soy	45	72.95 ± 2.46^{ab}	-0.62 ± 0.15^{bc}	6.65 ± 0.54^{a}
Almond	39	66.48 ± 1.36^{bc}	0.06 ± 0.06^{a}	5.27 ± 0.27^{ab}
Almond	42	63.73 ± 1.18^{c}	0.19 ± 0.08^{a}	4.66 ± 0.28^{b}
Almond	45	63.41 ± 1.92^{c}	0.22 ± 0.12^{a}	4.26 ± 0.46^{b}
C.V(%)		3.77	0.28	10.68

Note. Dairy is the control made with 8 hours of fermentation and 43 °C; $^{a-c}$: means with different letter are statistically different (P ≤ 0.001); C.V(%): Coefficient of variation

Table 12

Color analysis results of the plant-based yogurts in 16 hours of fermentation.

Treatments		Color Lab		
Milk	Temperature	L*	a*	b*
Dairy	43	74.67±2.52[a]	-1.01±0.17[b]	3.55±0.17[c]
Soy	39	71.92±4.80[ab]	-0.65±0.30[b]	7.44±1.52[a]
Soy	42	70.50±3.12[abc]	-0.54±0.12[b]	6.79±0.53[ab]
Soy	45	70.2±5.14[abc]	-0.54±0.22[b]	6.83±1.03[ab]
Almond	39	64.74±0.98[bcd]	0.39±0.11[a]	5.07±0.20[bc]
Almond	42	64.23±1.48[cd]	0.33±0.06[a]	4.68±0.36[c]
Almond	45	63±1.28[d]	0.35±0.08[a]	4.6±0.32[c]
C.V(%)		3.77	0.28	10.68

Note. Dairy is the control made with 8 hours of fermentation and 43 °C; [a-d]: means with different letter are statistically different (P ≤ 0.05);

C.V(%): Coefficient of variation

Table 13

Color analysis results of the plant-based yogurts in 24 hours of fermentation.

Treatments		Colors lab		
Milk	Temperature	L*	a*	b*
Dairy	43	74.67±2.52[a]	-1.01±0.17[c]	3.55±0.17[c]
Soy	39	73.85±2.27[a]	-0.81±0.19[bc]	7.7±0.36[a]
Soy	42	72.97±3.18[a]	-0.75±0.29[bc]	7.75±0.88[a]
Soy	45	72.78±2.29[a]	-0.52±0.22[b]	7.56±0.65[a]
Almond	39	65.25±2.67[b]	0.28±0.19[a]	5.44±0.61[b]
Almond	42	63.93±1.30[b]	0.21±0.07[a]	4.87±0.35[bc]
Almond	45	64.69±1.53[b]	0.37±0.08[a]	4.82±0.33[bc]
C.V(%)		3.77	0.28	10.68

Note. Dairy is the control made with 8 hours of fermentation and 43 °C; [a-c]: means with different letter are statistically different (P ≤ 0.05);

C.V(%): Coefficient of variation

Oxygen

According to the ANOVA results included in Table 14, the most significant interaction is between time and temperature of fermentation ($F=7.78$, $p<0.001$) meaning that the dissolved oxygen (DO) in the yogurts was mainly affected by these factors. Different temperatures can affect the metabolic rate of microorganisms altering the consumption of oxygen also over time, the microbial activity varies (Plasquy et al., 2021).

The dissolved oxygen has an effect in yogurt production because DO retards the acidification of the product, the bacteria such as *L. delbrueckii ssp.* and *S. thermophilus* are facultative anaerobic bacteria that can grow in aerobic environments and when fermentation begins these bacteria reduce the amount of DO in yogurt (Horiuchi et al., 2009). Unfortunately, a decrease in DO levels in the yogurts compared to the initial DO levels of the dairy, almond and soy milks was not evident. Even though the bacteria in the yogurt should reduce the DO levels during fermentation, the introduction of air during the data collection could have counteracted this reduction, leading to DO levels that did not show a significant decrease. The only treatments that are statistically different from the control are the almond at 42 ˚C for 8 hours of fermentation, almond at 45 ˚C for 8 hours of fermentation, almond at 45 ˚C for 16 hours of fermentation and almond at 42 ˚C for 24 hours of fermentation. These results might be due to less bubble production and reduced air incorporation during the measurement of DO values. Since more movement of the equipment leads to more air being incorporated into the samples, it is possible that the equipment was kept steadier during the measurement of these yogurt samples, preventing additional air from being introduced.

Table 14

Dissolved oxygen (mg/L) results of the plant-based yogurts.

	Temperature of fermentation (˚C)	Substrates	Hours of fermentation 8	16	24
Treatments	39	soy	4.40 ± 0.33^{bcdefg}	4.70 ± 0.63^{abcde}	4.70 ± 0.32^{abcde}
		almond	4.20 ± 0.36^{cdefg}	5.30 ± 0.25^{a}	4.90 ± 0.25^{abcd}
	42	soy	4.40 ± 0.42^{bcdef}	4.80 ± 0.1^{abcde}	4.30 ± 0.22^{cdefg}
		almond	3.50 ± 0.37^{g}	4.70 ± 0.26^{abcde}	4.10 ± 0.56^{defg}
	45	soy	4.40 ± 0.51^{bcdefg}	4.30 ± 0.25^{cdefg}	5.20 ± 0.37^{ab}
		almond	3.70 ± 0.21^{fg}	4.0 ± 0.42^{efg}	4.70 ± 0.57^{abcde}
Control	dairy*				5.20 ± 0.31^{abc}
CV (%)					8.22

Note. *dairy: control made with 8 hours of fermentation and 43 °C; $^{a-g}$: means with a different letter are statistically different (P ≤ 0.001); mg/L: milligram per Liter; °C: Degrees Celsius CV (%): Coefficient of variation

Brix

The measurement of soluble solids in food products, known as Brix, is utilized to quantify the sugar content in yogurt (Adetuyi et al., 2008). According to Table 15 the interaction of temperature and milk is significant ($F=6.02$, $p<0.001$).

The dairy milk had more sugar than the almond and soy milk. The main sources of sugars for lactic acid bacteria in yogurt production depend on the type of milk used. In dairy milk, lactose is the primary substrate, which *S. thermophilus and L. delbrueckii subsp. bulgaricus* metabolize into lactic acid. In soy milk, the main fermentable sugar is sucrose, as well as the almond milk which contains sucrose, glucose and fructose in varying amounts. *S. thermophilus* can use sucrose for fermentation while *L. bulgaricus* cannot (Bernat et al., 2015; Lestiyani et al., 2014).

Table 15

Brix (˚) results of the plant-based yogurts.

	Temperature of fermentation (˚C)	Substrates	Hours of fermentation 8	16	24
Treatments	39	soy	$7.30±0.47^{cdefg}$	$8.10±0.51^{bc}$	$7.40±0.21^{bcdefg}$
		almond	$6.50±0.56^{efgh}$	$6.40±0.43^{fgh}$	$6.10±0.51^{h}$
	42	soy	$7.80±0.26^{bcd}$	$7.80±0.13^{bcd}$	$7.30±0.29^{cdefg}$
		almond	$6.40±0.36^{gh}$	$6.70±0.15^{defgh}$	$6.80±0.26^{defgh}$
	45	soy	$7.30±1.00^{cdefg}$	$7.60±0.27^{bcd}$	$7.50±0.26^{bcdef}$
		almond	$8.40±0.38^{b}$	$7.80±0.06^{bcd}$	$7.60±0.55^{bcde}$
Control	dairy*				$10.10±0.33^{a}$
CV (%)					5.69

Note. *dairy: control made with 8 hours of fermentation and 43 °C; $^{a-h}$: means with a different letter are statistically different ($P ≤ 0.001$); °C: Degrees Celsius; CV (%): Coefficient of variation; °: Degrees; CV (%): Coefficient of variation

Water Holding Capacity (WHC%)

The capacity of yogurt to retain water is referred to as its water-holding capacity. According to the ANOVA, the interaction between time, temperature and the type of milk is significant (F=4.30, p<0.001) so the impact of WHC varies depending on the specific combination of temperature and milk type.

In general, soy yogurts exhibit a greater water-holding capacity compared to almond yogurts. The capacity of yogurt to retain water is primarily determined by its solids and total protein contents (Senaka Ranadheera et al., 2012).

With a higher total solids content, yogurt tends to exhibit a greater water-holding capacity (WHC), as these solids are considered components available for binding with water. This phenomenon may account for the differences observed between soy and almond-based yogurts, as soy milk typically contains a higher total solids content. For instance, per serving (240mL), soy milk contains 8 g of protein, 4 g of fat, 9 g of carbohydrate, and more minerals, while almond milk contains 8 g of protein, 2.5 g of fat, 6 g of carbohydrates, along with fewer minerals.

When comparing these findings to a 2022 study, which also emphasized the superior water-holding capacity of soy-based yogurts (85.80±1.56%), it is important to note that the values obtained in this research are comparatively lower. This may be attributed to differences in measurement times; in this study, the values were assessed after freezing the samples which may have diminished their capacity to retain water. Water holding capacity decreases while frozen temperature storage (Li et al., 2023),

As shown in Table 16, there is a trend toward longer fermentation times, which has led to an increase in water holding capacity, also the soy yogurts with 39 ˚C and 16 hours of fermentation, 42 ˚C and 16 hours of fermentation, 39 ˚C and 24 hours, 42 ˚C and 24 hours and 45 ˚C and 24 hours are like the control. None of the almond yogurts were like the control. Almond has the lowest WHC which is why the industry usually uses hydrocolloids to reduce syneresis (Grasso et al., 2020).

Table 16

Water holding capacity results of the plant-based yogurts.

	Temperature of fermentation (˚C)	Substrates	Hours of fermentation		
			8	16	24
Treatments	39	soy	46.06±1.96^{d}	51.21±0.69^{ab}	54.46±0.86^{a}
		almond	30.26±1.10^{hi}	32.55±0.68^{fghi}	34.58±0.79^{fg}
	42	soy	46.14±2.17^{cd}	50.38±0.79^{abc}	54.69±0.57^{a}
		almond	30.08±0.64^{i}	31.44±0.78^{fghi}	34.47±0.39^{fgh}
	45		41.51±3.36^{e}	48.91±0.84^{bcd}	54.69±2.25^{a}
		almond	30.54±4.75^{ghi}	32.08±0.97^{fghi}	35.91±2.33^{f}
Control	dairy*				55.05±0.69^{a}
CV (%)					4.15

Note. *dairy: control made with 8 hours of fermentation and 43 °C; $^{a\text{-}i}$: means with a different letter are statistically different (P ≤ 0.001); WHC (%): Water holding capacity in percentage; °C: Degrees Celsius; CV (%): Coefficient of variation

Acidity

According to the ANOVA results contained in Table 17, all the interactions are significant in the acidity values ($F=111.38$, $p<0.001$). Acidity values are typically higher in soy yogurt than in almond yogurt. To avoid an overly sour taste, it is recommended to have acidity levels between 7.0 to 9.0 mg/g (Souza Oliveira et al., 2011). According to this information, the levels of acidity are above the recommended acid levels. It is important to state that the control showed lower acidity levels. This results also differ from those reported by Grasso (2020), which indicated that dairy yogurt has higher acidity compared to plant-based yogurts (Grasso et al., 2020). The statistical analysis indicates that at longer fermentation times the acidity increased.

The difference in acidity between the plant-based yogurts may be due to the presence of other acids after the fermentation process. In the fermentation of soy milk, it has been found that there is a presence of various organic acids such as citric acid, fumaric acid and malic acid (Leonardo

León, 2017). Additionally, it is possible that heterofermentation occurred in the plant-based yogurts. *B. lactis* can convert sugars into acetic acid and other volatile compounds, which can result in a more acidic yogurt (Pourbara et al., 2020). A study of various milks found that soy milk contains more raffinose, stachyose and verbascose than almond milk (Huang et al., 2023). This is evident as almond milk exhibits much lower acidity values than soy milk. In general, longer fermentation presents an increase trend of acidity and lower fermentation temperatures show a trend of higher acidity levels. The longer fermentation times lead for the bacteria to produce more acid and the lower fermentation temperatures may be due to the work of *L. delbrueckii* that produces acids in low temperatures which is related to the decrease in pH.

Table 17

Acidity (g/100 mL) results of the plant-based yogurts.

	Temperature of fermentation (˚C)	Substrates	Hours of fermentation		
			8	16	24
Treatments	39	soy	3.28±0.21[d]	3.81±0.13[bc]	4.84±0.12[a]
		almond	0.74±0.09[h]	2.25±0.22[f]	2.47±0.09[f]
	42	soy	2.82±0.05[e]	3.60±0.03[c]	3.93±0.05[b]
		almond	0.73±0.10[h]	1.61±0.04[g]	2.23±0.06[f]
	45	soy	2.49±0.09[f]	2.97±0.10[e]	3.87±0.12[bc]
		almond	0.27±0.05[i]	3.07±0.18[de]	0.60±0.06[h]
Control	dairy*				1.46±0.02[g]
CV (%)					4.53

Note. *dairy: control made with 8 hours of fermentation and 43 °C; [a-g]: means with a different letter are statistically different (P ≤ 0.05) according to the time of fermentation (columns); [xyz]: means with a different letter are statistically different (P ≤ 0.05) according to the temperature of fermentation (rows); g/100mL: grams of lactic acid per 100mL of sample; °C: Degrees Celsius ; CV (%): Coefficient of variation

Dairy yogurt and plant-based yogurt selection

The quality of yogurt is typically assessed based on pH, viscosity, and acidity (Table 18). In this experiment, none of the treatments matched the control´s acidity. However, considering the other two characteristics, soy yogurt with 39 ˚C and 24 hours of fermentation and 42 ˚C and 24 hours of fermentation are potential choices. The first treatment has a pH of 4.34 and a viscosity of 13,270 mPa/s and the second treatment has a pH of 4.63 and a viscosity of 12,705 mPa/s. The three treatments were like the dairy yogurt, which had a pH of 4.32 and viscosity of 14,448 mPa*s. The best option between the two soy yogurts would be the one with a lower temperature (39 ˚C) because it is a more efficient treatment requiring less energy.

For almond yogurt, none of the samples had the ideal viscosity, so thickeners will need to be used. However, the almond yogurts that achieved the ideal pH are those fermented for 39 ˚C and 16 hours, 39 ˚C and 24 hours, and 42 ˚C and 24 hours. Table 18 shows all the selected treatments.

Table 18

Selected Plant-based Yogurts and their Statistically Similar Properties to Dairy Yogurt.

Tempe-rature	Hours of fermen-tation	Yogurt	pH	Viscosity (mPa)	Color L*	Color a*	Color b*	Dissolved oxygen (mg/L)	Water Holding Capacity (%)
		*Dairy yogurt	4.32 ±0.06	14,448 ±1323	74.67 ±2.52	-1.01 ±0.17	3.55 ±0.17	5.2 ±0.31	55.05 ±0.69
39	24	Soy yogurt	4.34 ±0.02	13,270 ±654	73.85 ±2.27	-0.81 ±0.19	--	4.7 ±0.32	54.46 ±0.86
42	24	Soy yogurt	4.50 ±0.02	13,340 ±1192	72.97 ±3.18	-0.75 ±0.29	--	4.3 ±0.22	54.69 ±0.57
39	16	Almond yogurt	4.55 ±0.03	--	--	--	--	5.3 ±0.25	--
39	24	Almond yogurt	4.43 ±0.07	--	--	--	--	4.9 ±0.25	--
42	24	Almond yogurt	4.46 ±0.06	--	--	--	4.87 ±0.35	--	--

Note. Temperature (˚C): Temperature of fermentation; Hours: Hours of fermentation; mg/L: milligrams per Liter; mPa*s: millipascal second; °: degrees; %: percentage; g/100mL: grams per 100 milliliters; --: Not statistically similar

Conclusions

The transformation from milk to yogurt involves significant physicochemical changes, particularly in pH and viscosity, for the plant-based milks and dairy milk. During fermentation, the pH decreases, and the viscosity increases. Additionally, Brix values decrease in both soy yogurt and dairy yogurt. These changes result from the metabolism of lactic acid bacteria.

The physicochemical properties of yogurt are significantly influenced by the time of fermentation, the temperature of fermentation, and the type of milk used. The findings suggest that lower temperatures and longer fermentation times typically result in yogurts with lower pH values, while lower temperatures enhance the ability of *L. delbrueckii* to reduce pH. Additionally, almond-based yogurts generally have slightly higher pH and lower viscosity compared to soy yogurt.

On the other hand, when yogurts are fermented at higher temperatures and for shorter durations, they do not achieve the desired viscosity like traditional yogurts. Soy yogurts exhibit characteristics more like dairy yogurts, such as comparable viscosity and water-holding capacity.

In general, the best soy yogurt is the one made at 39 ˚C and 24 hours of fermentation because it is more efficient and the recommended 8 hours of fermentation would not achieve ideal yogurt characteristics. However, for the almond yogurt the ideal treatments would involve fermentation at 39 ˚C and 16 hours, 39 ˚C and 24 hours, and 42 ˚C and 24 hours of fermentation, this yogurts are more alike drinkable yogurts.

Recommendations

The research revealed the significant effect of time, temperature, and type of milk used in the fermentation of yogurts; therefore, future research should explore alternative plant-based milk like cashews and investigate the incorporation of thickening agents in plant-based yogurts such as almond yogurt. Additionally, conducting microbiological analysis to understand bacterial growth during fermentation would make a better understanding of the dynamics of bacterial population growth under varying conditions.

A sensory analysis is recommended for future research to relate the physicochemical analysis with the acceptability of the consumers of the selected treatments. Considering the utilization of raw plant materials instead of commercialized milk could further enhance the understanding of plant-based yogurt fermentation. Further investigation should include finding the acids produced during plant-based yogurt fermentation and comparing them with the dairy yogurt.

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Appendices

Appendices A

Almond milk: Silk brand

Appendices B

Soy milk: Great Value brand

Appendices C

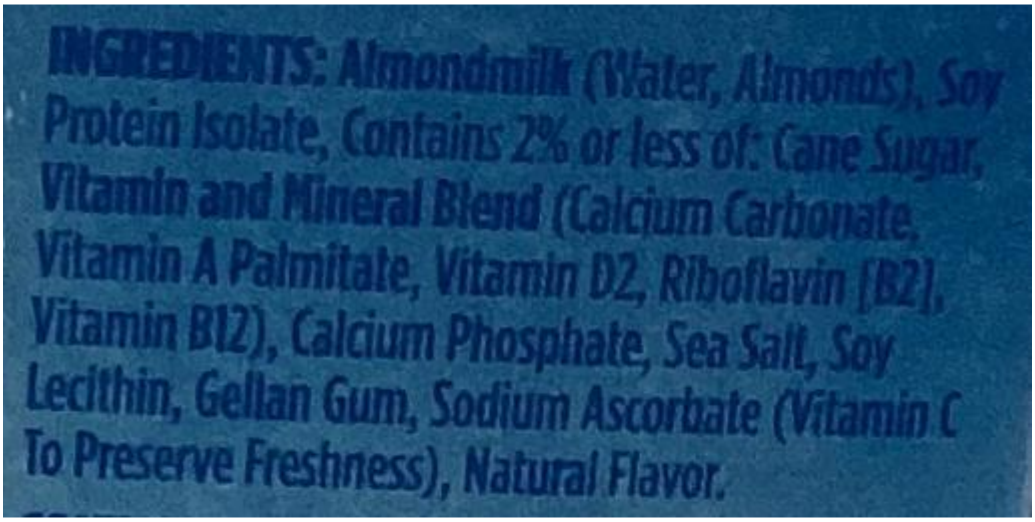
Dairy milk: Great Value brand

Appendices D

Yogurt Maker: Ultimate Probiotic Yogurt maker

Appendices E

List of ingredients of almond milk (Silk)



Appendices F

List of ingredients of soy milk (Great Value)

Appendices G

List of ingredients of dairy milk (Great Value)

Appendices H

pH, viscosity and dissolved oxygen in 8 hours of fermentation

Treatments		pH	Viscosity (mPa/s)	Dissolved oxygen (mg/L)
Milk	Temperature			
Dairy	43	4.32±0.02^{d}	14,448±1,323^{a}	5.20±0.31^{a}
Soy	39	4.80±0.04^{cd}	9,576±251^{b}	4.40±0.33^{ab}
Soy	42	4.86±0.04^{cd}	8,199±1,591^{bc}	4.40±0.42^{ab}
Soy	45	5.15±0.26^{bc}	6,556±1,222^{c}	4.4±0.51^{ab}
Almond	39	5.04±0.03^{c}	3,070±289^{d}	4.20±0.36^{ab}
Almond	42	5.74±0.44^{b}	2649±126de	3.50±0.37^{b}
Almond	45	6.59±0.61^{a}	78±22^{e}	3.70±0.21^{b}
CV(%)		4.17	12.75	8.22

Note. *Dairy: control made with 8 hours of fermentation and 43 °C; $^{a\text{-}e}$: means with a different letter are statistically different ($P \leq 0.001$); mPa/s: millipascals seconds; mg/L: milligrams Liter; °C: : Degrees Celsius; CV (%): Coefficient of variation

Appendices I

pH, viscosity and dissolved oxygen in 16 hours of fermentation

Treatments		pH	Viscosity (mPa/s)	Dissolved oxygen (mg/L)
Milk	Temperature			
Dairy	43	4.32 ± 0.02^{c}	$14{,}448 \pm 1{,}323^{a}$	5.20 ± 0.31^{a}
Soy	39	4.52 ± 0.10^{bc}	$11{,}095 \pm 330^{b}$	4.70 ± 0.63^{ab}
Soy	42	4.56 ± 0.10^{bc}	$10{,}459 \pm 964^{b}$	4.80 ± 0.1^{ab}
Soy	45	4.89 ± 0.07^{bc}	$11{,}414 \pm 2{,}212^{b}$	4.30 ± 0.25^{ab}
Almond	39	4.55 ± 0.03^{bc}	$3{,}927 \pm 639^{c}$	5.30 ± 0.25^{a}
Almond	42	4.97 ± 0.28^{b}	2279 ± 116^{c}	4.70 ± 0.26^{ab}
Almond	45	5.79 ± 0.18^{a}	2104 ± 157^{c}	4.0 ± 0.42^{b}
CV(%)		4.17	12.75	8.22

Note. *Dairy: control made with 8 hours of fermentation and 43 °C; $^{a\text{-}c}$: means with a different letter are statistically different ($P \leq 0.001$); mPa/s: millipascals seconds; mg/L: milligrams Liter; °C: : Degrees Celsius; CV (%): Coefficient of variation

Appendices J

pH, viscosity and dissolved oxygen in 24 hours of fermentation

Treatments		pH	Viscosity (mPa/s)	Dissolved oxygen (mg/L)
Milk	Temperature			
Dairy	43	4.32±0.02^{b}	14,448 ±1,323^{a}	5.20±0.31^{a}
Soy	39	4.34±0.02^{b}	1,3270±654^{a}	4.70±0.32^{ab}
Soy	42	4.50±0.02^{b}	13,340±1192^{a}	4.30±0.22^{ab}
Soy	45	4.63±0.02^{b}	12,705±738^{a}	5.20±0.37^{a}
Almond	39	4.43±0.07^{b}	3,666±321^{b}	4.90±0.25^{ab}
Almond	42	4.46±0.06^{b}	2,534±189^{b}	4.10±0.56^{b}
Almond	45	6.57±0.61^{a}	1,061±763^{b}	4.70±0.57^{ab}
CV(%)		4.17	12.75	8.22

Note. *Dairy: control made with 8 hours of fermentation and 43 °C; $^{a-b}$: means with a different letter are statistically different (P ≤ 0.001); mPa/s: millipascals seconds; mg/L: milligrams Liter; °C: : Degrees Celsius; CV (%): Coefficient of variation

Appendices K

Brix, water holding capacity and acidity in 8 hours of fermentation

Treatments		°Brix	Water holding capacity (%)	Acidity (g/100mL)
Milk	Temperature			
Dairy	43	10.10±0.33[a]	55.05±0.69[a]	1.46±0.02[b]
Soy	39	7.30±0.47[bc]	46.06±1.96[b]	3.28±0.21[a]
Soy	42	7.80±0.26[b]	46.14±2.17[b]	2.82±0.05[a]
Soy	45	7.30±1.00[bc]	41.51±3.36[b]	2.49±0.09[a]
Almond	39	6.50±0.56[c]	30.26±1.10[c]	0.74±0.09[c]
Almond	42	6.40±0.36[c]	30.08±0.64[c]	0.73±0.10[c]
Almond	45	8.40±0.38[b]	30.54±4.75[c]	0.27±0.05[c]
CV(%)		5.69	4.15	4.53

Note. *Dairy: control made with 8 hours of fermentation and 43 °C; [a-c]: means with a different letter are statistically different ($P \leq 0.001$); °: degrees; %: percentage; g/100mL:grams per 100 milliliters °C: : Degrees Celsius; CV (%): Coefficient of variation

Appendices L

Brix, water holding capacity and acidity in 16 hours of fermentation

Treatments		°Brix	Water holding capacity (%)	Acidity (g/100mL)
Milk	Temperature			
Dairy	43	10.10±0.33[a]	55.05±0.69[a]	1.46±0.02[e]
Soy	39	8.10±0.51[b]	51.21±0.69[ab]	3.81±0.13[a]
Soy	42	7.80±0.13[bc]	50.38±0.79[ab]	3.60±0.03[a]
Soy	45	7.60±0.27[bcd]	48.91±0.84[b]	2.97±0.10[c]
Almond	39	6.40±0.43[d]	32.55±0.68[c]	2.25±0.22[d]
Almond	42	6.70±0.15[cd]	31.44±0.78[c]	1.61±0.04[d]
Almond	45	7.80±0.06[bc]	32.08±0.97[c]	3.07±0.18[b]

Note. *Dairy: control made with 8 hours of fermentation and 43 °C; [a-d]: means with a different letter are statistically different (P ≤ 0.001); °: degrees; %: percentage; g/100mL:grams per 100 milliliters °C: : Degrees Celsius; CV (%): Coefficient of variation

Appendices M

Brix, water holding capacity and acidity in 16 hours of fermentation

Treatments		˚Brix	Water holding capacity (%)	Acidity (g/100mL)
Milk	Temperature			
Dairy	43	10.10±0.33[a]	55.05±0.69[a]	1.46±0.02[c]
Soy	39	7.40±0.21[b]	54.46±0.86[a]	4.84±0.12[a]
Soy	42	7.3±0.29[b]	54.69±0.57[a]	3.93±0.05[a]
Soy	45	7.50±0.26[b]	54.69±2.25[a]	3.87±0.12[a]
Almond	39	6.10±0.51[c]	34.58±0.79[b]	2.47±0.09[b]
Almond	42	6.80±0.26[bc]	34.47±0.39[b]	2.23±0.06[b]
Almond	45	7.60±0.55[b]	35.91±2.33[b]	0.6±0.06[d]
CV(%)		5.69	4.15	4.53

Note. *Dairy: control made with 8 hours of fermentation and 43 °C; [a-d]: means with a different letter are statistically different ($P \leq 0.001$); ° : degrees; %: percentage; g/100mL:grams per 100 milliliters °C: : Degrees Celsius; CV (%): Coefficient of variation